

## Alterations in Levels of Oxidative Stress Biomarkers, Some Serum Interleukins and Hormones in Infertile Women

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### Abstract

The aim of this study was to evaluate the effect of oxidative stress and some serum interleukins and hormones levels of women with primary infertility, and to reveal the correlation between them.

A total of 120 infertile women with different etiologies and 45 fertile women as a control were investigated, their age ranged between (16-45) years old. The levels of serum TAC, SOD activity, GSH, MDA, Mg, Zn, catalase activity, interleukin-1 $\beta$  and interleukin- 8 were estimated, also the levels of LH, FSH, prolactin, progesterone, AMH, inhibin B and leptin were estimated.

The current results were revealed that there was a significant elevation in the levels of MDA, interleukin- 1 $\beta$ , prolactin and leptin, and a significant decrease in TAC, SOD, GSH, Mg, Zn, catalase activity, AMH and inhibin B between the infertile and fertile groups. There was a positive correlation between SOD with AMH, inhibin B and Mg, and positive correlation between LH and FSH, TAC and inhibin B. there was a negative correlation between FSH and inhibin B. In conclusion, it suggested that inhibin B may play a role in the antioxidant–oxidant balance.

**KeyWords:** Infertility, Antioxidants, Interleukin, Antimularian hormone, Leptin

## Introduction

The miracle of life's possibility is gained by a healthy reproductive system. The female reproductive organs play a role in the reproductive process, which includes: menstrual cycle, conception, pregnancy and delivery (Noori, 2012). Female infertility may be caused by age, physical problems, hormone abnormalities, and lifestyle or environmental factors. Most problems deal with producing eggs, the ovaries may stop functioning before natural menopause or may not release eggs regularly or they may not release a healthy one (Olooto *et al.*, 2012a). Stress plays an integral role in the pathogenesis of infertility resulting from increased free radical generation and/or decreased levels of scavenging antioxidants (D'Hooghe and Debrock, 2002).

Antioxidants are a family of vitamins, minerals and other nutrients that help to protect the body from the oxidative damage. They work as a defense system. Glutathione is present inside and outside the cells in human body and its effects on oocyte and embryo quality. Follicular fluid GSH is found in high concentrations of different sized follicles; and may act as an antioxidant to protect oocytes from free radicals during oocyte growth and maturation (Liu *et al.*, 2002). Peritoneal fluid from patients with endometriosis has been shown to exhibit inadequate antioxidant defenses, including low total antioxidant capacity (TAC) and significantly reduced levels of individual antioxidant enzymes such as superoxide dismutase (SOD) (Lambrinoudaki *et al.*, 2009). Catalase enzyme has a major role in the first defense line of enzymatic antioxidant and very important enzyme

in reproductive reactions, despite catalase enzyme is distributed over the cell, it concentrates in peroxisomes and mitochondria (Franks *et al.*, 2008). Malondialdehyde (MDA) is an endogenous product of enzymatic and free radicals -induced lipid peroxidation (Niedernhofer *et al.*, 2008). Therefore, MDA levels measurement can be widely used as an important indicator of oxidative stress and lipid peroxidation (Jetawattana, 2005). Catalase activity level was significantly decreased whereas MDA levels were significantly increased in females with primary infertility when compared with those of the fertile group (Hussain *et al.*, 2013).

Zinc works as antioxidants in egg production and hormone regulation such as estrogen, progesterone and testosterone levels throughout the menstrual cycle (Rajeswari and Swaminathan, 2015). Magnesium is also essential for reducing complications of female reproductive system (DeRidder and Gossen, 1988), because it is essential for sex hormone production and function (Fukai and Murayama, 1984).

Interleukins as a group of cytokines are important for reproductive processes and work as immunoregulatory molecules responsible for modulating several biological responses including the ovarian function (Calogero *et al.*, 1998 and Mahdi, 2011).

Hormone abnormalities had been evaluated in infertile women; infertile women with a proper menstrual cycle had higher level of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The incidence of high prolactin levels with low levels of LH, FSH and progesterone may cause anovulation and hence infertility, due to the important role of LH and FSH in graffian follicle formation and

estrogen production (Olooto *et al.*, 2012b).

Anti-Mullerian hormone (AMH) is mainly secreted by the granulosa cells of primary, pre-antral, and small antral follicles, suggesting an important role of AMH in steroidogenesis and folliculogenesis. (Saleh *et al.*, 2015). Inhibin B is the primary regulator of FSH secretion during the follicular phase. The decrease in inhibin B levels lifts the inhibitory tone over the hypothalamic-pituitary- ovarian axis allowing FSH levels to increase and estradiol decrease (Vander, 2001). Leptin has an effect on the ovary and the hypothalamus, high level of leptin decrease estradiol production in the ovary, and leptin receptors are also located in the hypothalamus regulate GnRH, therefore leptin induces GnRH secretion (Jungheim, 2015).

## Materials and Methods

### A. Subjects and Samples

We investigated 120 infertile women and 45 fertile women as control, with age ranging between 18-45 years old, who attended the Sheray Naqeeb hospital in Kalar city from December 2014 to February 2015. The exclusion criteria include none of the women had previous ovarian surgery, no contraceptive methods, no receiving sex steroids or any drug known to affect ovarian function for at least 2 months, and all of them with primary infertility, They were subdivided into three groups according to the age, as following: Group 1: 55 women aged between 16-25 years, 40 of them infertile and 15 were fertile. Group 2: 55 women aged between 26-35 years, 40 of them infertile

and 15 were fertile. Group 3: 55 women aged between 36-45 years, 40 of them infertile and 15 were fertile. Venous blood had been collected from each woman after 24 hours of menstrual cycle, and the sera were separated by centrifuge at 3000 rpm for 10 minutes; the samples were stored at (-30) °C in deep freezer.

## **B. Biochemical Assays**

### **Estimation of Antioxidants**

SOD activity is estimated by using SOD Assay Kit-WST (Dojindo, Japan). SOD Activity was measured by inhibiting the reduction of nitroblue tetrazolium to produces a water-soluble formazan dye with the superoxide anion using microplate reader (StateFax, USA) at 450 nm. TAC concentration was estimated by using TAC Fast Track (LDN, Germany). This assay is based on the reaction of peroxides with peroxidase and the reaction of tetramethylbenzidine. Reduced glutathione (GSH) in the serum was estimated according to the method of Moron *et al.*, the reaction of GSH with 5,5-dithio-bis (2 -nitrobenzoic acid) [DTNB] gives an yellow colored compound that absorbs spectrophotometrically at wavelength of 412 nm. The analysis of MDA by 2-thiobarbituric acid assay has been employed in this study for the assessment of lipid peroxidation, and read the absorbance at 532 nm 9 Wallace and Kelsey, 2010). Catalase enzyme activity was measured following the method of L. goth (Goth, 1991). The activity was determined spectrophotometrically by measuring the decrease in H<sub>2</sub>O<sub>2</sub> absorbance at 240 nm. The inductively coupled plasma optical emission spectroscopy (ICP-OES) (Spectro Arcos, Germany) used for analysis of Mg and Zn.

## Estimation of IL- 1 $\beta$ and IL-8

The kits provided by (KOMA BIOTECH, Korea), they are a quantitative kits, and use Sandwich-ELISA assay. The optical density (OD) is measured using microplate reader (Dynex, USA) at 450 nm.

## Estimation of Hormones

LH, FSH, prolactin, and progesterone assays were achieved by electrochemiluminescence immunoassay (Roche-Hitachi Cobas e 411) using quantitative kits (Cobas, Japan). The kits of AMH and inhibin B (MyBioSource, USA) and leptin (LDN, Germany) were use sandwich-ELISA assay and read the optical density (OD) by using microplate reader (Dynex, USA) at 450 nm.

## C. Statistical Analysis

Duncan Post Hoc test was used to detect any possible correlation between the variables screened. Significant results were accepted at  $P \leq 0.05$ .

## Results

In the current study, the data was obtained demonstrated a significant decreasing in each of TAC and SOD activity, catalase activity and GSH levels, The data presents verified TAC means  $\pm$ SD at ( $p < 0.05$ ) in infertile and fertile groups were ( $1.30 \pm 0.36$  and  $1.91 \pm 0.30$ ), SOD activity were ( $4.03 \pm 2.59$  and  $9.46 \pm 2.01$ ), catalase activity ( $4.33 \pm 2.15$  and  $6.98 \pm 4.81$ ) and GSH level ( $0.41 \pm 0.26$  and  $0.59 \pm 0.23$ ) in respective, for more illustrations see Figure (1).

The results demonstrate a significant increase in MDA level in infertile group

as compared with fertile group ( $4.23 \pm 1.79$  and  $2.02 \pm 0.91$ ).

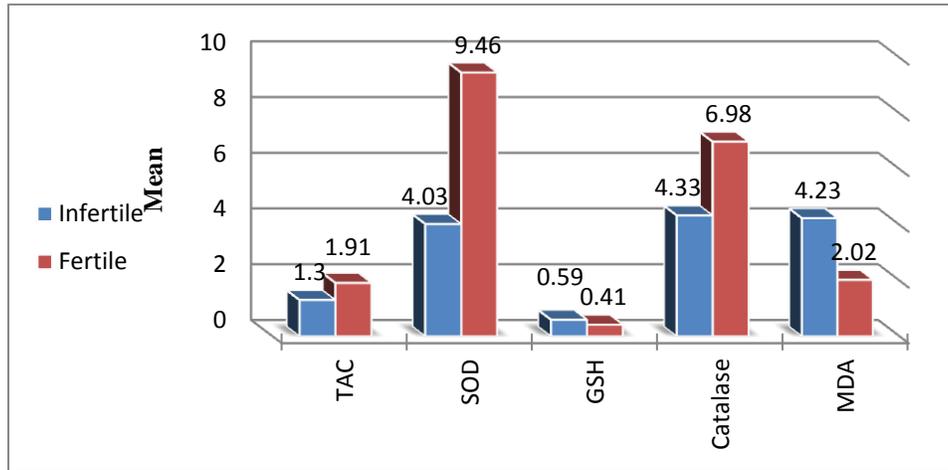


Figure (1): Levels of antioxidants in infertile and fertile groups

According to the data, it demonstrate that Mg and Zn were decreased significantly in infertile group as compared with the fertile group, the values of Mg in infertile and fertile groups were ( $20.59 \pm 3.18$  and  $23.90 \pm 2.84$ ) and Zn were ( $0.46 \pm 0.19$  and  $0.76 \pm 0.21$ ), as in Figure (2).

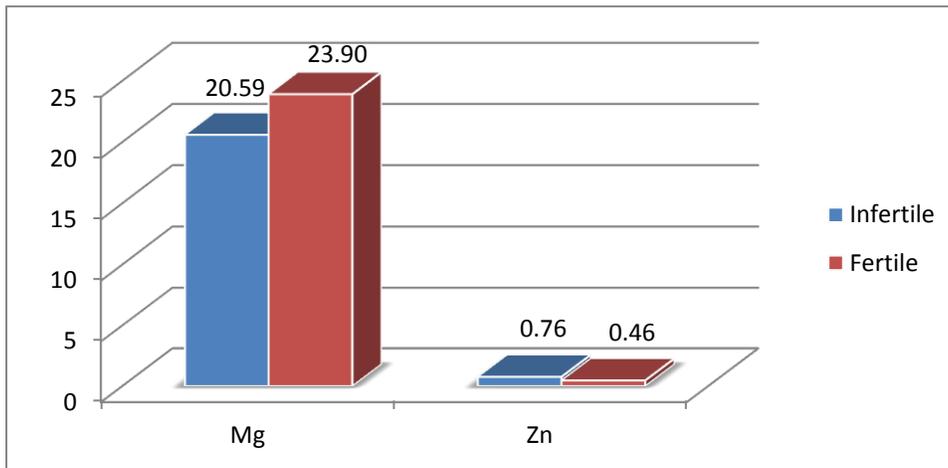
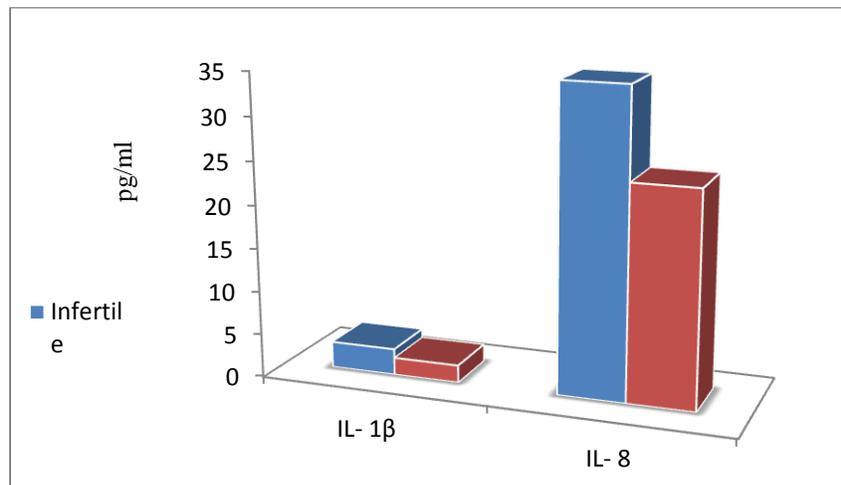


Figure (2): Levels of magnesium and zinc in infertile and fertile groups

The present data shows a significant elevation in the level of serum IL-1 $\beta$  between infertile ( $3.05 \pm 0.73$ ) and fertile group ( $2.03 \pm 0.53$ ), but IL-8 level was not

significant higher in infertile group ( $35.00 \pm 5.95$ ) as compared with fertile group ( $24.65 \pm 3.27$ ); as shows in Figure (3).



**Figure (3):** Levels of serum IL-1 $\beta$  and IL-8 in infertile and fertile groups

Our results shown in Figure (4) revealed that the levels of LH and FSH were not significant increased, while there was significant increase in prolactin levels of infertile group ( $27.98 \pm 4.33$ ) when compared with fertile group ( $10.09 \pm 2.51$ ).

According to the results that were shown in Figure (5), the level of progesterone was non-significantly elevated in infertile group comparing to fertile group, while the level of AMH was significantly decreased in infertile group ( $0.75 \pm 0.20$ ) comparing to fertile group ( $1.19 \pm 0.18$ ). As well as the level of inhibin B was significantly decreased in infertile group ( $39.95 \pm 6.45$ ) comparing to fertile group ( $73.32 \pm 7.19$ ). While, the level of leptin was significantly increased in infertile group ( $28.98 \pm 4.25$ ) as compared to fertile group ( $24.58 \pm 3.89$ ), as shown in Figure (6).

In addition, the results show regarding to age groups a significant and not significant variations among the three age groups of infertile women, for more clarification see table (1).

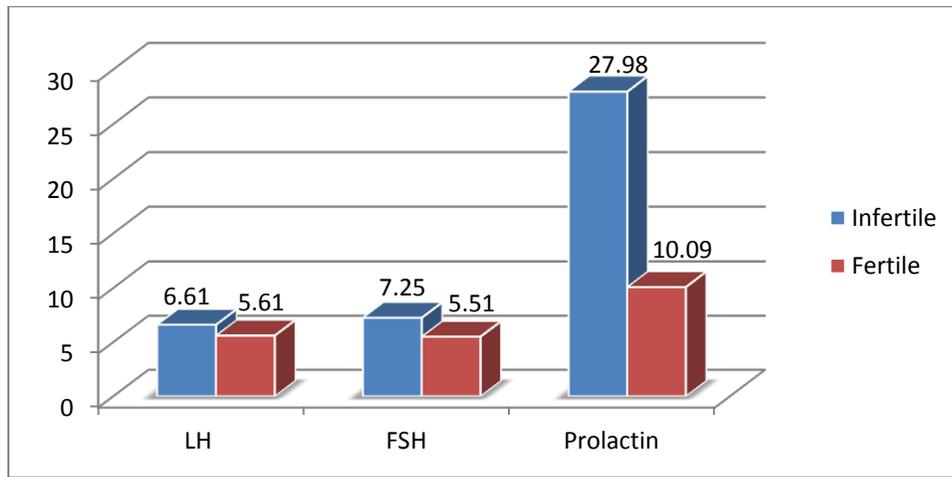


Figure (4): Levels of LH, FSH and prolactin in infertile and fertile groups

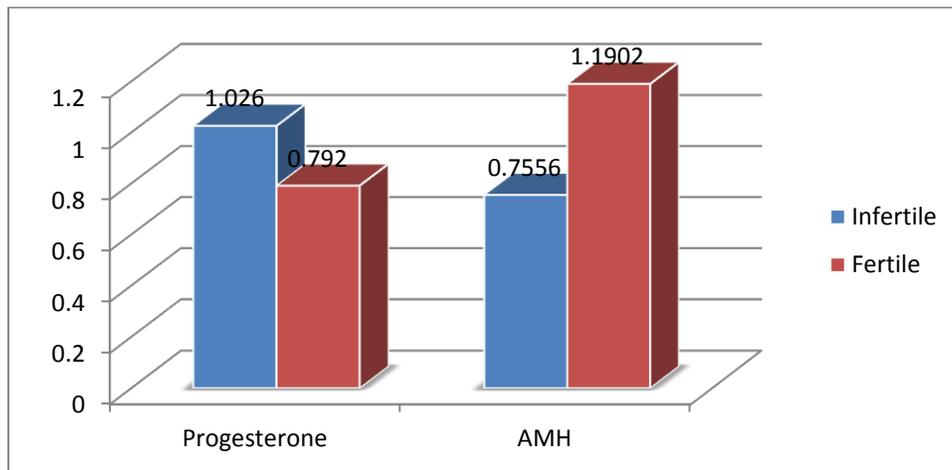


Figure (5): Levels of progesterone and AMH in infertile and fertile groups

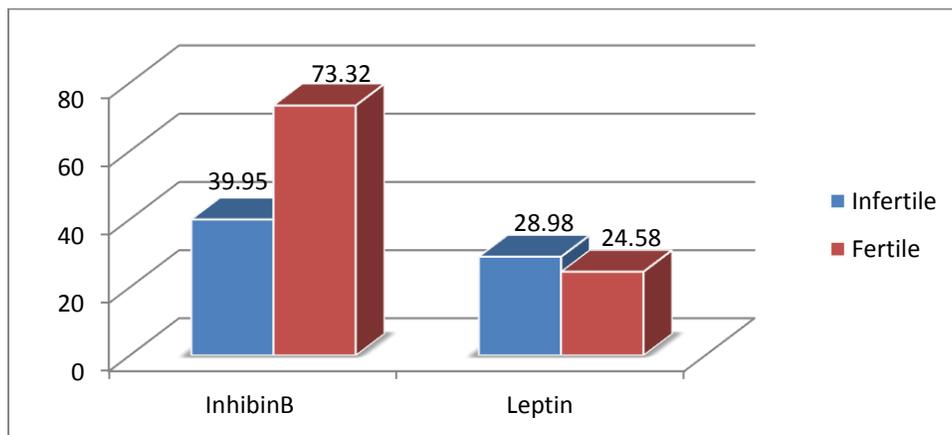


Figure (6): Levels of inhibin B and leptin in infertile and fertile groups

**Table (1):** The levels of studied variables in relation to age groups

Age group Variables	Group 1 n=40	Group 2 n=40	Group 3 n=40
TAC (mmol/L)	1.32 ±0.34 a	1.35 ±0.33 a	1.22 ±0.40 a
SOD (mIU/ml)	3.44 ±1.14 a	4.95 ±0.92 a	3.69 ±0.46 a
MDA (µmol/L)	3.82 ±1.01 a	3.82 ±1.11 a	4.51 ±1.05 a
Catalase (IU/ml)	4.60 ±1.22 a	4.83 ±0.25 a	3.57 ±0.79 b
GSH (mMolar/L)	0.45 ±0.21 a	0.45 ±0.10 a	0.33 ±0.08 a
Mg (mg/L)	20.35 ±2.40 a	20.49 ±3.07 a	20.92 ±3.94 b
Zn (mg/L)	0.46 ±0.11 a	0.30 ±0.08 a	0.61 ±0.20 a
IL-1β (pg/ml)	2.30± 0.74 b	2.72± 0.80 b	4.15± 1.15 a
IL-8 (pg/ml)	28.83± 3.43 b	47.31± 6.03 a	28.88± 5.69 b
LH (mIU/ml)	4.99 ±0.84 b	6.32 ±1.72 b	8.52 ±1.99 a
FSH (mIU/ml)	5.94 ±1.74 b	6.21 ±2.59 b	9.60 ±1.76 a
Prolactin (ng/ml)	25.53 ±4.17 b	41.20 ±8.16 a	17.23 ±3.75 b
Progesterone (pg/ml)	1.12 ±0.36 a	1.36 ±0.33 a	0.58 ±0.18 b
AMH (ng/ml)	0.89 ±0.19 a	0.78 ±0.63 a	0.58 ±0.31 a
Inhibin B (pg/ml)	47.50 ±6.89 a	37.80 ±6.34 a	34.53 ±4.01 a
Leptin (ng/ml)	27.81 ±4.80 a	27.09 ±3.68 a	32.05 ±2.97 a

In this study, the Pearson correlation was used to investigate the relationships between the variables of all values. There was a positive correlation between LH and FSH ( $r= 0.578$ ), TAC and inhibin B ( $r=0.368$ ), SOD and AMH ( $r=0.231$ ), SOD and inhibin B ( $r=0.364$ ), SOD and Mg ( $r=0.461$ ). A negative correlation also found between FSH and inhibin B ( $r=-0.442$ ).

## Discussion

The physiological role of ROS during ovulation and regulation of ovarian mesenchyme growth (Farzadi *et al.*, 2013), increased ROS causes oxidative stress

which is involved in numerous pathological conditions of female infertility (Gupta *et al.*, 2009).

These hormonal imbalances of (LH, FSH and prolactin) have an impact on ovulation and menstruation because FSH and LH are needed for follicular maturation at the beginning of the monthly cycle, hence LH stimulates theca cells to produce estrogens and FSH stimulates recruitment of secondary ovarian follicles and the secretion of estradiol from granulosa cells (Boehm *et al.*, 2015). The current study revealed that AMH has decreased significantly with infertility, generally this result goes along with a study on PCOS patients, which revealed the multiple follicles produce large amounts of estradiol that inhibits FSH release thereby preventing further follicular development. AMH may be involved in regulating the progression of follicles into growth phase, and abnormalities in AMH have also been proposed as contributors to the etiology of PCOS (Franks *et al.*, 2008).

The current data appeared a significant decreased in inhibin B levels between infertile and fertile groups; it also appeared decreasing in inhibin B corresponding with age. This result confirmed by other study concluded that serum concentrations of inhibin B decrease with age and premature ovarian failure; thus, inhibin B has largely been considered a marker of ovarian activity, rather than ovarian reserve (Domingues *et al.*, 2010). This diagnostic test can detect a decrease in AMH levels five years before a difference in the levels of FSH or inhibin B is noticed (Zheng *et al.*, 2008).

The results of current study showed significant differences in TAC levels between infertile and fertile women, which suggest that systemic antioxidant defense mechanisms are consumed in infertile women. This decreasing in TAC levels has been an indicator to reveal that there was not maturation in follicles thus no growing of good oocytes (Revelli *et al.*, 2009). SOD is the enzyme that reacts

with superoxide anion radicals to form oxygen and  $H_2O_2$ , its decline has been shown that women with infertility have insufficient antioxidant defense, this result was in accorded with several studies like the study of Szczepanska *et al.* (2003) that revealed low TAC and significantly reduced SOD levels in infertile women. This reduction in TAC may imply elevated ROS and increased ROS levels imply exhausted antioxidant defense, leads to its inability to scavenge ROS and neutralize their toxic effects (Nsonwu-Anyanwu *et al.*, 2015). MDA represents the end product of polyunsaturated fatty acid oxygenation, is commonly used as the lipid peroxidation level marker and the presence of oxidative stress (Grotto *et al.*, 2009). In our study MDA increasing may be explained by the increased ROS production as a result of higher lipid peroxidation, also according to previous studies there is a direct relation between increased MDA and decreased TAC (Farzadi *et al.*, 2013).

When we examined the impact of age on antioxidants levels, we found non-significant variation in TAC, SOD, MDA and GSH between the three age groups, whereas there was slightly decreased in antioxidants such as TAC, SOD and GSH levels with aging and increased in MDA levels. While catalase activity showed significant reduction in age group 3 comparing with the others, this result in accorded with the study of Appasamy *et al.* (2008) that found there was no significant relationship between age of the woman and plasma TAC and the study of Hussain *et al.* (2013) that was observed a negative impact of age on catalase values in infertile women which categorized into three age groups. The present results confirmed that magnesium concentration in serum was significantly decreased in infertile women as compared with fertile group; on the other hand, magnesium concentration expressed a significant elevation in the age group 3. This result corresponds to study of Howard *et al.* (1994) that demonstrated magnesium role in enhancing fertility and increasing pregnancy rate in patients with

unexplained infertility and low magnesium levels. The significant decreased magnesium concentration in the current study may participate in infertility because magnesium deficiency associates with variable complications of female reproductive system. Our results revealed that serum zinc concentration was significantly reduced in infertile group in comparison with the fertile group, this showed that zinc deficiency can play a negative significant role in female infertility and this was related to other previous studies demonstrated the same like the founding of Ceko *et al.* (2016), Zinc deficiency causes impaired synthesis and secretion of LH and FSH which leads to disruption of the pituitary-ovarian axis function and estrous cycle (Bedwal and Bahuguna, 1994).the data showed a significant elevation in the level of serum IL-1 $\beta$  between infertile and fertile groups. This result was not corresponded with the study of Kalu *et al.* (2007) on infertile women, who were found that serum level of IL-1 $\beta$  was not significantly different in the patients and controls. Indeed, IL-1 $\beta$  levels are reported to be decreased in endometrium of women with recurrent miscarriage, suggesting that a fail in activating the pathways downstream of IL-1 $\beta$  may contribute to pregnancy failure (Rossi *et al.*, 2005). Also it was seen that any increasing of follicular fluid IL-1 $\beta$  level was associated with fertilization. Previous studies suggested that ability to generate a healthy fetus and successful in vivo fertilization occur in the oocytes in follicles with lower concentration of IL-1 $\beta$  in follicular fluid (Revelli *et al.*, 2009). reason may due to IL-1 $\beta$  role in suppressing estradiol and progesterone release from granulosa and luteal cells (Mahdi, 2011).

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