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Role of trace element-Zinc and *in vitro* fertilization (IVF) outcome

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Abstract

Rationale: Couples facing problems of infertility might go for assisted reproductive technology (ART)to have a child. The success rate of in vitro fertilization (IVF) depends upon various factors.

Objective: To assess the role of trace metal-Zinc on IVF and its outcome.

Materials And Methods: Two hundred and three couples undergoing IVF treatment were enrolled and biological samples like follicular fluid (FF) from female and seminal plasma (SP)of male subjects were collected and Zn level was measured. IVF outcome was considered positive based upon level of β-HCG($\geq$ 100 IU/L)on day 15[th]after embryo transfer (ET).

Results: The Zn levels in FF were significantly higher in the subjects who have undergone Embryo transfer with positive IVF outcome ($\geq$ 100 IU/L β HCG; $p<0.05$)as compared to subjects who have not undergone ET and subjects with negative IVF outcome. Significantly higher level of seminal plasma Zn ($p<0.05$) was also observed in males of couples with positive IVF outcome. Zinc level was also significantly positively correlated to number of embryos cleaved.

Conclusion: The trace metal zinc, exhibited a positive role in ET done and IVF outcome.

Keywords: ART, Follicular fluid, Seminal plasma, Zinc, IVF, embryo transfer

Introduction

Assisted reproductive technologies involves infertility treatments by using woman's egg and a man's sperm to form embryo. These complex procedure assists couples facing infertility to achieve pregnancy. The success rate of ART varies depending upon different factors related to the couple's lifestyle, exposure, occupation, genetics etc. Trace metals are the few of the multiple factors that might affect the human reproductive system. Trace elements are necessary for cell functions at biological, chemical and molecular levels [1]. Such elements mediate vital biochemical reactions by acting as cofactors for various enzymes, and act as centers for stabilizing structures of enzymes and proteins. Elements like iron, zinc, and selenium are important components of enzymes where they attract or subtract molecules and facilitate their conversion to specific end products [1].

Zinc (Zn) is an essential trace element required for different physiological function of the body. It is known to be an important element in the reproductive cycle. The vital role of zinc is reported from ovulation, fertilization to delivery in females and formation and maturation of spermatozoa in males [2]. It plays an important role in growth and development [3] metabolism, cell mediated immune functions and oxidative stress and enzymatic, hormonal components[4] immunological [5] and many other physiological activities. In addition,Zn regulate the synthesis of DNA[6]. It also has anti-apoptotic [7] and antioxidant properties [8].Very recently Lee [9] reported that the actions of zinc are not straightforward due to its several roles in biological systems and zinc deficiency and excess cause cellular oxidative stress. Further, it was stated that adequate zinc stores in the body are essential during times of accelerated growth. However, zinc deficiency is common in developing countries and low maternal circulating zinc levels have been linked with pregnancy complications [10]. Hence, role of Zn has been explored with respect to IVF outcome.

Materials and Methods

The study is a part of a major project entitled "Investigations of factors affecting *in vitro* fertilization (IVF)" for which ethical approval of the study was obtained from Institutional Human Ethical Committee, National Institute of Occupational Health, Ahmedabad, India. A total of 203 couples undergoing *in vitro* Fertilization at IVF Center, Department of Gynecology and Obstetrics, IKDRC, Ahmedabad were enrolled. A written consent was obtained from each subject after explaining the objectives and ethical issues are involved. The predesigned Performa were filled through questionnaire interview that included demographic information, personnel, reproductive history etc.

All the women were kept on any one of ovarian stimulation protocol 1) long GnRH agonist, 2) short GnRH agonist or 3)GnRH antagonist protocol and controlled ovarian hyper stimulation treatment was given after evaluating serum hormone profile on day 2 of menstrual cycle and follicle development. When at least three follicles with a diameter of ≥18 mm were achieved, human chorionic gonadotrophin (hCG) injection 10,000 IU was administered intramuscularly for oocyte maturation and trigger. Mature oocytes were selected for sperm insemination. Embryo quality was assessed by embryoscope (Unisense Fertilitech, Denmark) before embryo transfer (ET). The cleavage rate was calculated as the number of oocytes cleaved out of total number of oocytes retrieved. On day 15th of ET, the level of β-HCG was measured, and positive outcome was considered when β-HCG levels was found to be ≥100 IU/L. Earlier, Parihar [11]and Kumar *et al.*[12] also used β HCG level of≥ 100 IU/L to predict the viable pregnancy.

The inclusion criteria of the subjects were couple attending IVF center with Male factor sub fertility(like oligospermia, astheno-zoospermia, terato-zoospermia), Female factor sub fertility,(endometriosis, PCOS, Fallopian tube pathology or abnormality, etc.), Idiopathic sub fertility, i.e. absence of abnormalities in both men and women but no spontaneous conception occurs within 1 year of unprotected intercourse. Exclusion criteria was Sperm or oocyte donors, Surrogacy, and Azoospermia.

During the oocyte retrieval, follicular fluid (FF) was collected and centrifuged at 5000 rpm for 15 min at 4ºC (micro centrifuge – Sigma) and supernatant was stored in the deep freeze at -40^{o}C. Pretreated seminal plasma was collected from the left-over sample from IVF centre. Seminal plasma was centrifuged at 10,000 rpm for 20 min at 4ºC and supernatant was diluted with equal volume of PBS (1:1). The follicular fluid was diluted 1:10 times and seminal plasma was diluted

1:100 times with triple distilled water for the estimation of zinc. Blood in non-heparinized vacutainers were centrifuged at 5000 rpm for 20 min (REMI centrifuge) to separate serum. The zinc level was measured in these samples using Flame Atomic Absorption Spectroscopy (Perkin Elmer, USA) at a wavelength of 213.9 nm.

For analysis purpose, the data were evaluated with respect to1) embryo cleavage rate (with FF-Zn and SP-Zn), 2) number of mature oocytes harvested (with FF-Zn), 3) endometrial thickness (with FF-Zn) 4) Reproductive impairment 5) ET status (done or not done) 6) IVF outcome (negative or positive)7) Sperm motility, sperm count (with SP- Zn).The data were analyzed with the help of GraphPad online software and SPSS version 16.0. The data were expressed mean±SE. Test for normal distribution was carried out on continuous variable, the data showed normal distribution. Student's t-test (for normally distributed) was performed to analyze statistical significance. Correlation between parameters was assessed by using Pearson's correlation coefficient and linear regression was obtained. Statistical significance was determined at p value ≤0.05 level.

Results

The mean age of female subjects was 31.74±0.3 (yrs) and their male partner was 35.09±0.36 (yrs). The data with regards to stimulation protocol adopted showed that over half of the subjects (51.7%) had undergone ovarian stimulation through short protocol. Whereas 27.6% and 20.7 % subjects had undergone ovarian stimulation by long and antagonist protocol respectively [Table 1].

Correlation between parameters related to IVF with Zn in subjects

Follicular fluid Zn was assessed with respect to embryo cleavage rate, number of mature oocytes and endometrial thickness by Pearson's correlation. The correlation was significant with regards to cleavage rate, and close to significance with number of mature oocytes. No significant correlation was observed between FF Zn and endometrial thickness. While SP-Zn do not have significant correlation with embryo cleavage rate [Table 2]. Further, SP-Zn was correlated with sperm count and sperm motility and no significant correlation was observed between sperm count and SP zinc (r=0.054, p=0.4904) level while SP zinc level was positively significantly correlated with sperm motility (r=0.2456, p=0.0017)[Figure 1].

FF and SP Zinc level with respect to Reproductive impairments

The data on Zn levels in both male and female were analyzed with respect to presence and absence of reproductive impairment. The results showed that there was no significant difference in FF-Zn and SP-Zn levels with respect to reproductive impairment [Table 3].

FF and SP Zinc level with respect to ET status and IVF outcome

Levels of Zn were analyzed with respect to subjects undergone ET/ or not undergone ET and IVF positive/negative outcome. The FF zinc was significantly higher in females who had undergone ET and positive IVF outcome as compared to subjects those who had not undergone ET and with negative IVF outcome [Table 4]. The zinc concentration in SP showed no statistically significant difference with respect to ET status (undergone or not undergone ET). Whereas Zn level was significantly higher in the groups with positive IVF outcome as compared to negative IVF outcome [Table 4].

Discussion

It is known that some of the essential metals like zinc, copper, selenium etc. are needed in trace quantity for various physiological functions in the body. Zinc is an essential component of more than 300 different enzymes and hormones and the role of zinc at the cellular level includes catalytic, structural and regulatory functions[4].Murarka *et al*. reported a positive role of zinc in both male and female reproduction and deficiency or excess of zinc might have hostile impact on sperm and egg physiology[13].The vital role of zinc in follicular fluid[14]and seminal plasma [15,16]in fertility has also been reported. In the present study, Follicular fluid zinc concentrations were significantly higher in the subjects with positive IVF outcome. While Sun *et al*.[14] observed that FF zinc was not significantly but positively associated with fertilization rate. Further, they reported that optimal concentration of zinc in the follicular fluid of infertile subjects, stimulated using agonist long protocol, turns out to be beneficial for oocyte growth and embryo development. A positive association of FF Zn was also observed with number of mature oocytes harvested and embryo cleavage rate, in the present study. Menezo *et al*. [17] stated that most of the zinc transporters, metallothioneins and metal regulatory transcription factor were expressed in oocytes, indicating an essential role for zinc, with potential linking to genome stability during early embryonic development.

Positive correlation was attained between sperm motility and SP zinc concentration, as the zinc concentration increases, % motility also increases. These results were in accordance with data of Fuse *et al*. [18], they also observed a positive correlation of zinc with sperm motility. In addition, Chia *et al.* [19] also demonstrated the positive correlation between sperm motility and seminal plasma zinc concentration. In the present study, a significant correlation was observed between SP zinc concentration with sperm motility, not with count and. Earlier, Abou-Shakra *et al*. [20] also observed no significant correlation between seminal plasma zinc level and sperm count. The zinc concentration in the SP was found to be significantly higher in the group with positive IVF outcome in present study. This is in confirmation with the recent finding of Kumar *et al.* [12].

Based upon this study, coupled with data available, it can be inferred that Zn is important factor for embryo cleavage, ET status and IVF outcome. Thus, the role of zinc in embryo growth in females and sperm quality in males could play a role in reproduction and IVF outcome. The clinical use of zinc supplementation may be explored in reproduction and IVF outcome.

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Legends Figure and Table

Table 1:Demographic and IVF variables of the study population

Variables		Female	Male
Mean Age (Yrs)		31.74±0.30	35.09±0.36
Stimulation protocol	Long protocol	56 (27.6%)	
	Short Protocol	105 (51.7%)	
	Antagonist Protocol	42 (20.7%)	

Table2: Correlation between different parameter and Zinc levels

Variables	Embryo cleavage rate			
	R^2	Pearson r	P value	95% CI
FF Zn	0.02257	0.1502	0.0342*	0.01135 to 0.2834
SP Zn	0.0002	0.0142	0.8457	-0.1284 to 0.1563
	Number of mature oocytes			
FF Zn	0.01721	0.1312	0.0668	-0.009168 to 0.2665
	Endometrial thickness			
FF Zn	0.0005845	0.02418	0.7645	-0.1335 to 0.1807

(*p<0.05)

Table 3: Follicular fluid and Seminal Plasma zinc concentration with respect to reproductive impairment

Variables		Follicular Fluid zinc (mg/L)	p-value	Seminal Plasma zinc (mg/L)	p-value
Reproductive impairment	Presence	0.43 ± 0.02(n=142)	0.0632	83.04 ± 5.552 n=128	0.3333
	Absence	0.40 ± 0.01(n=58)		74.03 ± 6.743 n=61	

Table 4: Follicular fluid and Seminal Plasma zinc concentration with respect to IVF details

Variables		Follicular Fluid zinc (mg/L)	p-value	Seminal Plasma zinc (mg/L)	p-value
ET status	Done	0.431 ± 0.010 (n=137)	0.0008***	76.18±4.58(n=137)	0.3224
	Not done	0.369± 0.013(n=64)		68.09±5.89(n=66)	
IVF outcome	positive	0.46 ±0.01(n=54)	0.0144*	88.61±7.53(n=54)	0.0105*
	negative	0.41 ± 0.01(n=83)		67.79±5.9(n=82)	

Data are mean ± SE

Fig 1: Linear Regression of sperm count and Percentage (%) Active motility with Seminal plasma zinc