Lead Exposure Through Secondhand Smoking and its Impact in ICSI Outcomes

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Abstract:

Background: Lead (Pb) is an important environmental agent to reproductive health. Purpose: The purpose of the study was to determine the relationship between the level of lead in both blood and follicular fluid (FF) and ICSI outcomes in passive smokers. **Methods**: A cross-sectional study was performed on 67 women (31 passive smokers, 36 non-exposed). The measurement of lead was done by Flame Atomic Absorption Spectrophotometry. **Findings:** Passive smokers were found to have a much higher lead level in blood (20.03 ± 3.01 µg/dL) and in FF (18.68 ± 3.92 µg/dL) than non-smokers ($p < 0.05$). The passive smoking group increase Metaphase I oocytes (1.23 ± 0.26) and Grade III embryos (0.65 ± 0.18) significantly ($p < 0.05$). There were no significant differences in MII oocytes, fertilization rates and clinical pregnancy rates. **Conclusion:** Secondary smoking is linked to higher levels of lead that could have a negative correlation with the maturation of the oocytes and the quality of the embryo during the ICSI cycles.

Keywords: Lead Pb , Passive smoking, Blood, Follicular fluid, ICSI outcomes .

Introduction

Over seven million deaths are attributed to direct tobacco use, while around 1.2 million deaths are the consequence of second-hand tobacco smoke (SHS). Among the numerous toxicants found in tobacco smoke, Lead is one of the most harmful elements due to its persistence and bioaccumulation [1]. Lead is extremely permanent in the environment and with its continued usage, its levels grow in almost all countries posing dangerous risks [2]. Exposure of people to lead and lead products mostly occurs in jobs involving lead, as a result of various causes including leaded gasoline, industrial operations such as smelting and burning of lead and lead-based plumbing, battery-recycling, grids, arms, pigments, and book printing[3]. At

the same time, the World Health Organization (WHO) (2017) has recently referred to the information on the Institute of Health Metrics and Evaluation, which suggested that lead exposure caused 494,550 deaths[4]. There are many published researches on the impact of lead on human reproduction, heavy metals, such as lead, mercury, cadmium, arsenic, and chromium, may cause birth defects. Although the mothers might not be affected or even unaware of the low-level lead exposure, their children may be subjected to negative impacts of these agents during pregnancy. Exposure to lead by women may cause infertility, miscarriage, premature rupture of membranes, preeclampsia, pregnancy-related high blood pressure, and preterm birth[5]. Although the toxicity of lead on overall health is established, there are very few studies on the particular level of lead concentrations in the microenvironment of the ovarian follicle (Follicular Fluid) and the direct influence of lead on meiotic proliferation (MI to MII transition) and direct embryonic cleavage during ICSI cases. This work is bound to fill this gap correlating lead levels in systemic circulation and the follicular compartment to the particular morphokinetic ART outcomes [6]. Exposed women might also have menstrual abnormalities, including amenorrhea, dysmenorrhea and menorrhagia [7].The exposure to lead is also associated with the hormonal imbalance which may cause reproductive difficulties and accumulation of lead affects some of the endocrine glands, effects of accumulation of lead in the ovary and its damage to folliculogenesis included the loss of primordial follicles and the excessive number of atretic antral follicles[8].

Method

1. **Patient:** This study included sixty-seven infertile women enrolled in (ART) programs who will undergo an ICSI cycle at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies a fertility and IVF clinic in Baghdad, Iraq, starting in October 2024 to March 2025. A thorough history assessment of every couple should be carried out. Exposure to passive smoking was collected in all the subjects and the data was collected in terms of the nature of the exposure (cigarettes, shisha and e-cigarettes), the duration of the exposure (in years), and the frequency of exposure on a daily and weekly basis. This was captured in order to assess the level of passive smoking exposure and potential association to reproductive problems.
   The exclusion criteria included women with systemic illnesses such as SLE, unmanaged diabetes, poorly controlled hypertension, thyroid disorders and

other endocrine disorders were excluded, as well as those with endometriosis. Additionally, females with acquired congenital abnormalities of the reproductive tract.

2. **Gathering Follicular Fluid and Blood Samples**: Following the controlled ovarian stimulation and the subsequent collection of oocytes (OPU), samples of blood and follicular fluid were gathered at the same time for analysis of biochemistry and trace elements. 2.5 milliliters of blood sample were collected by venipuncture, placed in EDTA tube, and used to evaluate blood lead, blood is stored at 2◦C. To prevent any blood contamination and flush media, five milliliters of follicular fluid was taken from the first follicle that was collected and put in Plane tubes. FF samples were centrifuged for 15 minutes at 3000 rpm and and placed in Eppendorf tubes then stored in deep freezer at - 20◦C until they were analyzed for lead. Blood and follicular fluid samples were collected and put in an ice-cooled container and sent to the Toxicology Consultation Unit of Al-Shaheed Adnan Hospital, Medical City, Baghdad, to undergo further preparation and analysis.
3. **Determining Elements Using Atomic Absorption Spectrophotometry (AAS):** Atomic absorption spectrophotometry (AAS) is widely used in clinical laboratories to measure the concentrations of elements like lead, as well as other metals**.** This method is based on the idea that a metallic atom within the sample absorbs light of a particular wavelength. However, the element does not get notably excited in the flame; rather, it simply separates from its chemical bonds (atomized) and remains in a neutral state (ground state). By the other hand in flame AAS, a liquid sample is passed through a nebulizer, which converts it into a fine aerosol before it is introduced into a flame. This flame, typically generated by a mixture of air–acetylene (approximately 2100-2400 °C) or nitrous oxide-acetylene (around 2600–2800 °C), supplies enough thermal energy to dissociate the analyte into individual atoms[9].
4. **Preparation of Blood and follicular fluid for lead measurement**: This was done through the stirring of blood sample over an hour in order to enhance blood cell (RBCs) and hemoglobin separation. The sample of blood was then put in a flat-bottom tube and 2.5 mL of trichloroacetic acid (TCA) was added. TCA reacts with hemoglobin and leads to precipitation of the protein and the development of a layer at the bottom of the tube. The mixture of blood and TCA

was stirred using a wooden stick and left to rest after 10 minutes. This was followed by centrifugation of the mixture after 10 minutes with 3500 rpm. The supernatant (filtrate) was taken and the level of lead was determined directly by flame atomic absorption spectrophotometry (AAS) at a wavelength of 283 nm. A final analysis of the follicular fluid was after centrifugation of the sample not diluted and the supernatant carefully collected and quantified at 283.3 nm wavelength. The acceptable level of lead in blood is (≤5 μg/dL)[10].

5. **Results and Statistical Analysis:** lead levels were examined in relation to ICSI outcomes, which included:
    - Total number of oocytes retrieved
    - Mature oocytes (MII)
    - Fertilization rate (FR%) FR% = (Number of fertilized oocytes ÷ Number of injected mature oocytes) × 100
    - Quantity and quality of embryos that were transferred
    - Data was assessed using SPSS v23.0, utilizing Student's t-test, Chi-square, and ANOVA as necessary. A p-value of ≤ 0.05 was considered statistically significant[11].

Results

1. **Fundamental traits of participants included in the current research:** A total of 67 females facing infertility issues were included in this cross-sectional study; among them, thirty-one were passive smokers while thirty-six were non-passive smokers. The findings were reported as the mean plus or minus the standard error of the mean (SEM). Concerning the hormones level the initial average levels were as follows: FSH at 5.93 ± 0.32 (mIU/ml), LH at 4.88 ± 0.32 (mIU/ml), AMH at 2.82 ± 0.26 (mIU/ml), Estradiol at 1404 ± 106 (pg/ml), prolactin at 19.63 ± 1.02 (ng/ml), and TSH at 2.02 ± 0.08 (mIU/ml). With respect to the parameters of ICSI, the average number of total oocytes was 13.24 ± 1.03, the average number of germinal vesicles was 1.63 ± 0.26, the average metaphase I oocytes was 0.88 ± 0.16, the MII was 9.43 ± 0.81, the count of abnormal oocytes was 0.87 ± 0.24, and the fertilization rate was 77.89 ± 2.61. Additionally, the total number of embryos was 5.24 ± 0.45, with grade I embryos averaging 2.96 ± 0.37, grade II embryos at 1.08 ± 0.18, and grade III embryos at 0.40 ± 0.09. In contrast, blood lead concentrations

were found to be 0.01888 ± 0.00044 ppm, while lead levels in follicular fluids were 0.01725 ± 0.00053 ppm.

2. **valuation of hormone levels in passive smokers versus non-passive smokers:** While hormonal levels were elevated in passive smokers, no notable differences were observed between passive smokers and non-passive smokers as illustrated in Table 1.

Table 1: A comparison of hormone levels in passive smokers versus non-passive smokers

Hormones	Passive smokers N.=31	Non-passive smokers N.=36	p value
TSH (mIU/ml)	2.27 ± 0.12	2.01 ± 0.10	0.095 Ŧ
AMH (ng/ml)	3.33 ± 0.39	2.37 ± 0.33	0.062 Ŧ NS
Prolactin (ng/ml)	20.15 ± 1.33	19.19 ± 1.52	0.643 Ŧ NS
E2 at day of trigger (pg/ ml)	1518 ± 182	1304 ± 212	0.318 Ŧ NS
FSH (mIU/ml)	6.54 ± 0.55	5.39 ± 0.35	0.073 Ŧ NS
LH (mIU/ml)	5.02 ± 0.52	4.77 ± 0.40	0.697 Ŧ NS

TSH: Thyroid-stimulating hormone; AMH: Anti-Müllerian hormone; E2: Estradiol; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; NS: Not significant (p> 0.05); Ŧ: t-test for independent samples**.**

3. **Comparison of the characteristics of embryos**:

Metaphase I oocytes were considerably more common among passive smokers, while Grade III embryos were notably more prevalent in passive smokers as demonstrated in the table 2.

Table 2: Comparison of ICSI features between individuals exposed to passive smoke and those not exposed to it.

ICSI parameters (Mean ± SE)	Passive smokers N.=31	Non-passive smokers N.=36	p value
Total oocytes count	12.29 ± 1.02	14.06 ± 1.71	0.397 Ŧ NS
Germinal vesicles	1.23 ± 0.29	1.97 ± 0.41	0.154 Ŧ NS
Metaphase I oocytes	1.23 ± 0.26	0.58 ± 0.18	0.040 Ŧ S
Metaphase II oocytes	8.42 ± 0.89	10.31 ± 1.28	0.247 Ŧ NS
Abnormal oocytes	1.19 ± 0.46	0.58 ± 0.20	0.208 Ŧ NS

Fertilization rates	73.91 ± 3.87	81.32 ± 3.48	0.158 ₸ NS
Total embryos	5.03 ± 0.63	5.42 ± 0.64	0.673 ₸ NS
Grade I embryo	2.87 ± 0.55	3.03 ± 0.50	0.833 ₸ NS
Grade II embryo	0.90 ± 0.22	1.22 ± 0.27	0.370 ₸ NS
Grade III embryo	0.65 ± 0.18	0.19 ± 0.09	0.023 ₸ S

NS: Not notable ($p > 0.05$); ₸: Independent sample t-test; €: Chi-square

4. **Comparison of lead concentrations in passive smokers versus non-passive smokers**:
   Levels of blood and follicular fluids were notably elevated in individuals exposed to passive smoking as shown in the table 3.

Table 3: Analysis of lead levels in passive smokers versus non-passive smokers.

Parameters	Passive smokers N.=31	Non-passive smokers N.=36	p value
Blood lead (ppm)	0.02003 ± 0.00053	0.01789 ± 0.00064	0.014 ₸ S
Follicular fluids lead (ppm)	0.01868 ± 0000.71	0.01603 ± 0.00071	0.011 ₸ S

₸: T test for independent samples; S: Statistically significant ($p \leq 0.05$); NS: Not statistically significant ($p > 0.05$)

5. **Analysis of lead concentrations among pregnant and non-pregnant women:** lead levels in both blood and follicular fluids were not significantly lower in pregnant females as illustrated in table 4**.**

Table 4: A comparison of lead concentrations in pregnant versus non-pregnant women.

Parameters	Pregnant females N.=15	Non-Pregnant females N.=52	p value

Blood lead (ppm)	0.01773 ± 0.00062	0.01921 ± 0.00053	0.163 ₸ NS
Follicular fluids lead (ppm)	0.01700 ± 0.00085	0.01733 ± 0.00064	0.798 ₸ NS

NS: Not noteworthy (p > 0.05); ₸: Independent samples t-test

6. Comparison of lead concentrations based on smoking type:

There were no notable variations in either blood or follicular fluids as shown in table 5.

Table 5: Comparison of lead levels according to the type of smoking

Parameters	Cigarettes smoking N.=13	Vape smoking N.=7	Hookah smoking N.=11	p value
Blood lead (ppm)	0.02015 ± 0.00093	0.02029 ± 0.00092	0.01973 ± 0.00093	0.916 ∀ NS
Follicular fluids lead (ppm)	0.01831 ± 0.00105	0.01871 ± 0.00061	0.01727 ± 0.00111	0.105 ∀ NS

NS: Not meaningful (p > 0.05); ∀:Variance analysis (ANOVA)

Discussion

In recent years, worries have been expressed regarding human infertility that could be linked to exposure to environmental pollutants [12]. Exposure to environmental pollutants before and after pregnancy begins, as well as during the initial stages of antenatal development, may impact the reproductive success of offspring [13]. [13]. This study was conducted to assess the relationship between blood and follicular fluid concentrations of heavy metals and trace elements on ICSI outcome. Thus the present study showed that environmental lead exposure and other heavy metals impact on oocyte, embryo quality and pregnancy success in women undergoing ICSI.

1. **Assessment of hormone levels in non-smokers compared to those exposed to passive smoking:** The analysis of hormonal profiles of exposed people to passive smoking and unexposed ones in this research did not show any statistically significant differences, however, some interesting tendencies could be evaluated that give powerful biological information. Lead has severe effects on endocrine functioning[14].

   The FSH p-value was 0.073, AMH 0.062 and TSH 0.095. The statistically insignificant differences notwithstanding, the small increases in FSH, AMH, and TSH might be the first signs of hormonal changes in passive smokers. These results should be viewed with caution since the sample size is small and the surrounding environments of heavy metal have complex components; but

it indicates that passive smoking could have a subtle influence on hormonal balance and the productivity of the reproductive system. Interestingly, the AMH levels were observed to be elevated in passive smokers which is not in line with previous results that indicate that smoking affects ovarian reserve adversely[15]. This unexpected result may be explained by the peculiarities of the study population as women with polycystic ovary syndrome (PCOS), poor ovarian response, and unexplained infertility-condition were involved in the research that is usually accompanied by the higher or growth of AMH levels.

2. **Comparison of ICSI characteristics between individuals exposed to passive smoking and those who are not affected by it:** These findings provide useful data regarding the extent in which passive smoking can result in impaired reproductive performance at both cellular and developmental levels. The number of Metaphase I (MI) oocytes was also found to be significantly higher among women who were exposed to passive smoking ($p = 0.040$) in comparison to those not exposed. The increased proportion of MI oocytes implies that the oocytes have not fully undergone meiotic maturation which is a crucial parameter of oocyte quality. MI arrest is usually an expression of either cytoplasmic or nuclear maturity, difficulties in the formation of the spindle, or a disruption in chromosome organization. Such effects may be attributed to the fact that environmental toxins like lead and other heavy metals found in tobacco smoke are known to have adverse effects and hence such findings are in line with previous studies, which have reported similar outcomes[16]. Also, women exposed to secondhand smoking exhibited significantly high cases of Grade III embryos ($p = 0.023$), indicating a decrease in the quality of embryos and a decrease in the possibility of embryonic development. Grade III embryos are typically characterized by asymmetric blastomere separation, augmented fragmentation and decelerated cleavage. Such anomalies may be linked to oxidative stress and the adverse impact of high lead content of passive smokers[17]. In summary, these results suggest that passive smoking does not significantly affect the overall quantitative outcomes of ICSI but does have a notable impact on the quality and maturation of oocytes and embryos, which are crucial factors for the success of assisted reproductive technology (ART). This highlights the importance of reducing passive smoke exposure for women undergoing fertility treatment.
3. **Evaluation of lead levels among pregnant and non-pregnant women:** In this study, it was noted that the occurrence of pregnancy was lower in individuals exposed to secondhand smoke compared to those who were not, though the difference was not statistically significant.

4. **Evaluation of lead and its levels based on smoking types:** No major differences in lead were observed between exposure to cigarettes, hookah or vaping. This implies that it is the extent of exposure to smoke in the environment and not the source that matters; any form of tobacco and nicotine product have the potential of producing heavy metals and fine particles that can be systemically absorbed.
5. **Study Limitations:** There are a number of limitations that can be regarded in this study. To begin with, the cross-sectional design restricts the possibility of making conclusive causal determination of the relationship between lead exposure and failure of ICSI. Second, the sample size is rather small and can impact the generalizability of the findings. Lastly, although we tried to remove the significant confounders, other environmental and lifestyle factors may still have an impact on the findings. Greater cohort longitudinal studies are justified in future**.**

Conclusion

In summary, the findings of our study indicate that passive smoking contributes to the lead exposure leading to oocyte-maturation arrest and inferior quality of embryos in the considered group. These pilot results suggest the urgency of longitudinal investigations in the future with bigger samples of individuals to confirm the causal aspect of environmental lead on ART success.

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Ethical Approval: This clinical study was conducted in accordance with the guidelines established in the Declaration of Helsinki. The study received approval from the Ethics Committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University (Approval Code: 0702-MF-2025S67-C1).

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