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RESEARCH ARTICLE

MEASUREMENT OF ZINC CONCENTRATION IN SERUM AND FOLLICULAR FLUID TO ASSESS ITS RELATION WITH OOCYTE AND EMBRYO QUALITY IN WOMEN UNDERGOING INTRA CYTOLASMIC SPERM INJECTION.

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Key words:-
Zinc, oocyte, embryo, ICSI.

Abstract

Background: Zinc is part of over 300 metalloenzymes found in all metabolic pathways. It is required for DNA, RNA and protein synthesis, cell mediated immunity, reproduction, and development of the epidermis and CNS.

Objectives: General population has relatively higher exposure to environmental pollution, the goal in this study is to assess the effect of Zinc concentration level in serum and follicular fluid on oocyte, embryo quality and pregnancy success in women undergoing ICSI.

Patient, Materials and Methods: This study included 70 infertile couples joined in assisted reproductive technology (ART) programs to enter ICSI cycle in high Institute for Infertility Diagnosis and Assisted Reproductive Technologies and Kamal AL-Samarai Hospital, center of fertility and IVF(Baghdad/Iraq) during the period from October 2016 to February 2017. The level of Zinc concentration will be measured and assess their effect on oocyte and embryo quality.

Results: No significant difference was observed in follicular fluid zinc (p- value 0.0859, $P < 0.05$) also there was No-significant difference was observed in serum zinc ($P < 0.05$) between pregnant and non- pregnant group. Serum zinc in pregnant group there was negatively correlated with (M2) type of oocyte, fertilization rate and number of embryo transfer but in no- pregnant group there was positive correlated with (M2) type of oocyte, fertilization rate and number of embryo transfer. Follicular fluid zinc in pregnant group shows negative correlated with M1, M2, rapture and abnormal type of oocyte, fertilization rate and number of embryo transfer and positive correlated with GV type. Follicular fluid zinc in pregnant group has negative correlated with fertilization rate and M2, GV type of oocyte and has positive correlated with number of embryo transfer and rapture, abnormal and M1 type of oocyte.

Conclusions: There was no Significant difference in follicular fluid and serum zinc between pregnant and no–pregnant. The

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Increasing number of oocyte lead to increase the fertilization rate, high number of embryo transfer(grade1) this lead to increase chance of pregnancy outcome.

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Introduction:-

Zinc (Zn) is a naturally occurring element. It found in the earth's crust in most rock-forming minerals. It is a lustrous, blue-white metal that burns in air with a bluish-green flame. Zn is the second to iron as the most an abundant trace element in the body[(1)]. It occurs in all living cells as a constituent of metalloenzymes involved in major metabolic pathway[(2)]**.** Zn can interact with almost all hormones and plays a significant role in homeostasis of hormones such as thyroid and steroid hormones, insulin, and pituitary hormones like prolactin [(3)]. Good dietary sources of Zn are meat, poultry, eggs, and sea food. Oysters are the richest sources of Zn, cereals and legumes also contain significant amounts of it, but because of the presence of phytic acid in these foods, it is less available than that supplied by foods of animal origin by reducing its absorption. Absorption of Zn is negatively correlated to phytic acid content [(4)]. Zinc plays a role in ovulation and the menstrual cycle, which means that zinc deficiencies can make it harder to get pregnant. Low zinc levels have been linked to hormonal imbalances, which can cause ovarian function problems, irregularities in menstruation or even anovulation (in which women don't ovulate). Even during preconception, zinc depletion has been shown to severely disrupt egg maturation. Down the road, this deficiency can impact fertilization and egg pre-implantation development. Indeed, zinc's role is critical in the initial stages of cell division – after the egg is fertilized by sperm – and for placental development. Low plasma zinc concentrations reduce placental zinc transport and may affect the supply of zinc to the fetus, putting at risk the fetus's natural growth trajectory [(5)].

Patient,Material And Methods:-

This study included 70 infertile couples enrolled in assisted reproductive technology (ART) programs to enter ICSI cycle in high Institute for Infertility Diagnosis and Assisted Reproductive Technologies and Kamal AL-Samarai Hospital, center of fertility and IVF (Baghdad/Iraq) during the period from October 2016 to February 2017. The average age of included women ranged between 18 and 42 years had primary and secondary infertility with duration between2-8year.

Collection of Serum and Follicular Fluid Sample :-

A-1 milliliters of blood sample were collected by venipuncture, from each infertile woman on day of oocyte retrieval, left in plan tube and was allowed to clot for 30 minutes and centrifuged to separate the serum for 15 minutes at 3000 rpm. Sera obtained from centrifugation are stored at - 20◦C in deep freezer before the analysis and use to evaluate serum zinc,

B- 1 milliliters of follicular fluid was obtained from the first retrieved follicle to avoid contamination of blood and flush medium, and collected in plane tube. Care was taken to avoid blood contaminated samples, FF samples were centrifuged for 15 minutes at 3000 rpm and stored at - 20◦C in deep freezer before the analysis and use to evaluate FF zinc.

Measurement of Serum Zinc:-

1-put 1ml of serum sample in plane tube and complete to 10ml from deionized water.
2-put in mixer for 1minute.

B- The result directly read by using flame AAS at wave length 213.9nm for zinc

Measurement of follicular fluid Zinc :-

1-put 1ml of FF sample in plane tube and complete to 10ml from deionized water.
2-put in mixer for one minute.

B- The result directly read by using flame AAS at wave length 213.9nm for zinc.

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. A P value < 0.05 was considered to be statistically significant [(6).]

Results:-

Table (1) show Compare between pregnant and non-pregnant in Zn in , The Zn level in serum expressed as (microgram/dl) of the total patients , pregnant , and non-pregnant group(67.56 ± 1.44 and 68.42 ± 1.64, respectively). In this study no significant difference was observed in Zn, Cu and Mg in serum between the pregnant and non-pregnant group. Zn level in FF is expressed as (microgram/L) of the total patients , pregnant , and non-pregnant group(84.58 ± 1.49 and 80.83 ± 1.50 , respectively). No significant difference was observed in Zn, Cu and Mg in FF between the pregnant and non-pregnant group.

Table 1:- Compare between pregnant and non-pregnant in Zinc in serum and follicular fluid:

The group	Mean ± SE of Zn in serum (microgram/dl)	Mean ± SE of Zn in FF (microgram/L)
Pregnant	67.56 ± 1.44	84.58 ± 1.49
Non-pregnant	68.42 ± 1.64	80.83 ± 1.50
T-Test	4.368 NS	4.295 NS
P-value	0.697	0.0859
Mean(M) ± Standard Error(S.E.)		

In this study, in pregnant group it was found that is No significant positive correlation was observed between the Zn in serum and M1($r = 0.08, P < 0.05$),GV($r = 0.15, P < 0.05$) and No. of embryo transfer ($r = 0.12, P < 0.05$), A significant negative correlation was observed between the Zn in serum and Fertilization rate (%) ($r = 0.32, P < 0.05$), No significant negative correlation was observed between the Zn in serum and M2($r = 0.18, P < 0.05$), and rapture ($r = 0.14, P < 0.05$). A significant positive correlation was observed between the Zn in serum and abnormal($r = 0.33, P < 0.05$), In no pregnant group No significant positive correlation was observed between the Zn in serum and M1($r = 0.11, P < 0.05$),GV($r = 0.006, P < 0.05$) , No. of embryo transfer($r = 0.08, P < 0.05$)and raptuer($r = 0.02, P < 0.05$), No significant negative correlation was observed between the Zn in serum and Fertilization rate (%)($r = 0.06, P < 0.05$), Abnormal ($r = 0.12, P < 0.05$) and M2($r = 0.07, P < 0.05$). In this study, in pregnant group it was found that is No significant negative correlation was observed between the Zn in FF and M1($r = 0.05, P < 0.05$) ,abnormal($r = 0.11, P < 0.05$) , No. of embryo transfer($r = 0.13, P < 0.05$) and rapture($r = 0.11, P < 0.05$),A significant negative correlation was observed between the Zn in FF and M2($r = 0.32, P < 0.05$), and Fertilization rate (%) ($r = 0.32, P < 0.05$).No significant positive correlation was observed between the Zn in FF and GV($r = 0.13, P < 0.05$). In no pregnant group no significant positive correlation was observed between the Zn in FF and M1($r = 0.08, P < 0.05$) abnormal ($r = 0.05, P < 0.05$) , rapture($r = 0.02, P < 0.05$) and No. of embryo transfer ($r = 0.13, P < 0.05$) . No significant negative correlation was observed between the Zn in FF and M2($r = 0.08, P < 0.05$) ,GV($r = 0.06, P < 0.05$) and Fertilization rate (%) ($r = 0.12, P < 0.05$). As show in(table2).

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Table 2:- Correlation between Zn in Follicular Fluid and serum with Type of oocyte Fertilization rate and number of embryo transfer in pregnant and non-pregnant.

Type of oocyte	Zn (microgram/L)in pregnant in FF	Zn (microgram/L)in non- pregnant in FF	Zn (microgram/dl)in serum of pregnant	Zn (microgram/dl)in serum of non-pregnant
M1	-0.05 NS	0.08 NS	0.08 NS	0.11 NS
M2	-0.32 *	-0.08 NS	-0.18 NS	-0.07 NS
Gv	0.13 NS	-0.06 NS	0.15 NS	0.006 NS
Abnormal	-0.11 NS	0.05 NS	0.33 *	-0.12 NS
Rapture	-0.11 NS	0.02 NS	-0.14 NS	0.02 NS
Fertilization rate (%)	-0.32 *	-0.12 NS	-0.32 *	-0.06 NS
No. of embryo transfer	-0.13 NS	0.13 NS	0.12 NS	0.08 NS
Significant * (P<0.05), NS : Non-significant, highly significant ** (P<0.01) Correlation coefficient-r				

Discussion:-

Environmental factors differ between areas with higher amounts of pollutants closer to sources of industrialization. Environmental factors, such as exposure to heavy metals, can cause reproductive dysfunction in women [7]. Zinc (Zn) is the unique trace intracellular element required for a number of cellular processes including cell proliferation, reproduction, immune function, and defense against free radicals [8], [9]. In the present study, the results showed that being serum Zn concentration with pregnant group was (67,56) microgram/dl among those with average serum Zn concentration in non-pregnant group (68,42) microgram/dl. It is less than the normal Value (80-150) microgram/dl so it's not highly effect on pregnant rate [10]. The results presented in this study confirm the findings of published reports in an article by Yan Sun et al [11]. Who have found no significant differences was detected in the follicular fluid concentrations of Zn on the day of oocyte retrieved ($P > 0.05$). In the present study Zn in pregnant group was statistically negative correlation with M2, rapture type of oocyte and Fertilization rate (%), positive correlation with M1, GV and abnormal type of oocyte and No. of embryo transfer, Studies have demonstrated that concentration of Zn Nutrition affects not only the number of oocytes that ovulate but also their quality, Fertilization rate (%) and No. of embryo transfer. While the only definitive measure of oocyte quality is its ability to form a blastocyst, and indeed viable young, numerous proxy measures of oocyte quality are used, including the attainment of metaphase II following in vitro maturation [12]. on the other hand, Zn in non-pregnant group was statistically positive correlation with M1, GV, rapture type of oocyte and No. of embryo transfer, Negative correlation with M2, abnormal type of oocyte and Fertilization rate (%) This agreement with Studies have demonstrated Low zinc levels have been linked to hormonal imbalances, which can cause ovarian function problems, irregularities in menstruation or even anovulation (in which women don't ovulate). Even during preconception, zinc depletion has been shown to severely disrupt egg maturation. Down the road, this deficiency can impact fertilization and egg pre-implantation development [13]. In the present study, the results showed that no significant difference between Follicular fluid Zn concentration between pregnant and non-pregnant group. Zn in pregnant group was statistically negative correlation with M1, rapture, abnormal type of oocyte, positive correlation with GV and M2 type of oocyte, Fertilization rate (%), and No. of embryo transfer the results presented in this study confirm the findings of published reports in an article [14]. Have found high concentrations of Zn can inhibit production of cAMP and P4 induced by FSH in chicken granulosa cell of dominant follicle, slowing down maturity of oocyte. on the other hand, Zn in non-pregnant group was statistically positive correlation with M1, abnormal, rapture type of oocyte, and No. of embryo transfer and No. of embryo transfer, negative correlation with GV and M2 type of oocyte and Fertilization rate (%). This agreement with result of study [15] that found maintaining Zn at an optimal level is critical to oocyte development and ovulation. beside statistically negative correlations observed between follicular fluid concentrations of Zn and the fertilization rate, M2 and abnormal type of oocyte a positive correlation trend was found between follicular fluid concentrations of Zn and the No. of MI oocytes and cleavage rate in IVF patients Collectively, these data imply that Zn may play important roles in fertilization and early embryonic growth.

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