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ROLE OF TRACE ELEMENTS AND OXIDATIVE STRESS IN POLYCYSTIC OVARY SYNDROME (PCOS) INFERTILITY

MASTER DEGREE THESIS

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THESIS NOTIFICATION

I declare that all the information in this study prepared in accordance with the thesis writing rules is completely cited to the source of all kinds of information and statements which are obtained and provided in the frame of scientific and academic rules and not belong to me.

Rzgar Tawfeeq KAREEM

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PREFACE

Infertility is the most concerned human health problem; it is defined as inability of a sexually active, non-contraception couple to achieve conception in one year Jungwirth et al (2012) conveys noteworthy individual, societal and money related results Ruder et al. (2008). Statistically female factors are responsible for 40%-50% of infertility cases while the others are due to male factor as well as combined female/male factors and unexplained infertility (Agarwal et al., 2008). clinical and biochemical hyperandrogenemia, amenorrhea, hirsutism, and infertility (Amini et al., 2015). Polycystic ovary syndrome (PCOS) is a standout amongst the most widely recognized endocri-enopathy in ladies, influencing around 5-10% of the populace. PCOS is a heterogeneous issue portrayed by menstrual anomalies

There is a growing evidence of possible role of free radicals and Oxidative stress (OS), in infertility (Jaiswar et al., 2006). The first one is recognized as molecular species have the ability of independent presence as well as comprising more than one unpaired electrons, making them comparatively active and paramagnetic. Free radicals are shaped as oxygen metabolism natural byproducts and assist the aim of burning bacteria and reject body matter. However, they become poisonous and begin injuring body tissues via a process called oxidative stress when free radicals are out of control. Furthermore, the second one has been demonstrated in many of the causes of infertility, such as endometriosis, PCOS, unexplained infertility, tubal infertility, and recurrent pregnancy loss (Agarwal et al., 2006).

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ABBREVIATIONS AND SYMBOLS

Abbreviation	Description
AOs	: Anti-Oxidants
CAT	: Catalyze
SOD	: Super Oxide Dismutase
LH	: Luteinizing Hormone
FSH	: Follicle-Stimulating Hormone
PCOS	: Polycystic Ovarian Syndrome
GRD	: Glutathione Reductase
OS	: Oxidative Stress
ROS	: Reactive Oxygen Species
LPO	: Lipid Peroxidation

ÖZET

YÜKSEK LİSANS TEZİ

PCOS INFERTILİTESİNDE YARIŞ ELEMANLARININ ROLÜ VE OKSİDATİF STRESİ

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Polikistik over sendrom, kadınlarda yaygın olarak görülen bir endokrinik rahatsızlık olarak kabul edilir. Üreme dönemindeki kadınların yaklaşık% 15'inde bu rahatsızlık görülür. Bu çalışma, bazı iz elementlerin (Magnezyum (Mg), Çinko (Zn), Bakır (Cu) ve Selenyum (Se)), bazı hormonların (LH, FSH ve Testosteron) yanı sıra LPO, CAT gibi enzimlerin Polikistik Over Sendromlu (PCOS) kadınlarda infertilite üzerine etkileri araştırıldı. İki grup kadın araştırıldı, birinci grupta 14 sağlıklı ve doğurgan kadın (kontrol), ikinci grupta ise 64 kısır kadın vardı. Verilerin analizi için, graphpad prizması 7.0 kullanıldı. Bu çalışmada elde edilen sonuçlara göre kısır kadınlardaki, Cu, Mg ve Se seviyelerinin daha düşük olduğunu, ancak Zn düzeylerinin kontrol grubuna göre daha yüksek olduğu bulunmuştur. Bununla birlikte, yalnızca Cu ve Mg istatistiksel olarak anlamlıydı ($p < 0.05$). Kontrol grubu ile PCOS'lu hastalar arasında LPO, SOD ve CAT düzeyleri açısından fark yoktu. Dahası, gruplar arasında LH, FSH ve F-testosteron düzeylerinde önemli farklılıklar vardı. Yapılan araştırma sonucunda, sağlıklı ve doğurgan grupta LPO'nun FSH ile anlamlı şekilde ilişkili olduğunu bulundu. Buna ek olarak, hem F-testosteron hem de testosteron ile ağırlık ve VKİ arasında anlamlı korelasyon vardı. Ayrıca, eser elementler ile ilgili, yalnızca Se uzun boylu. İnfertil grupta, eser elementler ile diğer incelenen faktörler arasında anlamlı bir korelasyon bulunmamıştır. Bununla birlikte, FSH ile LPO ve VKİ arasında anlamlı korelasyon vardı. Buna ek olarak, LH ve VKİ arasında anlamlı bir korelasyon vardı. Bu araştırmadaki sonuçlar, PCOS'a yeni bakış açıları elde etmek için daha ileri çalışmalarla incelenecektir.

Anahtar kelimeler: Eser element, PCOS, Oksidatif stres.

ABSTRACT

MS THESIS

ROLE OF TRACE ELEMENTS AND OXIDATIVE STRESS IN PCOS INFERTILITY

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The Graduate School of Natural and Applied Science of Siirt University
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In women, polycystic ovarian disorder is extensively acknowledged endocrine problem. It influences around 15% of women of reproductive period. This study aimed to inspect the role of some trace elements like magnesium (Mg), zinc (Zn), copper (Cu) and selenium (Se)), some hormones like (LH, FSH, Testosterone, and Free Testosterone) and LPO, CAT and SOD in the ladies with (PCOS). Two groups of women were investigated, the first group involved 14 fertile women (control) and the second group included 65 infertile women. For analyzing the data, graph pad prism 7.0 was utilized. The study found that the Cu, Mg and Se levels were lower, however Zn levels was higher in infertile or PCOS group in comparison to the control group. Nonetheless, only Cu and Mg were statistically significant ($p < 0.05$). There was no difference between control and patients with PCOS regarding the level of LPO, SOD and CAT. Moreover, there were noteworthy contrasts in the level of LH, FSH, Testosterone and Free Testosterone between groups. The investigation found that in fertile group, LPO is significantly correlated with FSH. In addition, there were significant correlation between both Free testosterone and testosterone with weight and BMI. Furthermore, regarding trace elements, only Se with tall. In infertile group, no significant correlation was found between trace elements and all the other examined factors. However, FSH was correlated significantly with LPO and BMI. Additionally, there was significant correlation between LH and BMI. The results in this research ought to be examined by further studies for obtaining new insights into PCOS.

Key words: Trace element, PCOS, Oxidative stress and infertility

1. INTRODUCTION

Infertility is the most concerned human health problem; it is defined as inability of a sexually active, non-contraception couple to achieve conception in one year Jungwirth et al. (2012) conveys huge individual, societal and monetary outcomes Ruder et al (2008). Statistically female factors are responsible for 40%-50% of infertility cases while the others are due to male factor as well as combined female/male factors and unexplained infertility (Agarwal et al., 2008).

(PCOS) is a standout amongst the most widely recognized endocrinopathy in ladies, influencing around 5-10% of the populace. PCOS is a heterogeneous issue described by menstrual anomalies, clinical and biochemical hyperandrogenemia, amenorrhea, hirsutism, and infertility (Amini et al., 2015).

There is a growing evidence of possible role of free radicals and Oxidative stress (OS), in infertility (Jaiswar et al., 2006). The first one is recognized as molecular species have the ability of independent presence as well as comprising more than one unpaired electrons, making them comparatively active and paramagnetic. Free radicals are shaped as oxygen metabolism natural byproducts and assist the aim of burning bacteria and reject body matter. However, they become poisonous and begin injuring body tissues via a process called oxidative stress when free radicals are out of control. Furthermore, the second one has been demonstrated in many of the causes of infertility, such as endometriosis, PCOS, unexplained infertility, tubal infertility, and recurrent pregnancy loss (Agarwal et al., 2006).

1.1. Female Infertility

Female infertility is defined as the inability to accomplish a pregnancy following one year of unprotected intercourse. It has been reported that, 4–6% of married couples in South Asia, 7–10% in China and 10% in USA have infertility problems (Oskay et al., 2010). Female infertility occurs due to many causes including:

- Uterine or cervical abnormalities
- Endometriosis
- fallopian tubes abnormalities
- Ovulation disorders

- Polycystic ovary syndrome (PCOS)
- Other causes (Genetic causes & physical factors e.g. weight, age)

Several circumstances of infertility might be avoided via the following steps:

- ✓ **Healthy lifestyle maintenance:** increase caffeine and alcohol intake and smoking, and excessive exercise are all related to fertility decrease, therefore ought to be evaded. Having a balanced and nutritious diet, fruits and vegetables and maintaining of normal body weight are related to improved fertility prospects.
- ✓ **Treatment or prevention present illnesses:** fertility prospects are increased by classifying and controlling long-lasting illnesses like diabetes, hypothyroidism and hyperthyroidism.
- ✓ Rapid treatment of STDs.
- ✓ **Early parenthood:** After the age 27, fertility begins to fall and drops at a somewhat bigger proportion beyond 35 years of age.

1.2. Polycystic Ovary Syndrome PCOS

(PCOS) is a condition portrayed by ovulatory brokenness, hyperandrogenism, and polycystic ovaries. It is the most well-known endocrine variation from the norm in conceptive matured ladies, with the Rotterdam Criteria, demonstrating the pervasiveness of PCOS at around 18 %. (Aponte, A and Agarwal, A, 2013).

PCOS is a syndrome of ovarian dysfunction. Its cardinal features are hyperandrogenism and polycystic ovary morphology. Its clinical Manifestations may incorporate menstrual inconsistencies, indications of androgen abundance, and corpulence. Clinically, diagnosing a woman as having PCOS implies an increased risk for infertility, dysfunctional bleeding, endometrial carcinoma, obesity, and many other diseases (Azziz et al., 2009). PCOS is an ovarian dysfunction resulted from androgens, which constrain Folliculogenesis and cause polyfollicular morphology. After that, it distracts the menstrual periods and result in anovulation.

1.3. Female Reproductive Hormones

The control of reproduction in females is complex. The anterior pituitary hormones cause the release of the hormones FSH and LH. In addition, estrogens and progesterone are released from the developing follicles. Estrogen is the reproductive hormone in

females that assists in endometrial regrowth, ovulation, and calcium absorption; it is also responsible for the secondary sexual characteristics of females (Davis, S. R., and Tran, J., 2001).

The ovaries are a primary site of testosterone synthesis. Whether there is direct secretion of testosterone by the adrenal glands is controversial. The ovaries and adrenals both produce androstenedione and dehydroepiandrosterone (DHEA), with the adrenals also being the main source of DHEA-sulfate (DHEAS). Androstenedione and testosterone increase in the middle third of the menstrual cycle, as well as in the luteal phase. Testosterone is further metabolized to the potent androgen dihydrotestosterone (DHT) or aromatized to estradiol (E2) in target organs and peripheral tissues.

Prolactin hormone secreted by the pituitary gland. The role of prolactin is to stimulate milk production in pregnant women. It affects a woman's ovulation and menstrual cycles, which is why women who are breastfeeding rarely get pregnant. High levels of prolactin (hyperprolactinaemia) is one of the real causes of female infertility. Prolactin affects the menstrual cycles by inhibiting the two hormones necessary for ovulation: follicle stimulating hormone (FSH) and gonadotropin releasing hormone (GnRH) (Ajibola, M., Oloruntoba, A. C., and Valeria, 2012).

1.4. Ovulation and Menstrual Cycle

Female infants have 6 to 7 million oocytes at 20 weeks of gestation, when progressive atresia occurs, resulting in 1 to 2 million oocytes at birth, approximately 25,000 at the age of 37 years, and 1000 at the age of 51 years (Rogerio A. Lobo, 2005). In the short period of maturity preceding rupture the Graafian follicle increases rapidly in size, a process characterized by changes in the theca internal cells and by a loosening of the ovum in its enveloping granulosa, the ovum itself attains the stage of maturation (Haterius, Hans O 1937). Ovulation created by the interplay between hypothalamic, and ovarian hormones, bringing about various changes in the female reproductive tract. It is traditionally divided into two phases (follicular and luteal) or three phases (follicular, ovulatory, and luteal), based on ovarian function (Naama W. Constantini, 2005). Menstruation is governed by 2 ovarian hormones, estrogen and progesterone. Estrogen affects different tissue types on many different levels, thereby initiating or mediating a

multitude of biological functions. Progesterone also acts on numerous tissues, but the effects are more limited and less studied (Miranda et al., 2009).

1.5. Antioxidants (AOs)

Antioxidants are equipped for balancing out or deactivating ROS before they assault cells. What's more, completely basic for keeping up ideal cell and foundational wellbeing and prosperity (PERCIVAL, MARK 1998). The interplay between ROS and AOs is important in maintaining health. Antioxidants are then subjected to extensive research as they protect cells against the damaging effects of (ROS) (Silva, et al., 2010).

People have advanced exceedingly complex AO frameworks (enzymatic and non-enzymatic), which work synergistically and in mix with each other to ensure the phones and organ frameworks of the body against ROS harm (Rahman, Khalid, 2007).

Currently, antioxidants utilization for managing women with PCOS has fascinated many interests. In these women, oxidative stress is developed by some features of PCOS like obesity and abdominal adiposity, excess of androgen, and insulin resistance.

1.5.1. Enzymatic Antioxidants

Enzymatic AOs are otherwise called characteristic AOs; they kill abundance ROS and keep it from harming the phone structure. Enzymatic AOs are made out of superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GRD). Other enzymes, such as glutathione transferase, ceruloplasmin or hemoxygenase may also participate in enzymatic control of ROS and their products (Kefer et al., 2009).

- **Super Oxide Dismutase (SOD): EC 1.15.1.1**

SOD are metal-containing enzymes that catalyze the conversion of two (O_2^-) into oxygen and H_2O_2, which is less toxic than ($O_2^{\bullet -}$) (Kefer et al 2009). spontaneously dismutase superoxide anion ($O_2^{\bullet -}$) to form O_2 and H_2O_2 (Bilaspuri, Amrit Kaur Bansal and G. S, *2010).*

$$\mathbf{2\,(O_2^{\bullet -}) + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2}$$

- **Catalase (CAT): EC 1.11.1.6**

This enzyme is present in the peroxisome of oxygen consuming cells and it is exceptionally efficient in advancing the change of H_2O_2 to water and O_2.

$$\mathbf{2\ H_2O_2 \xrightarrow{Catalase} 2\ H_2O + O_2}$$

In addition to that, they have also per oxidative functions, so as to convert peroxides (ROOH) into alcohol (ROH) and water. Catalase is mainly localized in the peroxisomes, but it is also found in the cytosol of human neutrophils (Tamer et al., 2003).

- **Glutathione Peroxidase (GPx): EC 1.11.1.9**

A selenium-containing AO enzyme belong to the classical catalytic triad of primary enzymatic AO scavengers, along with superoxide dismutase and catalase (Noblanc et al, 2011).GPx enzyme with glutathione as the electron donor removes peroxyl (ROO•) radicals from various peroxides including H_2O_2 (Suresh C. Sikka, 1996)

- **Glutathione Reductase (GRD)**: **EC 1.6.4.2**

Stimulates the reduction of GSSG to reduced glutathione, this ensures a steady supply of the reductive substrate (NADPH) to glutathione peroxidase (Agarwal et al., 2005). GRD then regenerates reduced GSH from GSSG as shown in the following equation.

$$2\ GSH + H_2O_2 \xrightarrow{\text{peroxidase- (Se)}} GSSG + 2H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{\text{Reductase}} 2GSH + NADP^+$$

1.5.2. Non – enzymatic Antioxidants

The non-enzymatic AOs present within semen include ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione, amino acids (taurine, hypotaurine), albumin, carnitine, carotenoids, flavenoids, urate and prostasomes. These agents principally act by directly neutralizing FR activity chemically (Kelton Tremellen, 2008).

1.6. Trace Elements

Trace elements form part of enzymes, involved in the synthesis of hormones and in the transport of oxygen, and act as antioxidants (Aguiar et al., 2012). The decrease in trace elements results in various cellular abnormalities because of their specific

biochemical changes, while the increase makes them toxic for cells (Sundaram et al., 2013).

Various minerals (copper, cobalt, selenium, manganese, iodine, zinc, and iron) can influence reproductive performance. Reproductive failure may be induced by deficiencies of single or combined trace elements and by imbalances (Hidiroglou M., *1979).*

Heavy metals influence the female endocrine system, and thus may play a role in the increasing infertility problem. Heavy metals induce modifications of neurotransmitters in the central nervous system and impair the pulsatile, hypothalamic release of gonadotropin-releasing hormone (GnRH). In the adrenal gland, heavy metals are deposited in the lipid-rich cortex and block various enzymatic pathways, causing hyperandrogenemia or partial hypoadrenalism (Gerhard et al., 1998). Long period, the trace minerals had been recognized to have potential to enhance metabolic illnesses like prediabetes (metabolic disorder, obesity and insulin resistance) or diabetes mellitus. The therapeutic utilization of trace minerals is distinguished through its targeting of cells and sites of activity. In insulin sensitivity and homeostasis of glucose, the activation of signals of insulin receptor (chromium), antioxidant features (zinc, selenium), or phosphatases inhibition (vanadium) seems to be vital.

1.7. Oxidative Stress

Oxidative stress results from an imbalance between production and removal of (ROS) leading to both a steady-state concentration of reactive intermediates higher than normal (Gerhard et al., 2004). Hence, ROS must be inactivated constantly to keep up just the little sum important to keep up typical cell work (Agarwal, Ashok and Saleh, Ramadan A., 2002) overproduction of ROS or dimension of AOs defense leads to OS state. OS are created in our body by different endogenous frameworks, introduction to various physicochemical conditions or pathophysiological states (Devasagayam et al., 2004). OS associated with an increased rate of cellular damage, it can lead to an increase in membrane permeability, loss of membrane integrity, enzyme inactivation, and structural damage to DNA, mitochondrial alterations, ATP depletion, and apoptosis (Gupta et al., 2010). These phenomena cause pathologic effects. Today, more than 70 pathologies are intrinsically associated with OS and its biochemical consequences

(Kusano, Carlos and Ferrari, Bucalen. 2008). An oxidative stress resulted from an imbalance between antioxidants and ROS causing cellular damage. Oxidative stress has been associated with some diseases like ageing atherosclerosis, cancer, ischemic injury, inflammation and neurodegenerative.

1.8. Consequences of Oxidative Stress State

1.8.1. Lipid Damage

ROS attacks poly unsaturated fatty acids (PUFA) in the cell membrane, leading to a cascade of chemical reactions called lipid peroxidation (Makker et al., 2009). The cell membrane which is composed of poly-unsaturated fatty acids is a primary target for reactive oxygen attack leading to cell membrane damage**.** The onset of lipid peroxidation (LPO) within biological membranes is associated with changes in their physicochemical properties and with alteration of biological function of lipids and proteins (Catala, Angel. 2012).

High levels of lipids (saturated and unsaturated fatty acids) found in the mammalian oocytes structure and in the energy metabolism during oocyte maturation (Wathes et al., 2007). ROS attack each cell component including lipids causing peroxidation of membrane phospholipids, destroying cell membrane and eventually cell death (Hussain et al., 2013).

1.8.2. Protein Damage

Proteins can undergo direct and indirect damage following interaction with ROS, including peroxidation, damage to specific amino-acid residues, changes in their tertiary structure, degradation, fragmentation denaturation, loss of function, cross-linking, aggregation and fragmentation of connective tissues as collagen. The consequences of protein damage as a response mechanism to OS are loss of enzymatic activity, altered cellular functions such as energy production, interference with the creation of membrane potentials, and changes in the type and level of cellular proteins, Protein oxidation products are usually aldehydes, keto compounds, and carbonyls (Kohen, R., & Nyska, A. 2002).

1.8.3. DNA Damage

DNA bases are susceptible to oxidative damage resulting in base modification, strand breaks, and chromatin crosslinking (Cocuzza et al., 2007). ROS also leads to loss of purines (purinic sites), damage of deoxyribose sugar, DNA-protein cross-linkage, and damage to the DNA repair system.

The mechanism of DNA damage by ROS may be due to ($OH^{\bullet}$) radical. ($OH^{\bullet}$) can attack guanine at its C-8 position to yield an oxidation product, 8 hydroxydeoxyguanosine (8-OHdG) (Kohen, R., & Nyska, A. 2002). Hydroxyl radical likewise assaults different bases and the deoxy-Rib sugar in DNA, creating huge harm (Halliwell, B. 2006).

1.9. Role of OS in Female Infertility

Albeit generally little is thought about components influencing fertility and early pregnancy misfortune, there is adequate confirmation to conjecture that dietary cell reinforcements and (OS) (Ruder et al., 2008). The possibility that reactive oxygen species (ROS) and oxygen radicals might be involved in human reproduction was suggested nearly 60 years ago (Oyawoye et al., 2003).

OS impacts the whole regenerative traverse of ladies' lives and significantly from there on (i.e. menopause). It has been proposed that the age-related decrease in ripeness is adjusted by OS. The obsessive impacts are applied by different components including lipid harm, hindrance of protein blend, and consumption of ATP (Agarwal, A., Gupta, S., & Sharma, R. K., 2005). In many of the causes of infertility like polycystic ovarian disease, endometriosis unexplained infertility, tubal infertility, and recurrent pregnancy loss, oxidative stress has played a significant role.

1.10. Hormonal Regulation Antioxidants

Hormones contribute in the cellular generation of free radicals and have a role in the OS status (Agarwal, A., Gupta, S., & Sikka, S., 2006). New relationships between Hormones and OS are proposed recently. It has been demonstrated the activities of Antioxidant enzymes have been shown to be regulated by nutrients and hormones (Azevedo, et al., 2001), and the levels of several hormones (e.g., growth hormone and

prolactin) have a correlation with different Antioxidant enzyme activities in different tissues (Mayo et al, 202). In addition, a relationship between reproductive hormones and plasmatic total antioxidant capacity has been observed (Angelini et al, 2011 and Mancini et al 2008).

1.11. Aim of the Study

OS has been considered recently as a cause of many diseases, female egg has a high content of lipid, peroxidation of these lipids due to low antioxidant levels may be related to ovary disorders including PCOS, which may enhance egg weakness and infertility. Change of food and nutrition style (deficiency of some trace elements) may cause low antioxidant potential and this is probably having relation with PCOS disease.

2. LITERATURE REVIEW

2.1. Polycystic Ovary Syndrome (PCOS)

In women, polycystic ovarian disorder is extensively acknowledged endocrine problem. It influences around 15% of women of reproductive period (Lali L. et al., 2012). It is defined by augmented androgen secretion from adrenal gland and ovary. Hirsutism, alopecia and/or acne are some characteristics of hyperandrogenic metabolic disorder (Wehr E. et al., 2009 and Swetha N. et al., 2013). There is an escalated danger of emerging hyperinsulinemia, insulin resistance, and the reduction in glucose tolerance in patients with polycystic ovarian syndrome (PCOS) (Anuradha K, Sreekumaran N and Lavanya R, 2006).

In patients with type two diabetes mellitus, insulin resistance and related Hyperinsulinemia, polycystic ovarian syndrome (PCOS) is predominant (Tsilchorozidou et al., 2004; R. Azziz, J.E. Nestler, and D. Denally 1997Ü; Conn JJ, Jacobs HS, Conway G.S., 2000; Previc G.M., 1997). In comparison to women without PCOS irrespective of their obesity, women with PCOS have higher risk of Hyperinsulinemia and insulin resistance (Carvajal et al., 2010; Dunaif A, Wu X., 2001). The origination of the hyperandrogenemia and insulin resistance may occur in the fetal life while the detection might be in the adolescence (Ibanez L. et al 2001; Rodin et al., 1998). In patients with PCOS, the glucose metabolism abnormality might happen at a more youthful age. In addition, it could display faster alteration from impaired tolerance to glucose to type two diabetes mellitus (Ehrmann et al, 1992). In the past decades, metabolic diseases such as prediabetes (obesity, insulin resistance and metabolic disorder) or diabetes mellitus are potentially enhanced by trace minerals. In glucose homeostasis and insulin sensitivity, the initiation of antioxidant features (zinc, selenium), signals of insulin receptor (chromium) and phosphatases inhibition (vanadium) are vital (Nicolas W, Jean Robert R., 2010). Dismutase enzyme, which is an antioxidant that helps in fighting the free radicals, consist mainly of manganese. Superoxide ions which are highly reactive to hydrogen peroxide (H_2O_2), is neutralized by Dismutase enzyme. After that, hydrogen peroxide (H_2O_2) is instantly altered to H_2O by catalase and other various peroxidases into the mitochondria matrix (Chu CJ. et al 2009; Hori H. et al., 2000).

Bone mutations, loss of fertility as well as convulsion might be caused by low level of manganese. This important component is available in all nuts, grains and seeds (Rockville, M.D., 2005).

2.1.1. The Symptoms of PCOS

Some general PCOS symptoms in woman are associated with hormonal problems. Occasionally, a functional ovarian cyst will be formed by the ovaries. Around a maturing egg, a pouch is formed on the ovary's superficial. Regularly, when the egg is released, the sac or pouch disappear. The sac becomes a functional cyst if the egg is not released or the sac surrounded the egg and fills with liquid. Literally, the word (polycystic) indicates that the ovary of a woman has multiple tiny cysts.

Generally, a tiny quantity of androgens is released by the ovaries, however in patients with PCOS, slightly higher amount of androgens is made by the ovaries. This caused the appearance of masculine indications such as extra body and facial hair and male pattern baldness.

Typically, even though not every patient identified with polycystic ovarian syndrome has evident cysts on their ovaries; doctors have searched for numerous cysts on the ovaries. If most of other common symptoms are experienced, PCOS can be identified (Percival, 1998).

The followings are the most common PCOS symptoms:

1- Infertility or problems in conceiving
2- Irregular menstrual, comprising amenorrhea
3- The resistance for insulin (associate with an escalated danger for diabetes)
4- Weight alterations, particularly weight gain and problem with weight loss.
5- Acne
6- Elevated testosterone levels
7- Hair thinning and male pattern baldness
8- Extreme hair growth in locations regularly women do not grow hair, for example on the abdomen and face. This situation is called Hirsutism.
9- tiredness
10- Alterations in mood.

2.1.2. The Causes of PCOS

Abnormally elevated male sex hormones level in comparison to female hormones is the fundamental cause of PCOS symptoms. Testosterone and DHEA-S Male are some kinds of androgens or sex hormones. although earlier studies have principally concentrate on the androgen testosterone, a research carried out by the University of Birmingham in 2017 exposed that a type of androgens, recognized as 11-oxygenated C19 steroids, considerably take a part androgen surplus in patients with polycystic ovarian syndrome (Silva at el., 2010).

Regularly, elevated concentration of the male sex hormones in polycystic ovarian syndrome (PCOS) women is termed hyperandrogenism. The egg discharges every month in the ovaries is prevented by high concentrations of testosterone. Thus, among women with PCOS skipped or unbalanced periods and trouble getting pregnant are common.

Occasionally, the case is regarded an irregular when the concentration these male sex hormones are not considerably high (Sheehan, M.T., 2004). Nevertheless, once male sex hormones are not equivalent to female sex hormones, this can still result in problems. Actually, the proportion of hormones appears to be the serious element. For one cause or another, male sex hormones can increase and female sex hormones can decrease

On PubMed Central (PMC), more than 5,000 studies regarding PCOS are accessible. Investigations continues to disclose more knowledge concerning the core causes of polycystic ovarian syndrome and other hormonal disproportions. This provides the information on the complexity this kind of disorder. Similar to the majority other disorders, joined with environmental and lifestyle factors, genetic factors engaged in the PCOS development.

In the following, several common causes of polycystic ovary syndrome are:

1- Long-lasting stress
2- The predisposition of genetic
3- Either too low or too much physical activity relying on the woman
4- Exposing to great quantities of chemicals disrupting endocrine
5- Deprived diet (particularly a high-glycemic diet that’s high in sugar and refined carbohydrates)

6- Disorders or imbalances of thyroid for example hypothyroidism
7- In some cases, big proportion of body fat, being overweight or obese
8- In some case, small proportion of body fat (regularly subsequent a constrained diet)
9- High concentration of insulin
10- High levels of inflammation

Even though polycystic ovarian syndrome (PCOS) is generally considered as a disorder that runs inside families, it does not denote somebody is destined to experience this disorder when she is predisposed genetically. When a family has polycystic ovarian syndrome (PCOS) history, the women in the family required to be cautious about their diet, lifestyle and stress level.

In addition, in polycystic ovarian syndrome, inflammation and insulin have a great role. The ovaries increase the production of male sex hormone by surplus insulin, which the hormone generated in the pancreas that permits cells to utilize glucose. This excess of insulin results in throws off the ovaries' usual capability for ovulation. Polycystic ovaries are also stimulated by Long-lasting inflammation in which higher androgens is produced. The Type 1 Diabetes and Polycystic Ovarian Syndrome: Systematic Review and Meta-analysis," recommended that screening for androgen surplus and PCOS ought to be part of the administration guidelines for women with type 1 diabetes (Héctor F. Escobar-Morreale and M. Belén Roldán-Martín, 2016).

Numerous individuals believe that typical PCOS woman are obese or overweight and have issues with insulin resistance. At some time, a big proportion of patients with PCOS handle weight gain. Nevertheless, females with normal weight or underweight some time develop hormonal disruptions, which cause polycystic ovarian syndrome.

Body weight and Diet are greatly associated with the level of hormonal health. For instance, in accordance to the PCOS Foundation, researches display that around 40% of all females between the ages of 20–50, who have diabetes and/or glucose intolerance, have polycystic ovarian syndrome. Regardless of the diversity of PCOS women, the overweight appears to obscure the symptoms of PCOS and hormonal problems. In addition, it is believed that the endocrine profile is improved, the ovulation and pregnancy possibility in women with PCOS is increased by weight loss.

Researches have revealed that overweight women can improve their ovulation and menstrual cycles by slight weight loss, for example plummeting 5% of initial body weight (Badawy, A., and Elnashar, A. 2011).

2.1.3. Insulin Resistance (IR)

Insulin Resistance is associated with the escalation of oxidant status. It can be discovered in around 50 % of females with PCOS (Azziz R. 2003). Because of the PCOS heterogeneous nature, ethnic variations and different diagnostic criteria, the extensive variety might happen. According to Verit FF, Erel O. (2008), in non-obese PCOS women, the level of oxidative stress and levels of total oxidant and antioxidant status is escalated increase as a result of Insulin resistance (IR). It indicated that Hyperglycemia decreases the levels of antioxidant and upsurges the peroxidation of lipid. Uzel et al. (1987) reported that the level of MDA, a marker of OS, negatively correlated with insulin sensitivity and GSH (antioxidant) levels. Thus it can be stated that antioxidant levels is decreased by insulin resistance while lipid hydro peroxide (LPO) is increased. Since hyperglycemia and greater levels of free fatty acids cause ROS generation, insulin resistance stimulates oxidative stress. It shown that an escalation in ROS production caused by hyperglycemia in females with PCOS (Rosen P. et al, 2001). The Dandona et al. (2001) stated that The ROS generation is inhibited by insulin infusion in obese women. Therefore, thru performing like an anti-inflammatory agent, insulin might protect against pro-inflammatory reaction to hyperglycemia. Hyperinsulinemia and insulin resistance are occurring in around 75% of overweight PCOS females (Nestler J.E., 1997). Nevertheless, when the insulin resistance is not depending on obesity, it has a great a role in polycystic ovarian syndrome. High level of oxidative is displayed in Young, nonobese PCOS women with high levels of triglyceride as the only dyslipidemic characteristics. Moreover, Glucose tolerance is reduced in around 40% of women with PCOS (Sundaram et al., 2013). Dahlgren et al., (1992) stated that in comparison to normal women (2-3%), women with PCOS display greater levels of type II diabetes (T2DM) (15%). Hyperinsulinemia and Insulin resistance are characteristics of metabolic syndrome. An escalated threat of dyslipidemia, hypertension, elevated endothelin, endothelial dysfunction, elevated plasminogen inhibitor type 1, as well as cardiovascular disease same as the threat

related to metabolic syndrome are exhibited in women with PCOS (Khan K.A., Stas S. and Kurukulasuriya L.R., 2006).

2.1.4. Hormonal Markers in PCOS Patients

As an approach for evaluating steroidogenesis, in PCOS women, hormonal markers are observed. Estrogen, FSH, insulin-like growth factor -1 (IGF-1), sex hormone-binding globulins (SHBG), total/free testosterone, androstenedione, anti-Mullerian hormone (AMH), dehydroepiandrosterone (DHEA) and DHEA metabolite DHEAS, and 17- hydroxyprogesterone are the utmost frequently faced markers (Diamanti-Kandarakis E, Piperi C. 2005; Bremer A.A., Miller W.L.,2008; Azziz R. et al., 2009).

In PCOS patients, the liver straightly down-regulate the synthesis of SHBG as a result of high insulin level and testosterone generation. It has been stated that a decent indicator of insulin resistance is low level of SHBG (Nestler J.E., 1991). Androgen bioavailability in serum is regulated by SHBG because it has tough binding affinity to dihydrotestosterone and testosterone (Nardo LG, Patchava S, and Laing I, 2008). An increase in bioavailable testosterone is resulted from declined SHBG levels. The decrease in the proportion bound to SHBG makes the evaluation undependable to some extend as the most common androgen calculated to diagnose hyperandrogenemia is serum bound testosterone (T) (Azziz R. et al., 2009). Consequently, the free androgen index (FAI), (FAI=T/SHBG * 100%) (Vermeulen A., Verdonck L., Kaufman J.M., 1999). Thru equilibrium dialysis, free T might be measured straightly as well (Azziz R. et al., 2009). No research has demonstrated their superiority as surrogate markers even though other androgens like androstenedione (A4) or whole testosterone might be used for diagnosing hyperandrogenemia. For instance, Knochenhauer et al., (1998) indicated that merely approximately 20% PCOS women irregularly had greater level of thyroxine (T4). In comparison to IGF-, Insulin binds to receptors of IGF-1 on theca cells with considerably greater affinities [Homburg R. et al 1992]. In women with PCOS, the secretion of Hepatic IGF-1 binding protein kjis inhibited, causing extreme free IGF-1. This is alleged to have a role in the irregular and rogenesis of theca cells alongside with high LH (LeRoith D. et al., 1995). mRNA of P450c17 is further increased by insulin and IGF-1, causing escalated androgen biosynthesis in adrenal glands and ovary (Khan

K.A., Stas S., Kurukulasuriya L.R., 2006). The circulating insulin concentration and ovarian androgen biosynthesis are reduced by the utilization of insulin-sensitizing agents like metformin (la Marca A. et al., 1999). Another actively examined hormonal marker in PCOS women is DHEA secreted from the adrenal zona reticularis. Nevertheless, because of its diurnal differences, intra-subject difference and low serum level, DHEA has several disadvantages as a surrogate marker (Azziz R., 2001). In medical investigation, up to 70 seventy percent of PCOS women manifest surplus DHEAS serum concentration (Lobo R.A., Paul W.L., Goebelsmann U., 1981; Jaquish C.E., 1996). Nonetheless, the level of DHEAS declines with age and it is regulated through the DHEA sulfotransferase activity (Azziz R., 2004; Kumar A., 2005; Hammer F. et al., 2005). Besides, the levels of circulating DHEAS might be influences by the ethnicity. For example, in comparison to Caucasian American controls, lower circulating levels of DHEAS is observed in Mexican American group (Kauffman R.P., 2006). As a result, merely 10 percent in PCOS women with elevated DHEAS measurements will truly have hyperandrogenaemia (Azziz R, et al., 2009). Therefore, measurements of DHEAS ought to be taken in consideration (Azziz R, et al., 2009).

2.2. Oxidative stress

Via stealing electrons from nucleic acids, lipids, carbohydrates, proteins, and other neighboring molecules, highly reactive and unbalanced free radicals accomplish stability (Agarwal A. et al., 2008), Therefore persuading cellular impairment. The RNS and reactive oxygen species (ROS) are the two main forms of free radicals. Throughout oxygen decrease as a by-product of natural metabolic pathways, normally free electrons create reactive oxygen species (Agarwal A. et al., 2006). At complexes I (at which NADH dehydrogenase performs), and III (at which the ubiquinol to ubisemiquinone to ubiquinone conversion happens) of the chain of electron transport (ETC), the majority of the mitochondrial generation of ROS happens (Inoue M. et al., 2003).

Through lipolysis and chemical energy production 98% of inspired oxygen is decreased while 2% is incompletely decreased, resulting in the formation of three main types of ROS which are superoxide radical [O_2-], hydrogen peroxide [H_2O_2], and hydroxyl [HO·] (Agarwal A. et al., 2008).

During the leakage of electron at the electron transport train, the Superoxide is created. Molecular oxygen usually is changed to water at complex IV. However, as they are being passed down the ETC through ATP production might obtain an additional electron (Cadenas E. and Davies K.J., 2000). From either oxidase enzymes or superoxide dismutation, Hydrogen peroxide is produced. Because the hydroxyl ion has three additional electrons, it is the most reactive form. Strand breakdowns and DNA damage are caused through alteration of pyramidines and purines. Alteration of key transcription factors can happen as the balance between oxidants and antioxidants is not present. Via mitochondrial superoxide dismutase, the superoxide radical can be altered to hydrogen peroxide. Therefore, for sustaining redox homeostasis, the antioxidants existence is imperative. The cell damage is resulted from reduced antioxidants quantity to counter the production of ROS (Inoue M. et al., 2003).

2.2.1. Oxidative stress role of antioxidants in PCOS

For counteracting probable for substantial cell damage by surplus ROS, the antioxidants scavenge surplus ROS. A balance between damaging oxidative stress and advantageous oxidant generation (regularly perform like cell signaling molecules), is created by the assistance of antioxidants. Non-enzymatic and enzymatic are the main classes of antioxidants. The SOD, catalase and GPx are some enzymatic antioxidants. However, tocopherol (vitamin E), GSH, carotene, taurine, L-carnitine, ascorbate (vitamine C) and coenzyme Q10 are some Non-enzymatic antioxidants (Agarwal A. et al., 2008). Copper/zinc SOD (Cu/Zn-SOD), Manganese SOD (Mn-SOD) and extracellular SOD (EC-SOD) 1 are three SOD isoforms in eukaryotes (Nestler J.E. et al., 1991).

It has been stated Antioxidants play significant roles in the female reproductive system and in the pathogenesis of female infertility by preventing or limiting the damaging impact of oxygen radicals. Alterations in concentrations of antioxidant in peritoneal liquid and serum have been examined in tubal infertility, idiopathic infertility and endometriosis women. Outcomes demonstrated that in PCOS women, examination of antioxidant concentrations is encouraging. For correlating PCOS and OS and the varied clinical appearances of metabolic syndrome comprising obesity, diabetes, and

cardiovascular diseases, numerous studies have measured antioxidant markers. (Jozwik M. et al 1999, Paszkowski T. et al., 1995).

2.2.2. Role of TAC in PCOS

The capability of serum for quenching free radical generation, defending the structure of the cell from molecular damage is the total antioxidant capacity. The spectrophotometric assay where longstanding 2, 2'-azino-di- [3-ethylbenzthiazoline sulfonate] (ABTS) radical cation is calculated, is one of the numerous discovery assays for TAC. Through the ABTS incubation with a hydrogen peroxide and peroxidase (metmyoglobin), ABTS radical is produced. Measuring the capability of lipid and aqueous antioxidants for inhibiting the oxidation of ABTS to ABTS+ is the principle of the assay (Jozwik M. et al., 1999). The capacity of the antioxidants for preventing ABTS oxidation in comparison with that of standard Trolox. The generation of hydroxyl radical *via* Fenton reaction is another approach for measuring TAC. This is commenced via the hydroxyl radical, and the brown-colored dianisidinyl radical cations are generated in the response intermediate of the assay (Paszkowski T. et al., 1995). For representing TAC, the capability of antioxidant supplemented samples in contradiction of these colored potent free-radical reactions is calculated as a whole. The outcomes were reported as millimoles of Trolox equivalent per liter as well (Verit F.F, and Erel O 2008).

According to Fenkci et al., (2003), in PCOS women, the TAC was considerably lower (n=30 mean age 25.80±0.63 years and mean BMI 24.3±1.1) in comparison to the age-, BMI-, and smoking status-matched controls (1.15±0.01 vs 1.30±0.02 mmol/L, p=0.001) [59]. This statement proposed that in PCOS women, risk of cardiovascular diseases is escalated by the oxidative status imbalance. Furthermore, id also suggested that fasting insulin level are negatively correlated TAC. This proposes that antioxidant defense system in PCOS might detrimentally be effected insulin resistance. Nonetheless, in Verit et al., (2008) study, the level of TAC was higher considerably in PCOS women (n=63 mean age 24.4±4.1 years and mean BMI 21.2±1.8) in comparison to age and BMI-coordinated controls (1.8±0.5 vs 1.1±0.2 mmol Trolox Eq/L, p<0.0001). These researches indicated that TAC was escalated in non-obese, normoinsulinemic PCOS women (fasting insulin 10.7±5.0 mIU/mL, no significant

difference compared with controls). Therefore, it is recommended high concentration of antioxidants in PCOS have detrimental impact. These outcomes disagreed with other research in the field. It is suggested that TAC was escalated for compensating for the upsurge the complete oxidative stress (19.1±7.6 vs 12.3±4.8 mmol H_2O_2 Eq/L, $p<0.0001$) even though the whole mechanism of this escalation is unknown (Verit F.F. and Erel O 2008).

It is probable for concluding that an imbalance between antioxidants and oxidants happen in PCOS even though outcomes of studies about antioxidant levels are conflicting. Additional research of oxidative stress protect in PCOS are required for clarifying the relationship between PCOS and antioxidants.

2.2.3. Role of SOD in PCOS

The exchange of superoxide, which a poisonous constituent that is converted to water by GPx to H_2O_2, is induced by SOD. The deficiency of endothelial dysfunction markers might be explained high levels of the SOD. Appropriate functioning of vascular system might prevent from an intrinsic oxidative load by the production of a suitable antioxidant reaction. According to Kusçu et al. (2009), in comparison to the control group, the levels of SOD were higher significantly in a PCOS group (8.0±0.7 vs 7.28±0.8, $p=0.001$). This report divided the PCOS women into IR and IR+ subgroups. In both subgroups, concentration of SOD was greater considerably in comparison to the control (7.99±0.7 vs 8.22±0.8 vs 7.28±0.8, $p=0.009$ and 0.03, correspondingly). Because of the mechanisms of the body's defense system, this raise may have been occurred. This report utilized comparatively young subjects (average age 23.8±4.37 years) with higher capability for coping with greater concentration of production of ROS.

The study of Sabuncu et al., (2001) expressed that high concentration of SOD (average value 94.62 MU/mol Hb) in PCOS patients with average BMI 31.4 ($p<0.05$). The study suggested that the escalation in levels of SOD could be because of a compensatory reaction to oxidative stress. It is displayed by the Zhang et al., (2008), the serum level of SOD in PCOS women was lower considerably compared to the control group (Zhang D. et al., 2008). Nevertheless, in this study the characteristics of other

patients was not captured. This makes it hard to comment on the lower level of SOD in this chosen PCOS group.

2.3. Role of Trace Elements in Female Reproductive Function

Numerous researches have been carried out about the role of dietary trace elements on reproductive function during the last forty years. Nevertheless, according to a review of the literature, the research has been profoundly weighted toward male in comparison the function of female reproductive. There are insufficient researches that have a lot of evidence on the influence of micronutrients, comprising trace elements on female fertility (Buhling K.J. and Grajecki D., 2013). Numerous research taking into consideration the period of preconception, or highlights the common reproductive health as it comes to the function of ovary, comprising hormone combination and advancement of follicular even though there are numerous animal and human research concentrating on the dietary consumption of trace elements throughout pregnancy.

Around thirty years, Xu et al., (1997) stated that one of the significant factors that leads to mysterious infertility is the disorder in the metabolism of trace elements. New researches have stated that alterations in the trace elements levels, Mg, Zn, and Co, have imperative roles in female infertility, nevertheless relationship between Fe and infertility is still unexplained (Pathak P., Kapil U., 2004). For improving treatment and diagnosis, a superior knowledge of the role of trace elements in the fundamental mechanisms in infertility, and more rigorous researches illustrating the effectiveness of nutritional factors are required (Ebisch I.M.W. et al., 2007). It is evident that there is a robust connection between successful conception and healthy pregnancy progression and nutritional status of trace elements prior to conception, in spite of the scarcity of studies (Cetin I, Berti C. and Calabrese S., 2010).

This report lately commenced measureable XRF imaging of bovine ovaries and carried out ICP-MS and XAS evaluates so as to obtain a superior knowledge of the role of trace elements in the ovary, (Ceko M.J. et al., 2015; Ceko M.J. et al 2015). Especially, the study attained information on Co, Mg, Zn and Se. (Azziz R. et al., 2009) reported that the role of zinc in the hindrance or clinical administration of PCOS and/or diabetes is ambiguous. Nonetheless, small number of researches pointed out a promising result in diabetic patient’s subsequent zinc addition whereas others did not

accomplish comparable outcomes (Dendougui F. and Schwedt G., 2002). The basis of this study is the role of trace elements and their potential in the ovary. However, some ovaries exhibit evidence of other trace elements such as chromium, nickel and titanium, even though these were not particularly high level. Therefore, these components were not further inspected.

2.4. Oxidative Stress and Ovarian Function

For energy necessities of the gametes, aerobic metabolism using oxygen is vital. In addition, physiological procedures inside the ovary, the free radicals have a substantial role. Numerous researches have indicated ROS contribution in the follicular-fluid folliculogenesis, steroidogenesis and environment (Shiotani M. et al., 1991; Sugino N. et al., 2004). Riley et al., (1991) intensively covered the role of antioxidant enzymes, copper zinc superoxide dismutase (Cu–Zn SOD), manganese superoxide dismutase (MnSOD), glutathione peroxidase and ROS in the maturation of oocyte. For explaining these complicated roles in ovulation and luteal function in the human ovary, mRNA expression, immunohistochemically localization and thiobarbituric acid approaches were utilized (El Mouatassim S., Guerin P., and Menezo Y., 1999).

The steroidogenic capacity and midluteal corpus luteum both in vivo and in in vitro, are influenced by Oxidative stress. Corpora lutea collected from pregnant and non-pregnant women was utilized in an interesting investigation, was monitored that throughout regular circumstances Cu–Zn SOD expression parallels the levels of progesterone, with an increase from initial luteal to midluteal phase and decline throughout regression of the corpus luteum. Nonetheless, in comparison to those of midcycle corpora lutea, the mRNA expression of Cu–Zn SOD in the corpus luteum though pregnancy was greater. Probably, because of the escalated human chorionic gonadotropin (HCG) concentration, this factor improved SOD expression through pregnancy. In addition, it might be the cause of apoptosis of the corpora lutea. Likewise, as the antioxidant enzymes glutathione peroxidase and MnSOD are expressed merely in metaphase II oocytes, they are regarded the markers for maturation of cytoplasmic (Tamate K., Sengoku K., and Ishikawa M., 1995). Declined oocytes developmental potential from unwell vascularized follicles has been accredited to low intrafollicular

oxygenation as well (Chui D.K. et al., 1997; Van Blerkom J., Antczak M., Schrader R., 1997).

Researches approved the significance role of oxidative stress in functions of ovary. This is attributed to lipid peroxidation intensification in the pre-ovulatory Graafian follicle (Jozwik M., Wolczynski S., and Szamatowicz M. 1999) as well as that low maintenance of hydro peroxides concentration inside follicle glutathione peroxidase (Paszkowski T. et al., 1995). The pathophysiology of polycystic ovarian illness is significantly affected by inflammatory process and oxidative stress. Reducing the oxidative stress levels might increase effectiveness of drugs like Rosiglitazone (Sabuncu T. et al., 2001; Yilmaz M. et al., 2005).

3. MATERIALS AND METHODS

3.1. Sample Collection

The study conducted in Sulaimanyah city, Iraq. consist of infertile women (main group n=64) who attend doctors to treat infertility due to PCOS, and healthy women in fertility age (15-35) (n=14), PCOS diagnosed by 2003 AE-PCOS Society 2006 criteria {Clinical and/or biochemical signs of hyperandrogenism & Ovarian dysfunction (Oligo-anovulation) (**Both criteria needed**)}, blood samples drown in the laboratory of the hospital in serum separation tube then centrifuged at 4000 rpm for 5 min, serum aspirated and transferred to deep freeze (-80 ºC) in the university laboratories to save it for long time.

The sub group can be determined lately according to (BMI), as samples distribution showed which also done for healthy women too.

3.2. Instruments and Chemicals

3.2.1. Instruments

All instruments are available in the university labs including:

- Deep freeze -80 C, (Forilabo, France)
- Different centrifuges (high speed 15000 rpm, cooled centrifuge, etc.). (Eppedorf, Germany).
- pH meter (WTW, Germany)
- Computerized spectrophotometer. (GP, UK)
- Microplate reader ELISA, (BioTek, USA)
- Incubators and water baths (LabTech, Korea)
- ICP-MS Thermo scientific ICAP Q Model

Table 3.1. ICP-MS operating conditions

Instrument parameter	
RF power (W)	0.95
Nebulizer gas flow (L min−1)	1.12
Auxiliary gas flow (L min−1)	0.80
Cooling gas flow (L min−1)	14.00
analytical parameter	
Acquisition mode	Peak jumping, simultaneous pulse count/analogue detector system
Dwell time/isotope	30 ms for 103Rh, 105Pd and 195Pt, 10 ms for 63Cu, 87Sr, 68Zn, 115In, 179Hf, 206Pb
Channels per mass	3 for 103Rh, 105Pd and 195P, 1 for 63Cu, 87Sr, 68Zn, 115In, 179Hf, 206Pb
Number of repetitions/sample	3

Table 3.2. The standard of ICP-MS

Category	Concentration average	Concentration RSD	Standard concentration
24Mg (KED)	51.515 ppb	3.9 %	50.000 ppb
63Cu (KED)	51.882 ppb	2.8 %	50.000 ppb
66Zn (KED)	49.455 ppb	3.5 %	50.000 ppb
77Se (KED)	43.113 ppb	5.2 %	50.000 ppb

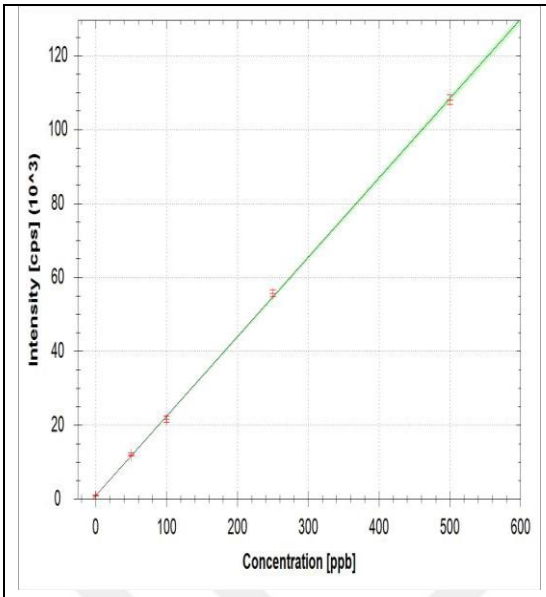
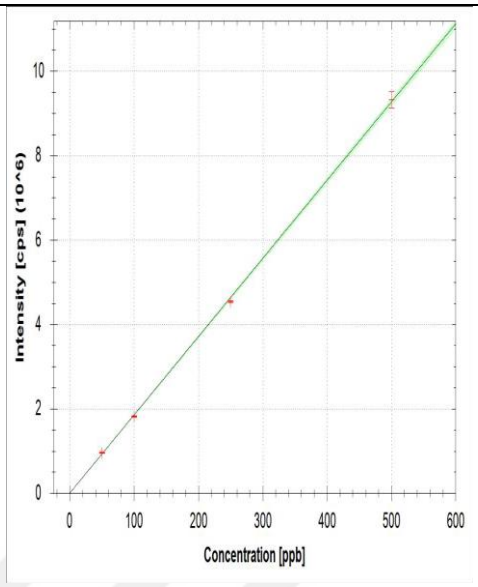
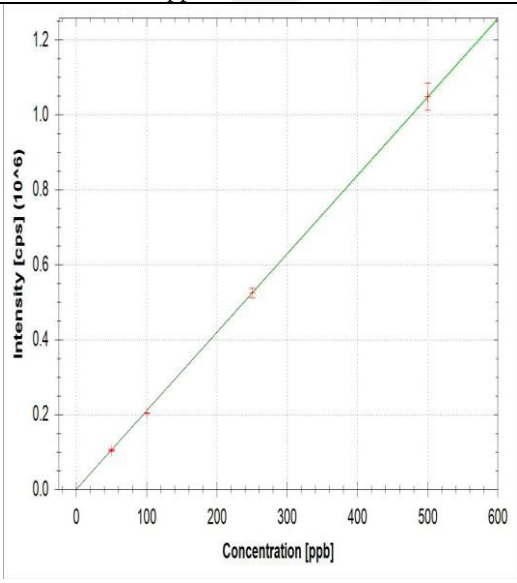
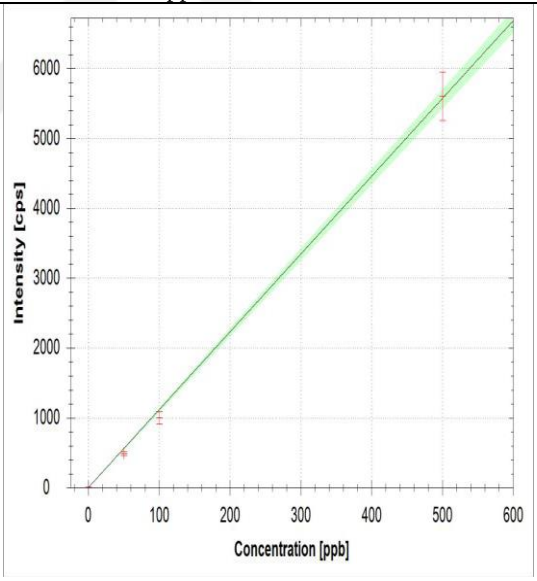
	
$_{24}$Mg (KED) f(x) = 214.8717*x + 876.6999 R^2 = 0.9997 BEC = 0.028 ppb LOD = 0.0154 ppb	$_{63}$Cu (KED) f(x) = 18529.2640*x + 520.0128 R^2 = 0.9997 BEC = 4.080 ppb LOD = 0.9297 ppb
	
$_{66}$Zn (KED) f(x) = 2093.5376*x + 296.6717 R2 = 0.9999 BEC = 0.142 ppb LOD = 0.0438 ppb	$_{77}$Se (KED) f(x) = 11.1339*x + 3.3333 R2= 0.9990 BEC = 0.299 ppb LOD = 1.5557 ppb

Figure 3.1. ICP-MS operating conditions of Mg, Cu, Zn and Se elements

3.2.2. Chemicals

All kit must provide from a research companies (Biolabs, USA + oxford, UK + MonoBind, USA). Other chemicals are found in the research lab of Garmian University.

The other reagents were as follows: H_2O_2 (30%, J.T. Baker,z.a.), HNO_3 (65%, Merck, supra pure). One millilitre of serum, HNO_3 and 1 ml of H_2O_2 were utilized. Firstly, the serum and the 6 ml of HNO_3 were mixed in a test tube. After leaving the mixture in the room temperature for 1 minute, the H2O2 was added. Then, the test tube was closed up and inserted into sunflower oil that heated up to 200 ^oC for 2 hours until it is completely digested. After that, the test tube was taken out from the oil and left in the room temperature to cool down. The filter papers were utilized for the filtration process. Next, D.W was added to the mixture until it becomes 15 ml. Finally, the mixture is put in the Falcon tube in a normal freezer to the day of analysis.

3.3. Methods

In order to achieve the aims of this research, the following tests are suggested related to oxidative stress in serum.

3.3.1. Estimation of CAT Enzyme in Serum

The CAT activity assay performed using spectrophotometric determination of hydrogen peroxide (H_2O_2) which form stable complex with ammonium molybdate that absorbs at 405 n (Goth L., 1991)

The assay includes incubation of serum with 1.0 ml substrate (65 µmol per ml H_2O_2 in 60 mmol/l phosphate buffer, pH 7.4) at 37 °C for 60 second. In this method, serum CAT activity is linear up to 100 kU/l. If the CAT activity exceeded 100 kU/l. The enzymatic reaction was stopped with 1.0 ml of 32.4 mmol/l ammonium molybdate ($(NH_4)_6\ Mo_7O_{24}\ .4\ H_2O$) and the yellow complex of molybdate and hydrogen peroxide is measured at 405 nm against reagent blank.

One unit CAT decomposes 1 µmole of hydrogen peroxide/l minute under assay conditions. CAT activities are expressed as kilo unit per litter (kU/l).

3.3.2. Estimation of SOD Enzyme in Serum

Superoxide dismutase reacts with pyrogallol and prevents its auto oxidation. The rate of autoxidation of pyrogallol measured from the absorbance at 420 nm (orange colour). The activity of superoxide dismutase was expressed as units per

minute. One unit of the enzyme is defined as the amount of enzyme which inhibits the rate of pyrogallol auto oxidation by 50% (Marklund, S. and Marklund, G. 1974).

3.3.3. Estimation of Lipid Peroxidation

Lipid peroxidation is measured by reaction of thiobarbituric acid (TBA) with melon di aldehyde (MDA) In the presence of heat and acid, TBA reacts with MDA. Under heat and low pH in a ratio 1:2 to form a pink colour allegedly $[TBA]_2$-malondialdehyde. The intensity of the colour at 532 nm corresponds to the level of lipid peroxidation in the sample (Rao et al., 1989).

3.3.4. Estimation of Serum Hormones

All hormones assayed according to the same principle, hormones have been assayed using ELISA technique. ELISA Test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes an anti-Hormone antibody for solid phase (microtiter wells) immobilization and an anti-hormone antibody in the antibody-enzyme conjugate solution. After incubation, the wells are washed with wash buffer to remove unbound labelled antibodies. Colour developing solution is added and incubated, resulting in the development of a blue colour. The colour development is stopped with the addition of 2N HCl, and the absorbency is measured spectrophotometric ally at 450 nm. The intensity of the colour formed is proportional to the amount of enzyme present and is directly related to the amount of Hormone in the sample. By reference to a series of hormone standards assayed in the same way, the concentration of hormone in the sample is quantified (Singh, S.K. Sawhney and Randhir, 2009).

Table 3.3. Statistical data of infertile women

Sample	Age year	Tall (Cm)	Weight (Kg)	LPO(mM)	CAT (K/ml)	SOD	Free Testosterone (ng/dl)	Testosterone (ng/dl)	LH (IU/L)	FSH (IU/L)	Mg (ppm)	Cu (ppm)	Zn (ppm)	Se (ppb)	BMI
1	18	174	48	0.727	9.6989	1.375	2.491	118.024	34.860	11.000	17.622	2.656	1.554	31.440	15.900
2	21	160	41	0.9618	27.443	0.125	2.021	95.710	18.710	6.200	24.335	3.834	4.171	62.865	16.000
3	16	170	48	0.689	23.843	0.750	2.405	113.960	42.560	14.790	21.342	3.402	1.487	49.395	16.600
4	19	166	46	0.879	41.426	0.625	2.329	110.360	22.660	7.430	27.281	3.049	4.404	31.440	16.700
5	19	163	45	0.450	14.723	0.250	2.435	115.358	44.580	15.030	13.872	0.948	21.821	13.470	16.900
6	20	163	48	0.405	26.339	1.125	2.110	99.950	40.060	14.090	25.793	3.480	1.664	13.470	18.100
7	28	160	48	0.658	49.411	0.500	1.958	92.780	31.310	10.260	15.530	2.109	3.592	31.440	18.800
8	20	165	53	0.619	14.874	0.625	2.933	138.958	56.420	17.950	12.129	2.329	0.889	17.970	19.500
9	19	160	50	0.696	15.451	0.750	2.026	96.014	21.350	8.860	20.404	2.868	9.739	22.455	19.500
10	17	154	50	0.586	23.843	0.750	1.936	91.710	20.470	9.000	17.492	1.664	0.974	17.970	21.100
11	22	166	59	0.790	17.047	1.125	2.076	98.332	50.960	17.180	13.472	2.281	1.093	22.455	21.400
12	21	167	60	0.809	14.875	0.625	2.221	105.230	61.160	19.680	10.296	1.489	1.078	0.000	21.500
13	20	166	60	0.446	26.209	0.500	2.841	134.610	55.300	19.830	29.126	3.231	1.403	35.925	21.800
14	25	168	62	0.318	15.769	0.875	4.187	198.360	40.260	15.750	9.812	1.048	4.246	22.455	22.000
15	32	170	65	0.730	12.377	1.125	1.905	90.240	26.730	8.700	14.515	1.744	0.823	17.970	22.500
16	40	160	58	0.643	9.066	1.250	2.983	141.300	37.800	12.330	13.049	1.317	1.509	0.000	22.700
17	17	162	60	0.809	13.600	0.875	3.644	172.630	38.120	8.910	10.608	1.014	0.626	13.470	22.900
18	18	168	65	0.287	42.749	1.125	2.079	98.520	32.580	11.034	14.189	1.603	2.912	13.470	23.000
19	28	171	68	0.550	8.486	0.375	3.546	167.980	29.050	9.690	19.953	1.933	1.012	71.850	23.300
20	23	162	62	0.509	4.036	0.750	4.618	218.760	40.090	13.260	14.053	1.093	0.769	8.985	23.600
21	18	165	65	0.751	99.271	1.375	1.907	90.360	19.200	6.360	12.726	1.986	0.813	40.410	23.900
22	17	158	60	0.869	39.852	0.750	2.709	128.320	20.570	6.965	17.340	3.086	2.091	26.940	24.000

Table 3.3. Statistical data of infertile women (contd.)

Sample	Age year	Tall (Cm)	Weight (Kg)	LPO(mM)	CAT (K/ml)	SOD	Free Testosterone (ng/dl)	Testosterone (ng/dl)	LH (IU/L)	FSH (IU/L)	Mg (ppm)	Cu (ppm)	Zn (ppm)	Se (ppb)	BMI
23	36	159	61	0.658	31.511	0.500	2.800	132.660	48.030	15.970	9.083	1.300	0.652	0.000	24.100
24	24	160	62	0.465	20.216	0.500	2.301	109.008	21.590	7.130	14.548	2.578	1.162	22.455	24.200
25	19	163	65	1.000	35.000	1.625	1.988	94.180	18.490	6.020	22.624	2.242	1.056	49.395	24.500
26	20	158	62	0.556	26.743	1.500	3.011	142.657	40.060	12.410	15.701	1.385	10.997	17.970	24.800
27	24	162	65	1.179	20.216	0.625	1.968	93.230	18.050	6.030	33.624	3.839	7.597	26.940	24.800
28	26	172	74	0.800	19.483	1.375	3.044	144.230	57.690	19.320	23.668	3.961	2.012	31.440	25.000
29	32	160	65	0.696	17.047	0.375	2.635	124.830	22.800	6.450	16.267	1.808	0.914	31.440	25.400
30	19	160	65	0.318	17.047	0.875	2.971	140.740	36.050	13.010	19.556	2.195	1.070	35.925	25.400
31	28	160	65	0.556	10.291	1.000	2.900	137.380	36.580	11.290	19.556	2.195	1.070	35.925	25.400
32	51	160	65	0.843	99.271	0.250	3.305	156.560	32.870	11.310	12.573	1.900	1.179	22.455	25.400
33	31	157	63	1.1724	9.6989	0.625	2.458	116.460	30.760	8.940	16.151	2.627	1.003	31.440	25.600
34	18	169	73	0.658	10.291	1.000	2.885	136.664	28.770	10.210	11.388	1.225	0.770	13.470	25.600
35	23	165	70	0.617	24.771	1.625	2.420	114.670	22.060	7.080	24.383	2.234	2.986	40.410	25.700
36	32	165	70	0.807	14.723	0.375	2.605	123.430	22.660	6.650	13.447	2.253	0.888	26.940	25.700
37	25	162	68	0.850	99.271	0.125	3.296	156.130	35.370	11.680	5.437	0.527	0.408	4.485	25.900
38	30	167	75	0.770	26.201	0.500	2.351	111.380	32.100	9.570	13.989	1.866	1.015	49.395	26.200
39	21	170	76	0.994	11.050	0.500	2.506	118.735	21.740	6.380	16.912	1.843	1.317	26.940	26.300
40	30	160	68	0.408073	200.304	0.375	2.923	138.490	62.750	18.954	27.409	1.444	4.894	44.910	26.600
41	28	160	68	0.625	37.523	0.750	2.631	124.660	25.780	8.340	44.579	3.126	5.273	0.000	26.600
42	25	155	64	0.696	19.190	0.250	3.525	167.003	48.980	15.320	15.945	1.859	0.987	22.455	26.600
43	33	160	69	0.465	7.100	0.750	3.051	144.550	29.520	9.489	24.072	3.508	1.225	35.925	27.000
44	27	160	69	0.809	18.310	0.875	3.230	153.022	30.090	10.030	23.713	2.479	29.078	26.940	27.000

Table 3.3. Statistical data of infertile women (contd.)

Sample	Age Year	Tall (Cm)	Weight (Kg)	LPO(mM)	CAT (K/ml)	SOD	Free Testosterone (ng/dl)	Testosterone (ng/dl)	LH (IU/L)	FSH (IU/L)	Mg (ppm)	Cu (ppm)	Zn (ppm)	Se (ppb)	BMI
45	28	163	72	0.414	13.261	0.750	2.497	118.313	28.800	9.330	7.005	1.531	2.252	4.485	27.100
46	33	166	75	0.658	99.271	0.625	2.246	106.430	36.660	12.530	14.505	1.463	0.757	13.470	27.200
47	23	168	78	0.2872	23.843	0.875	2.815	133.374	37.510	12.810	8.472	1.145	0.567	17.970	27.600
48	49	163	75	0.5423	20.216	0.750	1.943	92.080	32.800	10.500	15.780	1.380	0.826	13.470	28.200
49	20	163	76	0.735	41.469	0.500	3.893	184.440	34.210	9.430	0.000	0.000	0.000	0.000	28.600
50	36	163	76	0.446	26.339	0.750	2.396	113.534	40.019	14.220	14.619	2.159	2.483	17.970	28.600
51	32	156	68	0.821	54.886	1.000	2.311	109.510	23.040	9.360	9.299	1.067	0.593	13.470	28.700
52	24	165	78	0.630	69.144	1.500	2.805	132.874	21.380	7.030	15.599	2.445	0.984	13.470	28.700
53	18	165	80	0.701	11.756	0.625	2.188	103.684	23.650	6.010	18.232	2.077	0.851	4.485	29.400
54	42	169	85	0.727	65.351	1.000	2.267	107.420	38.660	8.510	14.085	1.901	4.837	8.985	29.800
55	45	160	77	0.869	14.857	0.625	2.088	98.920	31.430	10.360	17.934	2.477	1.263	35.925	30.100
56	29	163	82	0.613	13.450	1.125	2.255	106.847	25.450	7.630	14.342	1.845	2.162	21.564	30.900
57	35	148	68	0.655	123.975	0.750	2.118	100.330	19.380	6.734	20.175	2.185	1.047	13.470	31.000
58	39	169	90	0.556	82.379	0.750	2.195	104.011	20.930	5.160	19.520	1.761	29.744	13.470	31.500
59	38	161	83	0.1835	26.201	0.750	2.099	99.460	20.710	6.970	10.496	2.417	1.435	0.000	32.000
60	33	156	78	0.371	24.771	0.125	3.619	171.430	35.940	11.760	15.971	2.006	1.293	26.940	32.100
61	32	165	90	0.787	12.356	0.875	2.043	96.750	19.550	6.540	20.788	2.067	1.107	26.940	33.100
62	26	165	93	0.700	17.047	0.500	4.001	189.530	28.290	9.880	9.856	1.214	0.619	26.940	34.200
63	23	165	102	0.994	22.535	1.500	3.100	146.863	26.690	8.410	12.761	1.379	0.940	44.910	37.500
64	31	170	120	0.727	82.379	0.500	2.225	105.420	26.450	8.540	22.740	1.802	26.926	22.455	41.500
65	19	165	45	1.1724	9.624	0.250	2.908	137.758	51.570	11.620	25.201	3.423	2.120	40.410	165.000

Table 3.4. Statistical data of fertile women

	Age year	Tall (Cm)	Weight (Kg)	LPO(Mm)	CAT (K/ml)	SOD	Free Testosterone (ng/dl)	Testosterone (ng/dl)	LH (IU/L)	FSH (IU/L)	Mg (ppm)	Cu (ppm)	Zn (ppm)	Se (ppb)	BMI
1	20	160	38	0.802	37.379	1.000	0.960	17.020	16.140	18.580	35.570	4.080	2.930	31.440	14.800
2	21	168	45	0.554	24.0881	0.250	0.980	19.970	13.260	16.820	17.330	2.430	1.030	22.460	15.900
3	20	160	48	0.465	14.875	0.625	0.660	6.310	6.170	7.110	18.390	2.910	1.220	22.460	17.600
4	24	165	56	0.287	8.320	0.625	1.630	36.740	34.010	33.630	26.640	1.830	2.230	31.440	20.600
5	24	168	60	0.730	23.257	0.625	0.700	8.120	11.130	12.060	15.260	2.160	5.380	40.410	21.300
6	21	160	55	0.547	42.749	0.500	1.430	32.110	29.580	21.950	24.670	2.560	1.870	8.990	21.500
7	19	165	60	0.61086	14.857	2.000	1.070	28.750	25.050	21.170	22.540	1.900	1.240	49.400	22.000
8	43	173	71	0.810	57.237	1.375	0.800	10.080	13.750	12.080	35.240	5.640	1.200	179.630	23.700
9	29	165	68	0.673	37.496	0.750	3.750	93.160	17.040	12.830	24.480	3.600	1.440	35.930	25.000
10	33	164	70	0.700	126.748	1.125	2.590	55.090	11.570	10.870	21.650	1.730	0.890	17.970	26.000
11	39	154	62	0.585	35.260	0.750	1.201	27.040	27.570	23.590	14.930	2.280	1.020	8.990	26.100
12	47	165	72	0.875	34.231	0.250	3.390	78.000	19.000	16.770	14.750	2.420	1.030	35.930	26.400
13	31	163	72	0.880	26.396	2.500	1.220	28.360	16.670	13.130	19.120	2.200	1.110	22.460	27.100
14	32	160	71	0.573	11.410	1.375	3.240	81.480	18.060	19.210	20.130	1.880	0.990	44.910	27.700

4. RESULTS AND DISCUSSION

4.1. Statistical Analysis of fertile and infertile women

Results analysed using (Graphpad prism Ver. 7.0) software program. Differences examined by t test table and differences considered significant at $p \leq 0.5$, person correlation used to find a correlation between 2 parameters.

4.1.1. Biochemical parameter of fertile and infertile women

Researcher seeks for a significance differences between means of different groups at ($p \leq 0.05$), and regression between two different parameters. Tables designed as follows.

Table 4.1. Biochemical of fertile and infertile women

Biochemical parameter	Fertile group (n=14)		infertile group (n=64)		Mean difference	t statistics	P	95% Confidence interval for differences	
	Mean	Standard Deviation	Mean	Standard Deviation				Lower	Upper
SOD	0.98	0.64	0.77	0.37	0.21	1.64	0.10	-0.044	0.466
CAT K/ml	35.31	29.6	33.56	34.35	1.75	0.17	0.81	-17.96	21.46
LPO	0.67	0.21	0.64	0.64	-0.023	0.37	0.70	-0.14	0.098

displays the level of oxidative parameters such as LPO, CAT and SOD in fertile and infertile group. The mean level of LPO in fertile group was slightly lower (0.64) compared to infertile group (0.67). Furthermore, the value of CAT and SOD were (35.31 and 0.98) in fertile and (33.56 and 0.77) in infertile group respectively. İt can be observed that there are no statistically significant differences between the means.

4.1.2. Trace elements parameter of fertile and infertile women

The comparisons of the concentration of essential trace elements such as Zn, Se, Cu and Mg between the fertile and infertile groups are demonstrated in Table 4.2. It can be seen that the mean concentration of Zn, Se, Cu and Mg in fertile group were 1.68, 39.46, 2.68 and 22.19 respectively. Furthermore, in infertile group the level of Zn, Se, Cu and Mg were 3.54, 23.79, 2.07 and 17.07. It can be seen that while the levels of Mg, Se and Cu were lower, the Zn concentration was higher in the fertile group than infertile.

Table 4.2. Trace elements of fertile and infertile women

Trace elements	Fertile group (n=14)		infertile group (n=64)		Mean difference	t statistics	P	95% Confidence interval for differences	
	Mean	Standard Deviation	Mean	Standard Deviation				Lower	Upper
Mg (ppm)	22.19	6.73	17.07	7.037	5.12	2.48	0.015	1.016	9.226
Cu(ppm)	2.687	1.08	2.07	0.83	0.61	2.37	0.019	0.1002	1.135
Zn (ppm)	1.684	1.209	3.545	6.463	1.86	1.06	0.28	-5.331	1.61
Se (ppb)	39.46	42.17	23.79	15.45	15.67	2.37	0.02	2.507	28.84

4.1.3. Serum hormone levels of fertile and infertile women

Table 4.3. Serum hormone levels of fertile and infertile women

Hormone	Fertile group (n=14)		infertile group (n=64)		Mean difference	T statistics	P	95% Confidence interval for differences	
	Mean	Standard Deviation	Mean	Standard Deviation				Lower	Upper
Free testosterone ng/dl	1.68	1.079	2.64	0.61	0.96	4.57	<0.0001	0.54	1.381
Testosterone ng/dl	37.3	28.55	125.5	29.07	88.19	10.33	<0.0001	71.19	105.2
LH IU/L	18.5	7.86	32.78	11.46	14.28	4.43	<0.0001	7.86	20.7
FSH IU/L	17.13	6.711	10.6	3.79	-6.52	5.00	<0.0001	-9.12	-3.93

Furthermore, the LH, FSH, testosterone and free testosterone level are demonstrated in table 7. The mean of LH, FSH, free testosterone and testosterone in the fertile group were 18.5, 17.13, 1.68 and 37.3 respectively. Nevertheless, the mean of LH, FSH, Free testosterone and testosterone in the infertile group were 32.79, 10.6, 2.64 and 125.5 accordingly. This table displays that in comparison to fertile group, the

mean of LH and Free testosterone were greater in infertile group. Nonetheless, the mean of FSH was smaller in infertile compared to the fertile group.

4.1.4. Age, tall, weight and BMI levels of fertile and infertile women

Table 4.4. exhibits the means of age, tall weight and BMI in fertile and infertile group. The body mass index was measured as weight divided by height squared (kg/m^2) for every single subject. The mean of age in fertile group was marginally higher (28.79) compared to infertile group (26.69). Furthermore, it is shown that the fertile women are higher than infertile (163.6 and 163.2 respectively). It can be observed the mean weight of fertile group is (60.57) while the mean weight of infertile group is (67.6). Moreover, the body mass index (BMI) in fertile women is lower compared to infertile women.

Table 4.4. Age, tall, weight and BMI levels of fertile and infertile women

Parameters	Fertile group (n=14)		infertile group (n=64)		Mean Difference	T statistics	P	95% Confidence interval for differences	
	Mean	Standard Deviation	Mean	Standard Deviation				Lower	Upper
Age	28.79	9.15	26.69	8.147	2.09	0.85	0.39	-2.79	6.978
Tall Meter	163.6	4.636	163.2	4.766	0.34	0.24	0.80	-2.44	3.12
Wight Kg	60.57	11.05	67.6	14.03	-7.02	1.75	0.08	-14.99	-14.99
BMI K/meter2	22.55	4.201	27.67	17.99	5.11	1.05	0.29	-4.557	14.79

4.1.5. OS parameters of fertile and infertile women for different BMI

Table 4.5. explains the mean and standard deviation of SOD, CAT and LPO in fertile and infertile women for BMI lower and higher. It is shown that the mean of SOD in women with higher BMI than 25 is bigger than both fertile and infertile women with low BMI. However, while the mean of CAT (the infertile group with higher BMI is (41.78), it is (35.31 and 25.13) in fertile and infertile group with lower BMI. Additionally, the level of LPO in all the groups are close (0.64, 0.672 and 0.67).

Table. 4.5. OS of fertile and infertile women for different BMI

Biochemical parameter	Fertile group (n=14)		infertile group (n=64)				P	95% Confidence interval for differences	
			>25		<25			Lower	Upper
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation			
SOD	0.98	0.64	0.8482	0.3929	0.7128	0.3583	0.12	-0.20	0.47
CAT K/ml	35.31	29.6	25.13	18.45	41.78	6.869	0.20	-15.75	36.11
LPO	0.64	0.166	0.672	0.207	0.67	0.22	0.93	-0.18	0.14

4.1.6. Trace Elements Concentrations of Fertile and Infertile Women for Different BMI

Table 4.6. Trace elements concentrations of fertile and infertile women for different BMI

Trace elements	Fertile group (n=14)		infertile group (n=64)				P	95% Confidence interval for differences	
			>25		<25			Lower	Upper
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation			
Mg	22.19	6.73	17.65	6.316	16.62	7.608	0.045	-0.94	10.03
Cu	2.687	1.085	2.267	0.944	1.916	0.709	0.019	-0.26	1.10
Zn	1.68	1.209	3.291	4.52	3.742	7.70	0.546	-6.25	3.044
Se	39.46	42.17	25.5	17.46	22.45	13.79	0.059	-3.66	31.57

The trace the concentrations of elements of fertile and infertile women for different BMI is demonstrated in Table 4.6. While the mean level of Mg, Cu and Se

are higher in infertile group with lower BMI compared to infertile group with higher BMI, the mean of Zn level is higher in lower BMI group.

4.1.7. Hormone Levels of Fertile and Infertile Women for Different BMI

In Table 4.7., the means level of LH, FSH, testosterone and free testosterone are exhibited in different body mass index (BMI). It is shown that while the F-testosterone and testosterone are higher in bigger BMI group, the mean LH and FSH are opposite.

In the current research, the concentrations of trace elements such as Zn, Se, Cu and Mg, and oxidative stress parameters (LPO, CAT and SOD) and some hormones like LH, FSH and F-testosterone in serum from fertile and infertile subjects were measured for identifying the effect of trace elements and oxidative stress on PCOS.

In regards with the level of Mg, Se and Cu levels, there are statistically significant differences between the two groups (p 0.015, 0.02 and 0.019) respectively. However, there are no significant differences between the two groups in the concentration of Zn (p 0.2).

Table 4.7. Hormone levels of fertile and infertile women for different BMI

Hormones	Fertile group (n=14)		infertile group (n=64)				P	95% Confidence interval for differences	
			>25		<25			Lower	Upper
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation			
Free Testosterone ng/dl	1.68	1.079	2.58	0.71	2.69	0.53	<0.0001	-1.46	-0.34
Testosterone Ng/dl	37.3	28.55	122.6	33.78	127.7	25.21	<0.0001	-108.1	-62.56
LH IU/L	18.5	7.866	35.31	13.47	30.86	9.41	<0.0001	-25.28	-8.347
FSH IU/L	17.13	6.711	11.83	4.492	9.677	2.894	<0.0001	1.905	8.697

In the literature, there is contradictory information on the association between PCOS and Se. Arnaud et al. reported higher Se concentration in subjects with PCOS even though other researchers stated comparable levels of Se normal and PCOS subjects (Ghosh, D. et al., 2002;Rabinowitz, M.B., 1983). However, Ghosh, D. et al., (2002) reported that concentrations of Se considerably declined with escalating the features of metabolic syndrome. In this research, the concentration of Se in fertile women was higher compared to infertile group.

Because of the good characteristics of Cu, it is an element that has a wide variety of utilizations. Nonetheless, this wide utilization escalates environmental and human exposure and therefore the possible danger associated to their poisonousness (Linder, M.C., 2012; Zheng, G., 2015) stated that the concentration of Cu was smaller in non- PCOS subjects compared to the PCOS subjects. Similar results were reported by the (Kurdoglu, Z., et al., 2012).

Perhaps, reduced capacity of the antioxidant can be increased by the deficiency of Zn in patients with PCOS. The Zheng, G., (2015) revealed that even though mean level of Zn were lower in the control group the PCOS group, the variation was not significant statistically (P>0.05). It seems that supplementation of Zn is advantageous for patients with diabetes for correcting lipid metabolism, controlling levels of glucose and preventing cardiovascular problems (Song, Y et al., 2005).

For human body, Mg is another essential element. Zheng, G., (2015) discovered similar mean of Mg levels in between the controls group and patients with PCOS with a ($p > 0.05$). Kauffman et al., (2011) noticed no significant difference between the controls and PCOS patients regarding its level even though Muneyyirci-Delale et al., (2001) demonstrated a lower serum Mg level in PCOS patients. However, this study found that the lower level of Mg in the infertile group compared to fertile.

In addition, the table 4.8. shows that the means of SOD, CAT and LPO are not significantly different (p value were 0.70, 0.81 and 0.10 respectively). Similar outcomes were found by Polak et al (2001), Jaiswar et al (2006) and Zhang *et al.* (2008), who found higher level of CAT and SOD in control than in infertile or PCOS group. In contrast, Sabuncu *et al.* (2001) and Kusçu *et al.* (2009) indicated that levels of SOD were greater significantly in a PCOS group compared with a control group. Moreover, in agreements with the results of this study, Lacey et al (2008) and Chattopadhayay et al., (2010) discovered lower level of LPO in control group compared to PCOS patients.

It is clear that the p value for all the hormones were <0.05. Coskun et al., (2013) reported that the levels FSH were significantly lower in PCOS women than the control group. In addition, it also revealed that PCOS subjects have higher levels of LH than the control group.

Moreover, Al-Jeborry, M., 2017 declared that the mean level of FSH and LH were higher in PCOS group compared the control, however the differences were no

statistically significant. In consistent with our study, Chakraborty, et al., (2013) found lower level of LH and testosterone in control group compared to PCOS patients. Similar to our results, Moti, et al., (2015) reported higher level of LH and lower level of FSH in PCOS patients compared to control.

4.2. Corelation Analysis of Fertile and Infertile Women

Results analysed using (graphpad prism Ver. 7.0) statistics software program. Differences examined by t test table and differences considered significant at $p \leq 0.5$, person correlation used to find a correlation between 2 parameters.

4.2.1. Correlations of Fertile Women

Table 4.8.-12. explain the correlation between age, tall, weight, Mg, Cu, Zn, Se, LPO, CAT, SOD, F-testosterone, testosterone, LH, FSH and BMI in fertile and infertile women.

Table 4.8. Correlations of biochemical for fertile women.

Biochemical parameter	FSH		Mg		Cu		Zn		BMI	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation		Pearson Correlation	P Value	Pearson Correlation	P Value
LPO	-0.534*	0.049	0.162	0.580	0.378	LPO K/ml	-0.534*	0.049	0.162	0.580
CAT k/ml	-0.359	0.208	0.166	0.571	0.057	CAT	-0.359	0.208	0.166	0.571
SOD	0.028	0.925	-0.331	0.248	-0.196	SOD	0.028	0.925	-0.331	0.248

Table 4.9. Correlations of biochemical for fertile women

Biochemical parameter	Age		Tall		Weight		Free Testosterone		Testosterone		LH	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
LPO	0.494	0.073	0.249	0.391	0.386	0.173	0.119	0.685	0.085	0.773	-0.423	0.131
CAT K/ml	0.329	0.251	0.116	0.693	0.289	0.316	0.203	0.486	0.129	0.659	-0.261	0.367
SOD	-0.050	0.866	-0.134	0.64	0.364	0.201	0.364	0.201	-0.064	0.829	0.235	0.418

This tables shows the correlation between biochemical parameters and age, tall, weight, Mg, Cu, Zn, Se, F-testosterone, testosterone, LH, FSH and BMI in fertile women. It clear that the even though biochemical parameters have positive and negative with all the other factors, only LPO has significant correlation with FSH (P value =0.049).

Table 4.10. Correlations of trace elements for fertile women

Trace elements	Age		Tall		Weight		LPO		CAT		SOD	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
Mg ppm	-0.144	0.624	0.114	0.697	-0.339	0.236	0.162	0.580	0.166	0.571	-0.331	0.248
Cu ppm	0.032	0.913	0.286	0.322	-0.220	0.450	0.378	0.182	0.057	0.847	-0.196	0.501
Zn ppm	-0.004	0.989	0.382	0.177	-0.062	0.834	-0.401	0.155	-0.168	0.565	-0.021	0.943
Se ppb	0.406	0.150	0.661*	0.010	0.279	0.333	0.325	0.257	0.102	0.730	-0.130	0.659

Table 4.11. Correlations of trace elements for fertile women

Trace elements	Free Testosterone		Testosterone		LH		FSH		BMI	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
Mg ppm	-0.093	0.753	-.0111	0.707	0.003	0.992	0.066	0.822	-0.408	0.147
Cu ppm	-0.184	0.530	-0.199	0.495	-0.214	0.462	-0.235	0.418	-0.341	0.233
Zn ppm	-0.196	0.503	-0.210	0.470	0.398	0.159	0.526	0.054	-0.190	0.516
Se ppb	-0.133	0.651	-0.165	0.572	-0.219	0.452	-0.219	0.452	0.057	0.846

The correlation between trace elements and age, tall, weight, LPO, CAT, SOD, F-testosterone, testosterone, LH, FSH and BMI in fertile women in Table 11. It can be seen that the correlation is statistically significant between Se and tall (P value= 0.01).

Table exhibits the correlation between serum hormone levels age, tall, weight, Mg, Cu, Zn, Se, LPO, CAT, SOD and BMI in fertile women. The Table 4.12. demonstrated that both F-testosterone and testosterone significantly correlated with weight and BMI. Furthermore, there was also significant correlation between FSH and LPO.

Table 4.12. Correlations of Serum hormone levels for fertile women

Serum hormones	Age		Tall		Weight		LPO		Catalase		SOD	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
Free. Testosterone (ng/dl)	0.433	0.122	-0.069	0.814	0.562*	0.036	0.119	0.685	0.203	0.486	-0.136	0.644
Testosterone (ng/dl)	0.386	0.173	-0.106	0.718	0.555*	0.039	0.085	0.773	0.129	0.659	-0.064	0.829
LH (IU/L)	-0.022	0.942	-0.305	0.288	0.022	0.940	-0.423	0.131	-0.261	0.367	0.235	0.418
FSH (IU/L)	-0.120	0.683	-0.260	0.370	-0.172	0.556	-0.534*	0.049	-0.359	0.208	0.028	0.925

Table 4.13. Correlations of Serum hormone levels for fertile women

Serum hormone	Mg		Cu		Zn		Se		BMI	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
Free.Testosterone (ng/dl)	-0.093	0.753	-0.184	0.530	-0.196	0.503	-0.133	0.651	0.591*	0.026
Testosterone (ng/dl)	-0.111	0.707	-0.199	0.495	-0.210	0.470	-0.165	0.572	0.599*	0.024
LH (IU/L)	0.003	0.992	-0.214	0.462	0.398	0.159	-0.219	0.452	0.129	0.661
FSH (IU/L)	0.066	0.822	-0.235	0.418	0.526	0.054	-0.219	0.452	-0.078	0.790

4.2.2. Correlations of Infertility Women

In Table 4.14 and Table 4.15., the correlations between biochemical parameters and age, tall, weight, Mg, Cu, Zn, Se, F-testosterone, testosterone, LH, FSH and BMI in infertile women are demonstrated. It is found that LPO is correlated significantly with FSH (P value = 0.017).

Table 4.14. Correlations of biochemical for infertile women

Biochemical parameter	Age		Tall		Weight		Free Testosterone		Testosterone		LH	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
LPO	-0.071	0.579	0.019	0.881	-0.060	0.638	-0.163	0.198	-0.163	0.198	-0.185	0.144
CAT K/ml	0.219	0.082	0.078	0.540	0.236	0.061	-0.163	0.199	-0.163	0.199	-.169	0.183
SOD	-0.190	0.132	0.225	0.074	0.118	0.353	-0.164	0.195	-0.164	0.195	-0.138	0.275

Table 4.15. Correlations of biochemical for infertile women

Biochemical parameter	FSH		Mg		Cu		Zn		BMI	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
LPO	-0.298*	0.017	0.049	0.702	0.117	0.359	-0.096	0.450	-0.073	0.568
CAT K/ml	-0.157	0.215	-0.125	0.325	-0.142	0.264	0.061	0.634	0.204	0.106
SOD	-0.104	0.415	0.040	0.754	.089	0.484	0.095	0.456	.062	0.625

Table 4.16. and Table 4.17. indicates the correlation between trace elements and age, tall, weight, LPO, CAT, SOD, F-testosterone, testosterone, LH, FSH and BMI in infertile women. The resulted indicated that there is no statistically significant correlation between trace elements and the other investigated factors.

Table 4.16. Correlations of trace elements for infertile women

Trace elements	Age		Tall		Weight		LPO		CAT		SOD	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
Mg ppm	-0.114	0.372	0.047	0.711	-0.010	0.937	0.049	0.702	-0.125	0.325	0.040	0.754
Cu ppm	-0.098	0.443	0.159	0.209	-0.033	0.798	0.117	0.359	-0.142	0.264	0.089	0.484
Zn ppm	0.065	0608	0.007	.0955	0.083	0.514	-0.096	0.450	0.061	0.634	0.095	0.456
Se ppb	-0.161	0.204	0.225	0.074	0.001	0.996	0.224	0.075	-0.112	0.379	-0.005	0.972

Table 4.17. Correlations of trace elements for infertile women

Trace elements	Free Testosterone		Testosterone		LH		FSH		BMI	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
Mg ppm	-0.065	0.607	-0.065	0.607	0.043	0.739	0.024	0.853	-0.028	0.823
Cu ppm	-0.133	0.294	-0.133	0.294	0.000	0.999	0.009	0.945	-0.082	0.519
Zn ppm	0.079	0.535	0.079	0.535	-0.049	0.700	-0.076	0.550	0.075	0.554
Se ppm	0.028	0.825	0.028	0.825	-0.063	0.622	-0.078	0.538	-0.067	0.599

The Table 4.18. and Table 4.19. express the correlations between serum hormone levels age, tall, weight, Mg, Cu, Zn, Se, LPO, CAT, SOD and BMI in in fertile women. It is shown that there is significant correlation between FSH and LPO, weight and BMI (P value = 0.017, 0.028 and 0.013 respectively). Moreover, the results also show LH is significantly correlated with BMI (P value = 0.020).

Table 4.18. Correlations of Serum hormone levels for infertile women

Serum hormone	Age		Tall		Weight		LPO		CAT		SOD	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
Free estosterone (IU/L)	-0.053	0.675	0-.029	0.823	0.053	0.679	-0.163	0.198	-0.163	0.199	-0.164	0.195
Testosterone (IU/L)	-0.053	0.675	0-.029	0.823	0.053	0.679	-0.163	0.198	-0.163	0.199	-0.164	0.195
LH (ng/dl)	-0.067	0.599	0.158	0.212	-0.244	0.052	-0.185	0.144	-0.169	0.183	-0.138	0.275
FSH (ng/dl)	-0.097	0.448	0.115	0.364	-0.274*	0.028	-.298*	0.017	-0.157	0.215	-0.104	0.415

Table 4.19. Correlations of Serum hormone levels for infertile women

Serum hormone	Mg		Cu		Zn		Se		BMI	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
Free Testosterone (IU/L)	-0.065	0.607	-0.133	0.294	0.079	0.535	0.028	0.825	0.070	0.584
Testosterone (IU/L)	-0.065	0.607	-0.133	0.294	0.079	0.535	0.028	0.825	0.070	0.584
LH (ng/dl)	0.043	0.739	0.000	0.999	-0.049	0.700	-.063	0.622	-0.290*	0.020
FSH (ng/dl)	0.024	0.853	0.009	0.945	-0.076	0.550	-0.078	0.538	-.0308*	0.013

5. CONCLUSION

We concluded that trace elements (Zn, Cu and Se) are involved in female infertility via their involvement in PCOS disease. Trace elements related to female weights as the result showed significant differences in trace element levels for different BMI of infertile women. Many studies Attributed PCOS disease to the oxidative stress status (Yeon Lee, et al., 2010; Fenkci, V. et al., 2003). The reduce of antioxidant defenses in the biological systems rises the oxidative stress status which lead to elevation of free radicals. uncontrolled Free radical production destroys lipids, proteins and DNA in the biological system (Floyd, R. A., and Carney, J. M. 1992).

Selenium, cupper and magnesium exhibit antioxidant defense power (Klotz, et al., 2003; Laires, M.J., et al., 2004). The decrease in these trace elements reduces antioxidant defense in PCOS women, which turn in increasing free radical and consequently damage destroy of lipids and proteins of the female ovary cells. The destroy of lipids and proteins in the female ovary negatively effects the eggs health causing premature death and deterioration of the menstrual cycle.

The menstrual cycles controlled by female reproductive hormones (LH and FSH), the disturbance of the menstrual cycle increase of this hormone the secretion controlling hormones (LH and FSH) to induce ovulation this cause the increase of this hormones in PCOS (Fenkci, et al., 2003).

The trace element levels decreased with age, this may explain the disturbance of menstrual cycle of older females.

Also we concluded that trace elements induce the reproductive hormones, Se and Cu can be supplemented with drugs in the treatment of hormone associated disturbance of menstrual cycle disease such as PCOS or postmenopausal, this supplementation may beneficial in the treatment such disorders.

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