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# ASSOCIATION BETWEEN SERUM AND FOLLICULAR FLUID OF COPPER AND MAGNESIUM CONCENTRATION WITH EMBRYO QUALITY IN WOMEN UNDERGOING ICSI

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## ABSTRACT

**Background:** Trace elements show a number of biochemical and physiological functions thus affect the oocyte maturation and embryo quality, and may play a role in decreasing fecundity and fertility. **Objective:** To assess the effect of Copper and Magnesium concentration in serum and follicular fluid on oocyte, embryo quality and pregnancy success in women undergoing ICSI. **Material and Methods: Methods:** The study was conducted based on 70 patients recruited from August 2016 to February 2017 in High Institute of Infertility Diagnosis and Assisted Reproductive Technologies and in Kamal Al-Samarrai Hospital, center of fertility and IVF (Baghdad/Iraq)**.** These women underwent ICSI using the GnRH-agonist long or short protocol. Out of these 70 patients, 39 had successfully conceived (pregnant group) and the other 31 failed (non- pregnant group). Follicular fluid samples were collected from these women on the oocyte retrieval day. Concentration of Cu and Mg in serum and follicular fluid samples were measured by flame atomic absorption spectrophotometer (FAAS). The correlations between serum and follicular fluid concentrations of Cu and Mg and the number (No.) of MII oocyte retrieved, fertilization rate and number of embryo transferred were determined. **Results:** In all subjects studied, the mean of serum concentrations of Cu and Mg of pregnant and non-pregnant group were (165.23 ± 3.99 and 168.412 ± 1.64 µg/dl) and (1.112 ± 0.04 and1.120 ± 0.04 µg/dl) respectively, while the mean of FF concentration of Cu and Mg were (117.00 ± 2.35 and 114.32 ± 2.67 μg/dl) and (1.562 ± 0.03 and 1.575 ± 0.04 μg/dl) respectively. However, no difference was observed in serum and follicular fluid concentrations of Cu and Mg on the oocyte retrieval day between pregnant and non-pregnant groups. However, for pregnant groups combined, follicular fluid concentrations of Cu and Mg were positively correlated with No. of MII oocyte retrieved (r = 0.29, P<0.05) and (r=0.48, P<0.01) respectively. **Conclusion:** These data suggested, there was no significant difference between pregnant and non-pregnant groups undergoing ICSI cycle in Copper and Magnesium concentration on the day of oocytes retrieval in both serum and follicular fluid.

**KEYWORDS:** Copper (Cu), Magnesium (Mg), follicular fluid (FF), intracytoplasmic sperm injection (ICSI), embryo transfer (ET)

## INTRODUCTION

The trace elements in human tissues are essential for cell growth, maturity, and physiological functions, the latter of which include regulation of hormone production and secretion of hypothalamus, pituitary, and gonad, thereby critically affecting the oocyte matura tion and embryo quality and ultimately significantly impacting pregnant outcomes.[1-3] Excessive trace elements may also be harmful to cells.[4] Cu had reported to be present in human follicular fluid.[3,6,7] To date, however, there are only a few reports determining the trace element levels in serum and follicular fluid of patients who undergo IVF, and the data are not consistent. For example, it was reported that the level of Cu in serum were lower in women who underwent IVF than in the healthy control subjects, suggesting important roles of both Zn and Cu in the normal development of oocytes.[3] In contrast, Ng et al. investigated human follicular uid levels of Zn and Cu in 33 patients stimulated with clomi- phene citrate and human menopausal gonado- tropin in the IVF program and showed no signi - cant difference in Zn and Cu in those collected follicles of different sizes and no association.

### Copper (Cu)

Copper is a chemical element with the symbol Cu (from Latin: cuprum) and atomic number 29. It is a ductile metal with very high thermal and electrical conductivity. Pure copper is soft and malleable; a freshly exposed surface has a reddish-orange color. It is used as a conductor of heat and electricity, a building material, and a constituent of various metal alloys.[153]

Copper are essential to all living organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome c oxidize. In molliscand crustacea copper is a constituent of the blood pigment hemocyanin, which is replaced by the iron-complexed hemoglobin in fish and other vertebrates. The main areas where copper is found in humens are liver, muscle and bone. Copper compounds are used as bacteriostatic substances, fungicides and wood preservatives.[153] The adult human body contains (80 - 150) mg of Cu. Liver is the main storage site, relatively high amounts are also found in heart, brain and kidneys. Muscle and bone Cu concentrations are low, although they contain about (50%) of the total body Cu in relation to their large mass.[146] Cu is widely distributed in foods. The accumulation of Cu in plants is not affected by the Cu content of the soil in which they grow.[154]

Mainly copper is available in the liver, shellfish, dried fruit, milk and milk products, sunflower seeds, oysters, sesame seeds, tahini, and sun dried tomatoes.[155] The average content of metal in the plant usually ranges from 4 to 20 mg of copper per kg of dry weight. The average adult human of 70 kg weight contains about 100 mg. The daily requirement is about 2-5 mg of which 50% is absorbed from the gastrointestinal tract (GIT). Rest is excreted via bile and kidney. Copper accumulates in the liver, brain and kidney more than rest of body. Over 90% of plasma copper is associated with ceruloplasmin and 60% of red blood cell (RBC) is bound to superoxide dismutase.[156]

In human blood, copper is principally distributed between the erythrocytes and in the plasma. In erythrocytes, 60% of copper occurs as the copper-zinc metalloenzyme superoxide dismutase, the remaining 40% is loosely bound to other proteins and amino acids. Total erythrocytes copper in normal human is around 0.9-1.0 pg/ml of packed red cells.[157]

**Magnesium**

Magnesium is the second most common intracellular cation, doing an essential role in many physiologic functions. It is important in energy requiring metabolic processes, protein synthesis, membrane integrity, nervous tissue conduction, neuromuscular excitability, muscle contraction, hormone secretion and intermediate metabolism.[166] The normal plasma magnesium concentration is 1.5-2.3 mg/dl (1.2-1.9 meq/l; 0.62-0.94 m mol/l) with some variation between clinical laboratories.[167] Magnesium has been used as a tocolytic agent since 1969.[168] A randomized trial resulted magnesium sulfate could effectively prevent preterm birth.[169] Another study showed successful tocolysis in more than 92% of patients by using larger doses of magnesium sulphate.[170] In a study, 46% of pregnant mothers had deficiency of magnesium as exposed by serum level.[171]

It has been studied that intake of magnesium may be insufficient for many women during pregnancy. However, it uses for eclampsia and sometime for preeclampsia.[172] Almone and colleague findings have implicated magnesium as being an essential element for fetal well-being and supplementation of magnesium may be benefited to fetal outcome.[173] Magnesium supplementation during pregnancy was associated with significantly fewer maternal hospitalizations, a reduction in preterm delivery, and less frequent referral of the newborn to the neonatal intensive care unit. The results suggest that magnesium supplementation during pregnancy has a significant influence on fetal and maternal morbidity both before and after deliver.[174] Trials have documented that oral supplementation of magnesium in physiological amounts during pregnancy reduces pregnancy hypertension, miscarriage, premature birth and fetal growth Retardation.[175]

## PATIENTS AND METHODS

This is study included 70 infertile couples enrolled in assisted reproductive technology (ART) programs to enter ICSI cycle in High Institute of Infertility Diagnosis and ART/ Al-Nahrain University and Kamal Al-Samarrai Hospital, center of fertility and IVF (Baghdad/Iraq) during the period of August 2016 to the end of January 2017.

ll patients were informed about the study and signed a written informed consent. In addition, all couples should be subjected to a full history taking, complete general examination, complete gynecologic examination and infertility workup including: husband's semen analysis, hystrosalpingography and trans-vaginal ultrasound.

### Main Parameter Measures

• Total number of oocytes retrieved at the day of oocyte retrieval.
• Number of mature oocytes [Metaphase II (MII)].
• Fertilization rate (FR%). FR%=Number of fertilized oocytes/Number of oocytes which entered ICSI cycle
• Embryo transfer number.

Trace elements determined

1- Copper

Copper was analyzed in serum and follicular fluid by flame atomic absorption Spectrophotometer by waves length 324.7 nm, using hallow cathode lamp. the instrument was calibrated using aqueous standerds of (200, 150, 100, 50 μg/dl), 1ml of serum or follicular fluid placed in plane tube and added to 10 ml Distal water, then mixed and the concentration were read by FAAS.

2- Magnesium

Magnesium was analyzed in serum and follicular fluid by flame atomic absorption Spectrophotometer by waves length 285.2nm, using hallow cathode lamp. the instrument was calibrated using aqueous standerds of (0.5, 1, 1.5, 2 μg/dl), 50 μl of serum or follicular fluid placed in plane tube and added to 100 ml Lanthium chloride (LACL), then mixed and the concentration were read by FAAS.

## STATISTICAL ANALYSIS

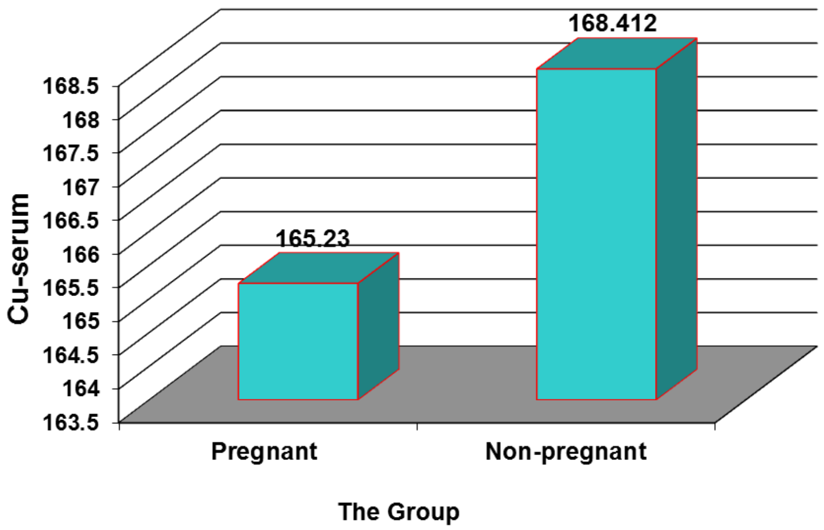
The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test (ANOVA) or T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage. Estimate of correlation coefficient between parameters in this study.[16]

## RESULT

### 4.3. Comparison Between pregnant and non-pregnant in Cu and Mg in serum

### 4.3.2. Serum copper

In this study, the mean of serum copper ± SE in both pregnant and non-pregnant groups were (165.23 ± 3.99 and 168.412 ± 1.64 μg/dl) respectively. the statistical analysis showed no significant difference ($P>0.05$) among the two groups. as shown in table (4.4) and figure (4-11).




**(Figure 4-11) Compare between pregnant and non-pregnant in Cu-serum.**

**4.3.3. Serum Magnesium**

the mean of serum Mg ± SE in both pregnant and non-pregnant groups were (1.112 ± 0.04 and 1.120 ± 0.04 µg/dl) respectively. the statistical analysis showed no significant difference (P>0.05) among the two groups. as shown in table (4-4) and figure (4-12).

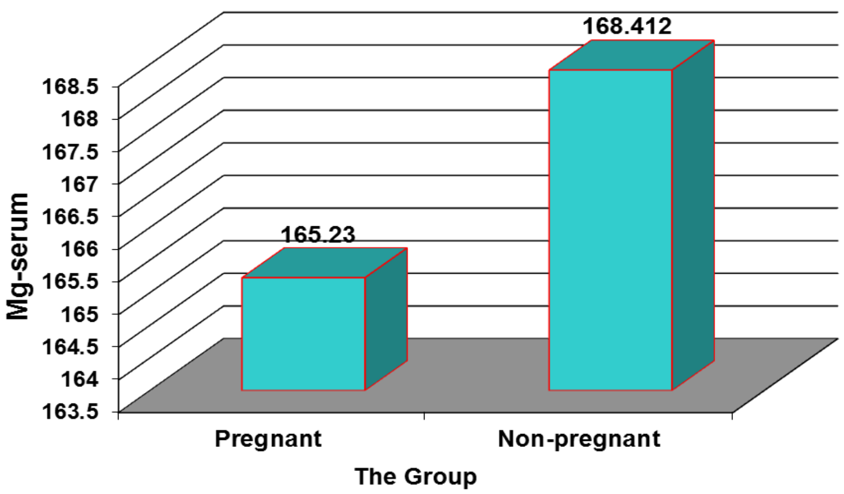



**Figure (4-12): Comparison between pregnant and non-pregnant in Mg-serum.**

**Table (4-4) Compare between pregnant and non-pregnant in Zn, Cu and Mg in serum.**

| The group | Cu | Mg |
|---|---|---|
| Pregnant | 165.23 ± 3.99 | 1.112 ± 0.04 |
| Non-pregnant | 168.412 ± 1.64 | 1.120 ± 0.04 |
| T-Test | 11.396 NS | 0.117 NS |
| P-value | 0.629 | 0.885 |

**Comparison between pregnant and non-pregnant in Pb, Zn, Cu and Mg in Follicular Fluid**

**4.4.3. Follicular Fluid Copper**

the mean of Follicular Fluid Cu ± SE in both pregnant and non-pregnant groups were (117.00 ± 2.35 and 114.32 ± 2.67 μg/dl) respectively. the statistical analysis showed no significant difference ($P>0.05$) among the two groups. as shown in table (4-5) and figure (4-15).

**4.4.4. Follicular Fluid Magnesium**

The mean of Follicular Fluid Mg ± SE in both pregnant and non-pregnant groups were (1.562 ± 0.03 and 1.575 ± 0.04 μg/dl) respectively. the statistical analysis showed no significant difference ($P>0.05$) among the two groups. as shown in table (4.5) and figure (4-16).

**Table (4-5): Compare between pregnant and non-pregnant in Pb, Zn, Cu and Mg in Follicular Fluid.**

| The group | Cu | Mg |
|---|---|---|
| Pregnant | 117.00 ± 2.35 | 1.562 ± 0.03 |
| Non-pregnant | 114.32 ± 2.67 | 1.575 ± 0.04 |
| T-Test | 7.098 NS | 0.1037 NS |
| P-value | 0.454 | 0.803 |

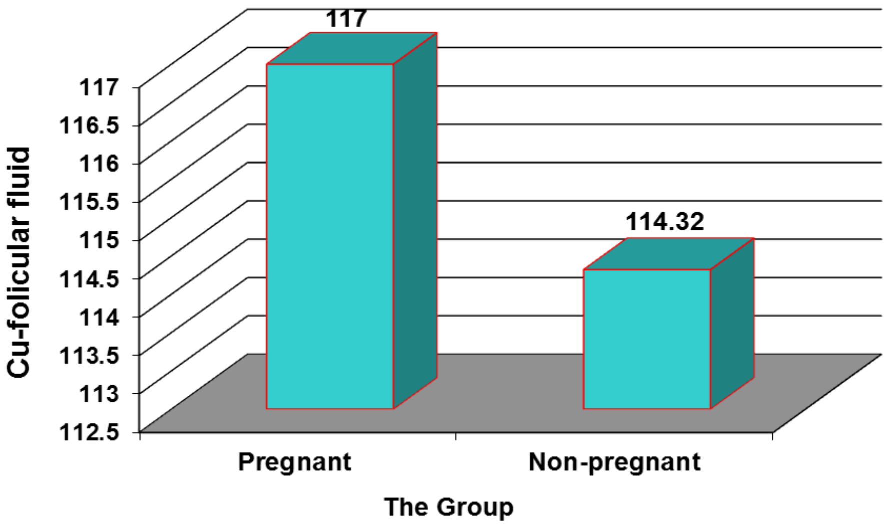



**Figure (4-15): Compare between pregnant and non-pregnant in Cu-follicular fluid.**

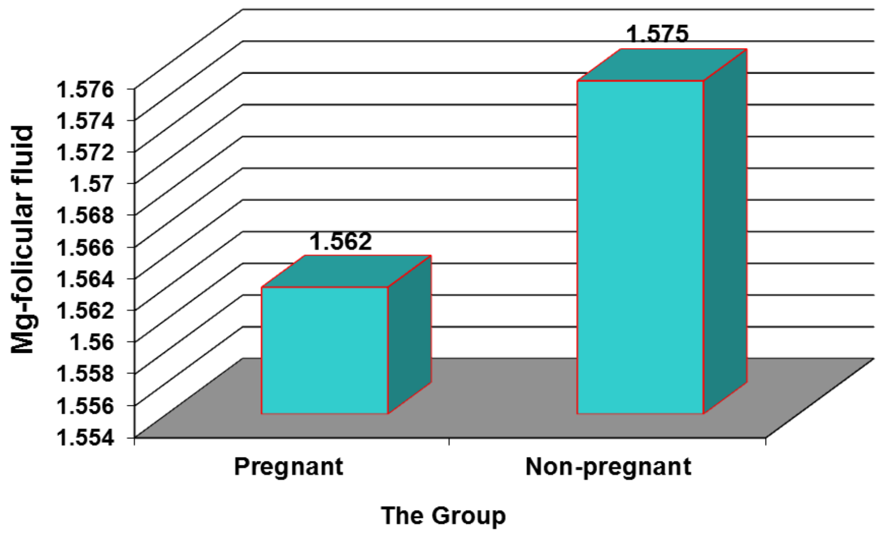



**Figure (4-16): Comparison between pregnant and non-pregnant in Mg-follicular fluid.**

**Correlation between Cu and Mg in serum with Cu and Mg in follicular fluid**

1- There was no significant between Cu and Mg in serum with Cu and Mg in follicular fluid (r=0.09, P>0.05), and (r=0.09, P>0.05) (Table 4-12)

2-There was no significant between Cu and Mg in serum with Cu and Mg in follicular fluid (r=-0.02, P>0.05), and (r=-0.02, P>0.05) respectively (Table 4-12)

**Table (5) Correlation between Pb in blood, Zn in serum with Pb, Zn in follicular fluid.**

| The group | Blood and Serum | Follicle fluid | Correlation coefficient-r |
|---|---|---|---|
| Pregnant | Cu | Cu | 0.09 NS |
| | Mg | Mg | 0.09 NS |
| | | | |
| | | | |
| Non-Pregnant | Cu | Cu | -0.02 NS |
| | Mg | Mg | -0.02 NS |
| ** (P<0.01), NS: Non-significant. | | | |

**DISCUSSION**

This study was conducted to assess the relationship between blood/FF concentrations of heavy metals/trace elements and ICSI outcome. These data suggest that blood lead levels are significantly lower in patients with ongoing pregnancy. Notably, multivariate analysis demonstrated that increments in blood lead and FF copper concentrations were significantly associated with lower likelihood of ongoing pregnancy. In Spearman's correlation analysis we detected some unexpected correlations, albeit weak, between blood and FF levels of some toxic metals and number of MII oocytes, Grade A embryos, IR, CPR and ongoing pregnancy rate. This may have occurred as a random effect due to extremely low levels of assessed toxic metals in blood or follicular fluid, all of which were detected at exceptionally low levels compared to toxic doses.

The small sample size, especially those with ongoing pregnan- cies, was the major limitation of the current study. However, considering the difficulty of assessing elements not measured routinely and found in extremely low levels in human body fluids or blood, we had to perform the statistical analyses with this.

**Associations between copper in serum and follicular fluid of pregnant and non pregnant women**

Cu is essential trace element in human body involved in regulating normal reproduction of mammals as well as different metabolic processes and enzymatic reactions.(225) The metabolic disorder, deficiency, and excessive intake of Cu also result in various diseases.[226] For instance, the lower levels of serum Cu are associated with decreases in fecundity of buffalo.[227] These decreases could be partially contributed to the effects of Cu on secretions of hormone produced by pituitary gland, e.g., growth hormone (GH), thyroid- stimulating hormone (TSH), LH and adrenocor- ticotrophin (ACTH), all of which are critical to oocyte development and ovulation.[228] Recently, Babaei et al.[229] found that antral follicles were the most susceptible to the Cu overexposure, leading to a signifcant decrease in the number of follicular cells, increased atresia, and decreased the number of corpora lutea. Possible mechanisms for Cu-overexposure induced follicular damage include Cu-increased cell apoptosis, vacuolization of the cytoplasm organelles, and detachment of cell membrane from its basement membrane.[229] However, supplement of Cu (0.46 and 0.68 mg/L) in culture media accelerates successful formation of 8-cell embryos, morulae, and blastocysts in bovine, while a long-term lack of Cu increased the number of apoptotic blastomeres.[230] In the present study, there is no significant difference in the mean number of serum and follicular fluid copper between pregnant and non-pregnant groups. This results agreement with results obtained by Ng et al.[224] who investigated human follicular fluid levels of Zn and Cu in 33 patients stimulated with clomiphene citrate and human menopausal gonadotropin in the IVF program and showed no significant difference in Zn and Cu in those collected follicles of different sizes and no association between the follicular fluid concentrations of Zn and Cu with the status and maturity of oocytes Thus, a clear association between the follicular fluid concentrations of Zn and Cu with IVF outcomes is not yet determined.

Also in the present study there is no significant correlation between M2 oocyte and serum zn and cu in pregnant (table 4-8) and non-pregnant group (table 4-8). And there was no significant correlation between fertilization rate and serum copper in pregnant (table 4-8) and non- pregnant group (table 4-8).

In follicular fluid copper, there was a significant correlation between M2 and FF Cu in pregnant (table 4-10) and non-pregnant (table 4-11). While there was no significant correlation between FR and FF Cu in pregnant (table 4-10) but there was a significant correlation between FR and FF Cu in non-pregnant (4-11) This results agreement with results obtained by Sun Y. et.al,[231] who confirmed that the follicular fluid concentration of Cu is positively correlated with the No. of MII oocyte retrieved, and fertilization rate. Together, these data suggest that an optimal concentration of Cu in follicular fluid may promote oocyte growth and improve the fertilization. it was reported that the levels of Zn in serum and follicular fluid as well as the levels of Cu in serum were lower in women who underwent IVF than in the healthy control subjects, suggesting important roles of both Zn and Cu in the normal development of oocytes.[231]

**Associations between Magnesium in serum and follicular fluid of pregnant and non pregnant women**

Magnesium, an essential trace element for the human body, is needed for proper bone formation and in various intracellular enzymatic processes.[232] Magnesium has established its role in obstetrics, it being an essential element to fetal wellbeing. Deficiency of magnesium may be possibly associated with pre-eclampsia and pre-term delivery and possibly with low birth weight.[233] It has been documented that magnesium deficiency during gestation significantly increases neonatal mortality and morbidity. Earlier supplementation trials of magnesium in developed countries during pregnancy have documented an association with significantly fewer maternal hospitalizations, a reduction in pre-term delivery, less intrauterine growth retardation, and less frequent referral of the newborn to the neonatal intensive care unit. The results further suggested a significant influence on fetal and maternal morbidity both before and after delivery.[234] A positive correlation between magnesium intake in the first trimester of pregnancy with the birth weight has been documented. However, magnesium supplementation during II and III trimester had no effect on the outcome of pregnancy.[235] Another study conducted in India documented a significant reduction in maternal mortality with the use of magnesium sulphate.[236] An introduction of a low dose of magnesium sulphate, to treat eclampsia during pregnancy has reduced mortality rates from 16% to 8% in Bangladesh. Similar findings have been documented from various other studies conducted in Bangladesh and China.[237] the Siberstein study reported that calcium and magnesium are the most abundant metals, followed by copper, zinc, and iron (present in concentrations of hundreds of ppb).[238]

In the present study, there was no significant difference in serum and follicular fluid Magnesium of pregnant and non-pregnant groups (tables 4-4, 4-5)

This results agreement with Previous studies in humans have also shown a decrease of Mg levels during the IVF cycle[239] In addition to this the authors found that magnesium levels in the follicular fluid were found to be higher than in serum.[239]

The distribution of magnesium in ovarian follicles during ART has been shown by Silberstein et al. The authors proposed that the concentration of cations in the follicular fluid were the consequence of blood transudation where larger follicles had the highest levels of magnesium.[238]

In the present study, there was a highly significant correlation between fertilization rate and M2 with FF Mg in pregnant. (4-10). But there was no significant correlation between M2 and FR with FF Mg in non-pregnant. (Table4-11).

Also, there was a significant negative connotation between M2 and FR with serum Mg of pregnant and non-pregnant (Table 4-8, 4-9).

Previous clinical studies have also dealt with the issue of fecundity and magnesium. In 2011 Bloom et al.[203] looked at pre-conceptional concentrations of magnesium. The authors concluded that higher magnesium concentrations showed a trend toward an increased probability for pregnancy. Similar results on the beneficial effects of selenium and magnesium supplementation in improving fecundity were reported already in 1994[241] These finding lend support to our observations.

In this present study we have observed that women with successful pregnancies have higher blood levels of magnesium. For this reason it appears sensible that magnesium levels should be optimized prior to ART. This has to be complemented by relaxation techniques that will reduce stress burden.[242]

**Table (4-10): Correlation between Pb, Zn, Cu and Mg in Follicular Fluid with Type of oocyte in pregnant.**

| Type of oocyte | Correlation coefficient-r | | | |
|---|---|---|---|---|
| | Pb | Zn | Cu | Mg |
| M1 | 0.01 NS | -0.05 NS | -0.34 * | 0.37 * |
| M2 | -0.14 NS | -0.32 * | 0.29 * | 0.48 ** |
| Gv | -0.08 NS | 0.13 NS | -0.02 NS | -0.06 NS |
| Abnormal | 0.32 * | -0.11 NS | 0.02 NS | -0.04 NS |
| Rapture | 0.59 ** | -0.11 NS | 0.004 NS | 0.11 NS |
| Fertilization rate (%) | -0.04 NS | -0.32 * | -0.02 NS | 0.55 ** |
| No. of embryo transfer | 0.29 * | -0.13 NS | -0.08 NS | 0.42 * |
| * (P<0.05), ** (P<0.01), NS: Non-significant. | | | | |