ASSOCIATION BETWEEN BLOOD AND FOLLICULAR FLUID OF LEAD AND OTHER MINERALS CONCENTRATION WITH EMBRYO QUALITY IN WOMEN UNDERGOING ICSI

Ruqaya Bashar Al-Smak¹*, Nawal khairy Al-Ani² and Muayyed Sarbit³

¹Dep. of Applied Embryology, High Institute of Infertility Diagnosis & ART/Al-Nahrain University, Baghdad-Iraq.

²PhD. Collage of pharmacy at Al-Nahrain University, Baghdad-Iraq.

³Lecturer in High Institute of Infertility Diagnosis & ART at Al-Nahrain University

High institute of infertility Diagnosis & ART Al-Nahrain University Baghdad, Iraq.

ABSTRACT

Background: Environmental pollution plays an important role in affecting the internal milieu of humans. The amount of trace elements in the environment is generally low, but these chemicals can interfere with physiological systems. Thus, the health and welfare of individuals could be compromised by exposure to environmental levels of pollutants. Trace elements, mainly the toxicones, can adversely affect reproductive system and its functions, through either direct or indirect effects on numerous organs and systems. Objective: To assess the effect of Lead concentration in blood and follicular fluid and Zinc in serum and Follicular fluid on oocyte, embryo quality and pregnancy success in women undergoing ICSI. Material and Methods: This study included 70 infertile women undergoing ICSI will be enrolled in assisted reproductive technologies (ART) program to enter their ICSI Cycle in High Institute of Infertility Diagnosis and Assisted Reproductive Technologies and in Kamal Al-Samarrai Hospital, center of fertility and IVF (Baghdad/Iraq) during the period from August 2016 to the end of February 2017. "Blood and follicular fluid lead" and "serum and follicular fluid Zinc ", were assessed on the day of oocyte retrieval by using Atomic Absorption Spectrophotometer (AAS). Results: There was no significant difference between pregnant and non-pregnant groups undergoing ICSI cycle in Lead concentration on the day of oocytes retrieval in both blood and follicular fluid (22.09 ± 0.29 and 23.45 ± 1.21 μg/dl) and (13.58 and 15.94 μg/dl)
respectively. Also there was no significant difference between pregnant and non-pregnant groups undergoing ICSI cycle in Zinc concentration on the day of oocytes retrieval in both serum and follicular fluid (67.56 and 68.42 μg/dl) and (84.58 and 80.83 μg/dl) respectively. But there was a highly significant correlation between Pb in blood with Pb in follicular fluid of pregnant and non-pregnant group (r=0.94, P<0.01), (r=0.96, P<0.01) respectively. Also there was a highly significant correlation between Zn in Serum with Zn in FF (r=0.46, P<0.01) of non-pregnant. **Conclusions:** There was no significant difference between pregnant and non-pregnant groups undergoing ICSI cycle in Lead concentration on the day of oocytes retrieval in both blood and follicular fluid. There was no significant difference between pregnant and non-pregnant groups undergoing ICSI cycle in Zinc concentration on the day of oocytes retrieval in both serum and follicular fluid.

**KEYWORDS:** Lead, Zn, ICSI, ART, FAAS.

**INTRODUCTION**

**Lead:** Is a chemical element in the carbon group, represented with the symbol Pb (from Latin: plumbum), having an atomic number of 82.[1] Lead is considered as one of the most hazards and cumulative environmental pollutants that affect all biological systems through exposure to air, water, and food sources.[2] Lead and other heavy metals create reactive radicals which damage cell structures including DNA and cell membranes.[3] Lead also interferes with DNA transcription, enzymes that help in the synthesis of vitamin D, and enzymes that maintain the integrity of the cell membrane.[4] Lead is one of the most significant reproductive toxicants, identified as a potential cause of infertility and abortion.[6] A high level of lead in animals resulted in reproductive failure.[5] Female reproductive function was more sensitive to lead exposure than the male function as reported earlier in rats, it induced impairment of the quality of female germ cells.[6] For the female, the main time in which lead affect the reproductive system is during pregnancy and lactation. This is a time of high physiological stress that yields mobilization of lead from reservoir.[7] Some studies of women living near smelter versus those living some distance away showed an increased frequency of spontaneous abortions, miscarriages and stillbirth.[8]

**Zinc (Zn):** Is a naturally occurring element. It found in the earth’s crust in most rock-forming minerals, Zn is the second to iron as the most an abundant trace element in the body.[9] Human body contains about (2-2.5)g of Zn, over 85% of the total body Zn is found in skeletal muscle and bone, while plasma Zn is only 0.1% of this total.[10] In females, zinc also seems to
be important in reproduction. Zinc deficiency in female rabbits resulted in disinterest in their male counterparts and in failure of ovulation.[11]

**Infertility:** Is a disease of the reproductive system clinically defined by the failure to achieve pregnancy after 12-24 months of regular unprotected sexual intercourse. It affects an estimated 48 million women in the world with the highest prevalence in South Asia, Sub-Saharan Africa, North Africa, Middle East, Central/Eastern Europe and Central Asia. Infertility is caused by many sources, including nutrition, diseases, and malformations of the uterus.[12]

**Assisted Reproductive Technique (ART):** Are methods used to achieve pregnancy by artificial or partially artificial means. ART used primarily for infertility treatment. Some forms of ART are also used with regard to fertile couples for genetic reasons (preimplantation genetic diagnosis PGD). ART are also used for couples who are discordant for certain communicable diseases such as Acquired Immune Deficiency Syndrome (AIDS) to reduce the risk of infection when a pregnancy is desired.[13]

**Intracytoplasmic Sperm Injection:** Is an IVF procedure in which a single sperm is injected directly into an egg cytoplasm, thus bypassing the zona pellucida(ZP) and the oolemma. The technique was developed by Gianpiero Palermo in 1991 at the Vrije universities Brussel, in the center for reproductive medicine headed by Paul Devroey and Andre Van Steirteghem. The ability of ICSI to achieve higher fertilization and pregnancy rates regardless of sperm characteristics makes it the most useful procedure yet with which to treat male factor infertility.[14] the indications for ICSI were expanded to include various patient populations with normal or mildly abnormal semen parameters. By all means, the male factor indications for ICSI are not well defined, apart from its absolute utility with surgically obtained spermatozoa in the presence of low motility, or in cases of severe defects with sperm concentration and motility.[15]

**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.
All 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, hysterosalpingography 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 determination**

1- Lead was analyzed in whole blood by flame atomic absorption spectrophotometer by waves length 283.2 nm, using hallow cathode lamp. The instrument was calibrated using aqueous standerds of (40, 30, 20, 10 μg/dl), 2.5 ml of blood in EDTA tube placed in a shaker machine for one hour, then the sample transferred to plane tube and added 2.5ml of Trichloroacetic acid (TCA) and to digested for 10 min. samples were centrifuged 3000 rpm for 5 minutes, lastly the resulting supernatant were transferred to new plain tube and the concentration were read by FAAS. Concentration of lead in Folicular fluid also determined according to the steps above except (shaker machine).

2- Zinc was analyzed in serum and FF by FAAS by waves length 213.9 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.

**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

1. Total number of oocytes outcome
In this study, the (mean±SE) of total number of oocyte outcome for pregnant and non-pregnant groups were (10.48 ± 1.07 and 7.35 ± 0.85) respectively. The statistical analysis showed a significant difference (P<0.05) among the two groups. As shown in figure (1).

2. Oocytes (Metaphase II)
In this study, the mean number of mature oocytes [metaphase II (MII)] ± SE in both pregnant and non-pregnant groups were (6.87 ± 0.96 and 4.32 ± 0.46) respectively. The statistical analysis showed a significant difference (P<0.05) among the two groups. As shown in figure (2).
3. **Fertilization Rate (FR%) and number of embryo transfer**

This study found that the mean number of fertilization rate ± SE in both pregnant and non-pregnant groups were (5.67 ± 0.62 and 4.32 ± 0.47) respectively. The statistical analysis showed no significant difference (P>0.05) among the two groups. But there was a highly significant difference (P<0.01) in number of number of embryo transferred ± SE in both pregnant and non-pregnant groups were (3.076 ± 0.21 and 2.193 ± 0.26) respectively. As shown in table (1).

**Table 1: Comparison between pregnant and non-pregnant in Fertilization rate and no. of embryo transfer.**

<table>
<thead>
<tr>
<th>The group</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fertilization rate (%)</td>
</tr>
<tr>
<td>Pregnant</td>
<td>5.67 ± 0.62</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>4.32 ± 0.47</td>
</tr>
<tr>
<td>T-Test</td>
<td>1.627 NS</td>
</tr>
<tr>
<td>P-value</td>
<td>0.1040</td>
</tr>
</tbody>
</table>

** (P<0.01), NS: Non-significant.

4. **Blood Lead**

In this study, the mean number of blood Pb concentration ± SE in both pregnant and non-pregnant groups were (22.09 ± 0.29 and 23.45 ± 1.21) respectively. The statistical analysis showed no significant difference (P>0.05) among the two groups. As shown in table (2).

**Table 2: Comparison between pregnant and non-pregnant in blood Pb.**

<table>
<thead>
<tr>
<th>The groups of study</th>
<th>Mean ± SE of blood Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>22.09 ± 0.29</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>23.45 ± 1.21</td>
</tr>
<tr>
<td>T-Test</td>
<td>2.740 NS</td>
</tr>
<tr>
<td>P-value</td>
<td>0.3288</td>
</tr>
</tbody>
</table>

NS: Non-significant.

5. **Serum zinc**

In this study, the mean of serum Zinc ± SE in both pregnant and non-pregnant groups were (67.56 ± 1.44 and 68.42 ± 1.64 μg/dl) respectively. The statistical analysis showed no significant difference (P>0.05) among the two groups. As shown in table (3).
Table 3: Comparison between pregnant and non-pregnant in serum Zn.

<table>
<thead>
<tr>
<th>The group</th>
<th>Mean ± SE of Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>67.56 ± 1.44</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>68.42 ± 1.64</td>
</tr>
<tr>
<td>T-Test</td>
<td>4.368 NS</td>
</tr>
<tr>
<td>P-value</td>
<td>0.697</td>
</tr>
<tr>
<td>NS: Non-significant.</td>
<td></td>
</tr>
</tbody>
</table>

6. Follicular Fluid of Lead and Zinc

The follicular fluid Pb and Zn in both pregnant and non-pregnant groups were (13.58 ± 0.58 and 15.94 ± 2.06 μg/dl), (84.58 ± 1.49 and 80.83 ± 1.50 μg/dl) respectively. The statistical analysis showed no significant difference (P>0.05) among the two groups. As shown in table (4).

Table 4: Comparison between pregnant and non-pregnant in Pb and Zn in FF.

<table>
<thead>
<tr>
<th>The groups of study</th>
<th>Mean ± SE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pb</td>
<td>Zn</td>
</tr>
<tr>
<td>Pregnant</td>
<td>13.58 ± 0.58</td>
<td>84.58 ± 1.49</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>15.94 ± 2.06</td>
<td>80.83 ± 1.50</td>
</tr>
<tr>
<td>T-Test</td>
<td>4.612 NS</td>
<td>4.295 NS</td>
</tr>
<tr>
<td>P-value</td>
<td>0.3095</td>
<td>0.0859</td>
</tr>
</tbody>
</table>

* (P<0.01), NS: Non-significant.

7. Correlation between Pb in blood, Zn in serum with Pb, Zn in follicular fluid of pregnant and non-pregnant groups

1- There was a highly significant correlation between Pb in blood with Pb in follicular fluid of pregnant (r=0.94, P<0.01). While there was no significant between Zn, in serum with Zn in follicular fluid (r=0.02, P>0.05). (Table 5)

2- There was a highly significant correlation between Pb in blood with Pb in follicular fluid of non- pregnant (r=0.96, P<0.01). There was a highly significant correlation between Zn in Serum with Zn in FF (r=0.46, P<0.01).

Table 5: Correlation between Pb in blood, Zn in serum with Pb, Zn in follicular fluid.

<table>
<thead>
<tr>
<th>The group</th>
<th>Blood and Serum</th>
<th>Follicle fluid</th>
<th>Correlation coefficient-r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pb</td>
<td>Pb</td>
<td>0.94 **</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td>Zn</td>
<td>0.02 NS</td>
</tr>
<tr>
<td></td>
<td>Pb</td>
<td>Zn</td>
<td>0.96 **</td>
</tr>
<tr>
<td>Non-Pregnant</td>
<td>Pb</td>
<td>Pb</td>
<td>0.46 **</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>Zn</td>
<td></td>
</tr>
</tbody>
</table>

**(P<0.01), NS: Non-significant.
DISCUSSION

During recent decades concerns have been raised about human infertility that might stem from exposure to environmental contamination. Exposure to environmental contamination prior and after the initiation of pregnancy, and during the early period of postnatal development could affect reproductive efficacy of offspring.\cite{17} Although few studies have been performed in women, several cases of lead poisoning have been associated with sterility, miscarriage, abortion, premature delivery and infant mortality.\cite{18} The present study showed that environmental lead exposure and other heavy metals affects on oocyte, embryo quality and pregnancy success in women undergoing ICSI.

**Associations between metals and oocyte maturity**

The present study suggest that trace exposure to toxic metals might influence oocyte maturation and fertilization rate, however the small sample size of this study, there was a significant difference in total numbers of oocyte outcomes, the statistical analysis showed the total number of oocytes outcome in pregnant group was higher than non-pregnant group as shown in (Fig.1). This result agreement with result obtained by Dorostghoal M. et.al,\cite{19} and Shirota et.al,\cite{20} the first one showed that neonatal lead treatment reduced the number of primary, secondary and antral follicles in the ovaries of offspring rats. The second one noted that the lead causes reduction in the number of growing follicles.

It was found a potential inverse association between blood Pb concentrations measured from female IVF patients and the probability of retrieving a mature oocyte (i.e., MII-arrest).\cite{21} In the present study there was a significant difference in mean number of mature oocytes between pregnant and non-pregnant groups as shown in (Fig. 2). This result agreement with results obtained by Avazeri N,\cite{22} who found Pb2+ inhibits the GV to MII-arrest transition via disruption of protein kinase activity.

**Associations between metals, fertilization rate and number of embryo transfer**

In present study, there is no significant difference in fertilization rate between pregnant and non-pregnant groups. This result may be due to the small sample size of the study. But there is a highly significant difference in the number of embryo transfer for pregnant women than non-pregnant women group, as shown in (Table 1). Bloom et al.\cite{23,24} investigated the association between toxic metals and embryo quality prospectively in a cohort of IVF patients. In the first report, they reported inverse relationship between blood Pb concentrations and embryo cleavage rate measured as embryo cell number. However, no
correlation was demonstrated between metal concentrations and embryo fragmentation. \cite{24} In the second report, they failed to detect an association between FF Hg, Cd or Pb and oocyte maturity. \cite{23}

**Associations between lead in blood and follicular fluid of pregnant and non-pregnant women**

The present study investigated the levels of lead within blood and follicular fluid in women undergoing ICSI cycle. The results show there is no significant difference in blood and follicular fluid lead between the pregnant and non-pregnant groups as shown in (Table 2). Both concentration are in upper limit of normal value which is < 25 μg/dl. \cite{25} This result agreement with result obtained by Bloom et al. \cite{26} who also reported there is no significant difference in the blood Pb levels in pregnant and non-pregnant women. Tali Silberstein et al. \cite{27} reported that Lead levels within follicular fluid were found to be significantly higher in non-pregnant compared to pregnant patients suggesting that elevated concentrations of the environmental toxicant lead adversely affect female reproduction.

The results of present study show a highly significant correlation between Pb in blood with Pb in follicular fluid of pregnant and non-pregnant women. (Table 5).

Tali Silberstein et al. \cite{27} reported that lead concentrations in the follicular fluid can exceed those in blood and even low concentrations of lead in follicular fluid are inversely associated with pregnancy. These positive correlations of environmental contaminant with negative outcomes point to the necessity for more detailed studies on couples. This results suggest that there may be a significant relationship between IVF success and the degree of lead contamination in the blood and follicular fluid and indicate that there is a need for more detailed analysis and larger study population to clarify the effects of lead on women reproductive outcomes. \cite{27}

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

Zinc is important in all biological systems. In vivo and in vitro reproductive studies have shown that zinc is essential for conception, for blastocyst development and implantation, organogenesis, fetal growth and parturition. \cite{28}
In the present study there is no significant difference in serum and follicular fluid zinc (Table 3,4) the mean in both pregnant and non-pregnant groups were in lower limit of normal range of concentration which is 80-120 μg/dl\cite{22} And this results agreement with result obtained by Soltan and Jenkins,\cite{29} which measured plasma zinc levels in 48 infertile and 35 control women and found no differences between these two groups. Ronaghy and Halsted,\cite{30} reported two women aged 19 and 20 years, suffering from nutritional dwarfism with delayed sexual maturation, these women had no breast tissue or pubic hair and had infantile external genitalia with extremely low blood plasma and erythrocyte zinc levels, after zinc supplementation, these women experienced their first menstrual period and developed breast tissue as well as pubic hair growth.

In the present study, there was a highly significant correlation between Zn in serum with Zn in FF of non-pregnant women group, but there was no significant correlation between Zn in serum with Zn in follicular fluid of pregnant women. (Table 5). Zinc concentrations in serum were significantly lower than FF concentrations. This results agreement with results obtained by Taupeau et.al,\cite{31} who suggested no correlation was found between zinc and oestradiol concentrations in serum and zinc concentrations in follicular fluid were significantly lower than serum concentrations. Ng et al.,\cite{32} investigated zinc levels in follicular fluid of 33 women undergoing IVF in Singapore and did not observe any correlation between follicular fluid zinc concentration and follicle volume.

CONCLUSIONS

1- There was a significant difference in total numbers of oocyte outcome and MII oocyte between pregnant and non-pregnant groups.

2- there was no significant difference in Fertilization rate between pregnant and non-pregnant groups, while there a highly significant difference in number of embryo transferred between the two groups.

3- There was no significant difference in blood lead and follicular fluid lead between pregnant and non-pregnant groups, also there was no significant difference in serum zinc and follicular fluid zinc between the groups.

4- A highly significant correlation between Pb in blood with Pb in follicular fluid of pregnant and non-pregnant groups, also there was a highly significant correlation between Zn in serum with Zn in FF of non-pregnant while there was no significant difference between Zn in serum with Zn in FF of pregnant.
REFERENCES


