

# Effect of High Trace Element Levels in Serum of Women Undergoing Intra-cytoplasmic Sperm Injection (ICSI) on Implantation Rate

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## Abstract

**Objectives:** To assess the effects of trace element concentrations in serum on implantation rate in women undergoing ICSI.

**Study design:** Across-sectional study was conducted between March 2018 to April 2019 in Kamal AL-Samarrai Hospital, center of fertility and IVF. One hundred and seventeen women underwent ICSI using GnRH-antagonist protocol were recruited. Concentration E2, P4 and two trace elements (Cu, Zn, and Cu/Zn) were measured in serum specimen. Women's were evaluated in two groups; the study group consisted of patients with successful implantation (n = 21) and women in the second group (n=96) experienced implantation failure.

**Results:** No significant differences were observed in age or BMI between the groups. Serum copper levels were significantly higher differences (P=0.001) in group of failure ( $198 \pm 107\mu\text{g/dL}$ ) than in group of successful implantation ( $133 \pm 46\mu\text{g/dL}$ ), zinc concentrations in group of successful and failure out of the maximum of normal range ( $137 \pm 40\mu\text{g/dL}$  versus  $135 \pm 34\mu\text{g/dL}$  respectively) but statistically significant differences were did not observed (P>0.05). When we calculated the copper/zinc ratio (Cu/Zn), it was significantly higher differences (P=0.001) in group of failure ( $1.59 \pm 1$ ) than in group of successful ( $1.03 \pm 0.4$ ). In conclusion, we observed that high serum copper concentrations and (high Cu/Zn ratio) are a risk factor for implantation failure.

**Key word:** Implantation failure .Copper. Zinc. Intra-cytoplasmic Sperm Injection.

## Introduction

The latest proposed definition of implantation failure is defined as a failure to achieve a clinical pregnancy after transfer of 4 or more good-quality embryos in a minimum of 2 in vitro fertilization (IVF) cycles in a woman under the age of 40<sup>1</sup>, and caused by inadequate uterine receptivity in two-thirds of cases, and by problems with the embryo in the other third and it is a cause of infertility<sup>2</sup>. Cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), and zinc (Zn) are essential for normal physiologic function and play significant roles in human and overall mammalian reproduction<sup>3</sup>. However, few human data are available to describe the role of essential trace elements in IVF/ICSI, and their impact remains inconclusive.

Zinc is a trace element acts an antioxidant functions through Cu/Zn superoxide dismutase (SOD) and essential in vital functions such as cellular division and differentiation, making it essential for successful embryogenesis<sup>4</sup>. It has been estimated that the total amount of zinc retained during pregnancy is ~100 mg<sup>5</sup>. Because zinc is transported across the placenta via active transport from the mother to the fetus. Studies have shown that the fetus has notably higher zinc concentrations compared to the mother, even in cases of preeclampsia<sup>6</sup>, indicating that the fetus, itself, can maintain adequate zinc homeostasis. Alteration in zinc homeostasis may have devastating effects on pregnancy outcome, including prolonged labor, fetal growth restriction, or embryonic and fetal death<sup>7</sup>. On the other hand, copper is a redox-active transition metal and can

participate in single electron reactions and catalyze the formation of free radicals, including undesirable hydroxyl radicals; it could contribute to oxidative stress characteristic of preeclampsia<sup>8</sup>. This illustrates that copper itself appears to be a pro-oxidant, but functions as an antioxidant when associated with Cu / Zn SOD. Cu/Zn SOD known to be expressed in both maternal and fetal tissues<sup>9</sup>. Copper-containing contraceptive devices have been suggested to deposit copper ions in the endometrium, resulting in implantation failure<sup>10</sup>. These findings led to the hypothesis that infertile patients with high serum copper concentrations may have experiences implantation failure due to the excessive accumulation of copper ions. Therefore, the aim of the present study was to investigate whether serum copper and Cu/Zn ratio concentrations are related to the implantation failure of human embryos in women undergoing intra-cytoplasmic injection.

## Materials and Method

### Subject selection

A cross-sectional study was conducted in a Center of Infertility Diagnosis and Assisted Reproductive Technology / Kamal AL-Samarai Hospital, (between March 2018 and April 2019). Women (n=117) were included in the present study. Patients aged between 20 -45 years old with BMI > 29 kg/m<sup>2</sup> and duration of non-reproductive (2-24years), out of these, 21 women who were successful implantation and 96 women who were implantation failure were compared. All patients provided written informed consent for the study, because our study involved the use of human data. Patients with gynecological disease such as endometriosis, endometrial polyps, fibroid in uterus and diabetes mellitus were excluded.

### Ovarian stimulation and ICSI procedure.

Most of patients underwent ICSI treatment with fresh oocytes and freshly ejaculated spermatozoa. In a controlled-ovarian stimulation, rFSH (Gonal-F; Serono; Switzerland or Menogon; Ferring, GmbH Company; Germany) was injected intramuscularly from 2<sup>nd</sup> day of menstruation to 2 days before oocyte retrieval, the dose was adjusted according to each individual case. A GnRH antagonist cetrorelix at 0.25 mg (Cetrotide®, Serono, Switzerland) or orgalutron (Organon company, The Netherlands) was administered when at least one follicle reached >14 mm. When more than two follicles were >18 mm, ovulation was

triggered with a subcutaneous injection of recombinant human chorionic gonadotrophin (HCG) (Ovitrelle at 250 µg) (Merck- Serono, Geneva: Switzerland), it was administered 24–28 h after the final FSH administration. Vaginal ultrasound-guided aspiration of oocyte-cumulus complexes was performed 36 h later<sup>11</sup>.

Oocyte denudation and ICSI were performed 3 hours after retrieval, and the *in vitro* culture was carried out in cleavage Gain medium (Fertipro/Belgium) under mineral oil until day 2 (2–5 cells stage) in automated incubators with 6% CO<sub>2</sub> at 37 °C, the growth of all the embryos from each patient (n=117) was continuously monitored. Embryo quality was assessed before embryo-transfer, and a maximum of three embryos transferred to all patients. Pregnancies were diagnosed by serum positive B-HCG levels (>100 miu/ml) 14 days after embryo transfer.

### Determination of parameters

Ethnicity was Iraqi only. Age, duration of non-reproductive, body mass index (BMI), endometrial thickness, E2 and P4, were assessed as possible confounders. Serum E2 and P4 were assessed using a solid-phase, competitive, enzyme immunoassay (Biomerieux/ France). Copper and zinc concentrations were measured in the day of embryo transfer by a clinical laboratory company (LTA/Italia). The trace elements were determined after digestion with chromogen. Briefly one volume of serum (50 µl) was mixed with two volumes of working reagent and for 5 min and then determined by atomic absorption spectrophotometer (Apel/Japan).

### Statistical Analyses

Statistical analysis carried out by using Vassar Stats Web Site for Statistical Computation (Lowry, 2013). Measurable data expressed as (M ± SE). The Student's *t* test (non-homogeneity, two-sided) was used for comparisons between two groups. The significance of differences estimated at two-tail P level less than 0.05

### Results

Table 1 shows the demographic features and clinical parameters of the two groups. No significant differences (P>0.05) were observed in age, BMI, duration of non-reproductive, endometrial thickness, E2, and P4 between two study groups. Copper concentrations (mean ± standard deviation) were significantly higher

( $P=0.001$ ) in group of failure ( $198 \pm 107\mu\text{g/dL}$ ) than in group of successful implantation ( $133 \pm 46\mu\text{g/dL}$ ), while zinc concentrations of successful and failure out of the maximum of normal range ( $137 \pm 40\mu\text{g/dL}$  versus  $135 \pm 34\mu\text{g/dL}$  respectively), but statistically significant differences were did not observed. When we calculated the copper/zinc ratio (Cu/Zn), it was significantly higher ( $P=0.001$ ) in group of failure ( $1.59 \pm 1$ ) than in group of successful ( $1.03 \pm 0.4$ ) as shown in Table 2.

**Table 1 Demographic features and clinical parameters between two groups.**

Character (M±SD)	Implantation Group		P value
	Successful (n=21)	Failure (n=96)	
Age (years)	$29.7 \pm 1$	$31.4 \pm 0.6$	0.232
Infertility duration (years)	$8 \pm 1.1$	$7.7 \pm 0.4$	0.777
BMI (kg/m <sup>2</sup> )	$29.2 \pm 3.3$	$28.4 \pm 3.6$	0.411
Endometrium thickness (mm)	$9.3 \pm 1.4$	$8.4 \pm 1.2$	0.081
E2(pg/ml)	$1610 \pm 1003$	$1503 \pm 914$	0.719
P4(ng/ml)	$35.4 \pm 21.4$	$48 \pm 37.2$	0.217

Values indicate the mean  $\pm$  standard deviation. BMI-body mass index, E2-Estridol, P4- Progesterone.

**Table 2 Comparison of copper and zinc levels in the serum of women with successful and failure implantation.**

Serum level (M±SD)	Normal range	Implantation Group		P value
		Successful (n=21)	Failure (n=96)	
Copper ( $\mu\text{g/dl}$ )	80-155	$133 \pm 46$	$198 \pm 107$	0.001
Zinc ( $\mu\text{g/dl}$ )	70-115	$137 \pm 40$	$135 \pm 34$	0.834
Copper: Zinc ratio (Cu/Zn)	.....	$1.03 \pm 0.4$	$1.59 \pm 1$	0.001

## Discussion

Our results suggest, for the first time, that high serum copper (low zinc) concentrations are related to implantation failure in Iraqi women.

In the present study, we found that group with failure implantation had significantly higher Cu concentrations and Cu/Zn ratios than groups of successful these results are consistent with previous studies by Tolunay and coworkers<sup>12</sup> when were found decreased ongoing pregnancy chance with increasing follicular fluid of Cu

concentrations. Prior research describes associations between essential trace elements in seminal plasma and semen quality parameters among infertile couples, and so is also likely to impact IVF outcomes<sup>13</sup>. Copper, it is essential trace element, has a role in hemoglobin synthesis and immune function and is a cofactor for Cu/Zn superoxide dismutase (Cu/Zn-SOD) and ceruloplasmin<sup>14</sup>. Cooper and zinc are important in restoring or maintaining the oxidant-antioxidant balance in blood and tissues also can provide protection of cells against oxidative stress caused by ROS, which would

lead to damage of DNA or other important structures such as proteins and cell membranes<sup>15</sup>. Studies indicated that an elevated Cu/Zn ratio was associated with increased oxidative stress<sup>16</sup>, and oxidative stress closely associated with inflammation status, and the maintenance of redox balance is known to modulate immune system homeostasis<sup>17</sup>. Chronic inflammation, as indicated by increased level of serum ceruloplasmin, is related to elevated level of C-reactive protein (CRP) and Cu<sup>18</sup>. Changes in plasma levels of Cu and Zn has also been demonstrated for certain diseases, but an imbalance of the Cu/Zn ratio seems to be a better indicator of infection, vascular complications, several cancers including in the uterine endometrium<sup>19</sup>, the pathogenesis of preeclampsia<sup>20</sup>, and prognosis of diseases than Zn or Cu status alone<sup>21</sup>. In addition, a previous study indicated that the Cu/Zn ratio may be a useful inflammatory–nutritional biomarker and predictor of mortality in elderly people<sup>22</sup>. Regarding Zn, studies found higher blood Zn was associated with lower fecund ability in a prospective study of women trying to conceive, though of borderline statistical significance<sup>23</sup>. Moreover, previous investigation reported that Zn deficiency increases the absorption of intestinal Cu<sup>24</sup> and that Cu significantly inhibits the influx of Zn across the intestinal brush border membrane<sup>25</sup>. At the molecular level, Zn increases the expression of Zn-finger protein A20, which can inhibit interleukin-1 $\beta$  (IL-1 $\beta$ ), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), cytokines associated with inflammation<sup>26</sup>.

Importantly, copper and zinc superoxide dismutase transcripts are present in human and mouse oocytes at germinal vesicle and metaphase II stages<sup>27</sup>. The authors concluded that antral follicles might be more susceptible to Cu overexposure and undergo atresia or produce more corpora lutea as a result of ovulation. Other possible mechanisms for follicular damage include induction of cell apoptosis, vacuolization of the cytoplasm, and detachment of cell membrane from its cell membrane<sup>28</sup>.

Again, Zn and Cu are well-known micronutrients that are important for the function of immune cells and the secretion of cytokines<sup>29</sup>. In addition, higher levels of Cu can significantly decrease the number of circulating neutrophils, antibody titer, CD4/CD8 ratio, and NK cell activity<sup>30</sup>.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Ministry of Education and all experiments were carried out in accordance with approved guidelines.

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