



Association of Serum and Follicular Zinc Levels With Stimulation Response in Intracytoplasmic Sperm Injection: A Prospective Cohort Study

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Abstract

Objectives: To evaluate the associations between serum and follicular zinc levels and the response to intracytoplasmic sperm injection (ICSI).

Patients and Methods: A prospective cohort study was conducted between May 2022 and May 2023 on 120 infertile women randomly selected based on specific criteria. The patients were classified into three groups according to stimulation response. Serum zinc level was measured in all patients on the day of ovarian stimulation, and follicular fluid zinc level was measured at the time of pickup. We monitored the patients to evaluate the correlation between serum and follicular zinc levels, as well as the reaction to ovarian stimulation. We assessed oocyte quality and quantity, fertilization rate, embryo quality, and clinical pregnancy rate.

Results: The participants were homogenized at comparable ages. The 120 patients were classified into three groups according to the number of oocytes retrieved after ovum pick-up: four oocytes were categorized as poor responders ($n = 40$), 4–15 retrieved oocytes were categorized as normal responders ($n = 40$), and >15 retrieved oocytes were categorized as hyper-responders ($n = 40$). The poor responders had significantly lower serum and follicular zinc levels than the others. There was a significant difference between the three groups (P value = 0.0001). There was a direct positive correlation between serum and follicular zinc levels. However, there was a moderately negative correlation between the serum and follicular zinc levels and the total gonadotropin dose. On the other hand, there was a slightly positive link between the amount of zinc in the serum and follicles and the response to stimulation in the ICSI cycle in terms of the number of oocytes, ovarian sensitivity index (OSI), follicular output rate (FORT), and follicle-to-oocyte index (FOI). There was a strong positive correlation between serum and follicular zinc levels and the fertilization rate and number of MII but a weak positive correlation with the number of MI. All significant correlations between serum and follicular zinc levels were found to be predictors of clinical pregnancy.

Conclusions: The serum zinc level at stimulation day was reflected in the follicular fluid zinc levels after stimulation and at the time of ovum pick-up. Both of them predict the success of an ICSI cycle, including the response to stimulation and the pregnancy rate. The serum zinc level can also indicate cases that may progress to clinical pregnancy. It is crucial to measure the blood zinc levels of women preparing to undergo ICSI. The start of ICSI program should be delayed until the serum zinc level is optimal, as it is a predictor of the response to stimulation and the outcome of the ICSI cycle. Encouraging adequate zinc intake prevents the potential impact of altered zinc levels on the success rate of these women's responses to stimulation.

Keywords: Zinc, Follicular fluid, Ovulation response, Embryo transfer, Intracytoplasmic sperm injection

Introduction

Intracytoplasmic sperm injection (ICSI) has become a popular method of assisted reproduction (1). Currently, the success of an ICSI cycle is determined by several cofactors, including the role of micronutrients (2). Infertility is strongly affected by the supply of trace elements. Reduced levels of these elements are known to hinder germ cell formation and the development of the embryo (3). Zinc is increasingly recognized as an essential micronutrient critical to various physiological processes, including cell growth, maturity, follicular development, and ovulation (2). The human body cannot synthesize zinc

and does not possess any zinc stores, necessitating a zinc-rich diet to maintain optimal zinc levels (4). Zinc is found mainly in meat, seafood, eggs, and dairy products and a lesser amount in plant-based foods such as cereals, legumes, and nuts (1). It is vital for the proper function of the reproductive system and its cells' proliferation and is needed in sperm and ovum formation, fertilization, conception, and delivery (5). The follicular fluid is also considered an environment for communication between oocytes and follicular cells during the development of follicles (6). Changes in the follicular fluid content may cause a change in the physiology of oocyte maturation and

Received 24 April 2023, Accepted 16 September 2023, Available online 16 July 2024

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Key Messages

- ▶ Measurement of the serum zinc levels in women planning to enrol in ICSI is essential and postponed starting until the serum zinc level is optimized as the serum zinc predicted response for stimulation and pregnancy rate, and clinical pregnancy. Encourage adequate zinc intake to prevent the potential impact of altered zinc levels on their response to stimulation and the success rate.

embryonic development or even an abnormality in this physiological process (6). The indicators used to evaluate ovarian response to ICSI are ovarian sensitivity index (OSI), follicular output rate (FORT), follicle-to-oocyte index (FOI), fertilization rate, and clinical pregnancy rate (7). The OSI, defined as the index that estimates the gonadotropin dose required to retrieve an oocyte, is a good parameter for determining the sensitivity to gonadotropins and predicting the outcome of pregnancy (7). Conversely, the FORT, the ratio between the number of pre-ovulatory follicles, is considered a sensitive marker for the quality and quantity of the response of the ovaries (8, 9). Meanwhile, the FOI is the ratio between the number of oocytes retrieved at pickup and the number of antral follicles at the beginning of stimulation (10). It reflects the dynamic nature of follicular growth in response to exogenous gonadotropins (11).

Study Aim

This study aimed to evaluate the associations between serum and follicular zinc levels and the response to ICSI.

Patients and Methods

Study Design

This prospective cohort study was conducted at the Al-Nahrain University Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Kamal Al-Samarrai Hospital of Infertility Diagnosis and Assisted Reproductive Technologies, and Al-Najaf Infertility and Andrology Centre in Iraq, among patients recruited via simple random sampling. The study was performed from May 2022 to May 2023.

Study Population

The participants comprised 120 infertile women who visited the outpatient clinic and decided to undergo the ICSI program.

The study included the following inclusion criteria to enhance precision in detecting the associations between zinc levels and ICSI success.

- Age of 20–35 years (to minimize the confounding effect of extreme ages on the result of ICSI).
- Adequate ovarian reserve; antral follicle count ≥ 5 or anti-Müllerian hormone (AMH) ≥ 0.5 ng/mL according to the Bologna Criteria (12)
- Body mass index (BMI) between 18.5 and 30 kg/m²,

to minimize the effect of extreme weight on response to stimulations and the result of ICSI,

- GnRH antagonist protocol,
- Fresh embryo transfer.

Women who did not meet the inclusion criteria were excluded from the study.

Exclusion Criteria

- Polycystic ovary syndrome patients,
- Malabsorption (gastrointestinal disease),
- History of recurrent implantation failure (to decrease confounding factors that may cause a decrease in the implantation rate and affect the success rate),
- History of recurrent miscarriage (as it will affect implantation and cause recurrent miscarriage),
- History of endocrine diseases to minimize the effect of these disorders on folliculogenesis and implantation,
- History of endometriosis, as these conditions affect the implantation rate and oocyte quality,
- Abnormal serum albumin level (40–53 g/L), as zinc uptake by endothelial cells modulates Albumin and facilitates the uptake of Zn²⁺ by erythrocytes,
- Uterus anomaly,
- Structural pathologies such as myoma and polyp,
- Supplementation that contains zinc in the previous 3 months,
- All male infertility factors, to minimize the cofounder effect on fertilization and embryo quality.

Full histories were recorded for every woman, specifically regarding the duration and causes of infertility. Drug histories were also evaluated. A pelvic examination was also performed. On days 2 or 3 of the cycle, hormonal investigations were done. For quantitative determination, all hormone levels were measured using a DiaSorin analyzer (LIAISON R, USA). An additional blood sample (5 mL) was also obtained from each woman to measure serum zinc level. The blood samples were placed in a labeled, sterile container. A centrifuge at 3000 rpm for 20 minutes was conducted for each sample, and the supernatant serum was then collected in a separate sterile polyethylene container and stored at 20 °C until analysis to measure the serum zinc level in 2 cc. Serum zinc levels ranging from 7.65 to 15.76 $\mu\text{mol/L}$ were considered normal (13).

The antagonist protocol was started for all patients enrolled in the program, and controlled ovarian stimulation (COS) was started, starting with gonadotropin (Gonal-F, Serono, Italy) on day 2 of the cycle. The dose, adjusted according to body weight, age, and number of antral follicles, started at 150–300 mIU/d. Follicular development was monitored via transvaginal ultrasound every 2–3 days from stimulation until ovulation, on the 7th day of stimulation. Gonadotrophin antagonist 0.25 mg (Merck-Serono, Germany) was subcutaneously injected once a day until at least three follicles measured greater than 18 mm in diameter, at which point 250 μg

of recombinant human chorionic gonadotropin (hCG) was supplied. At 35–36 hours after hCG administration, the ovum was picked up under transvaginal ultrasound guidance.

At the time of pickup, the follicular fluid samples (10 mL) were carefully collected from follicles 18–24 mm in diameter during oocyte retrieval using a 17-G oocyte recovery set (Wallace, USA). From the follicular fluid (without any media or blood contamination), the follicular fluid cells were pelleted via centrifugation at 3000 rpm for 20 minutes. They placed 2 cc in a sterile tube for freezing.

Colorimetric Method

For determination of zinc in serum and follicular fluid by reacts Zinc with the chromogen present in the reagent, forming a colored compound whose colored intensity is proportional to the zinc concentration present in the sample. The color intensity at 560 nm is directly proportional to the zinc concentration present in the sample, which was measured via spectrophotometry at 560 nm (BTS-350, Biosystem, USA). All assays were performed in the same laboratory.

Working Reagent

The working reagent was prepared by adding 2 ml of reagent (0.4 mM of Nitro-Paps and preservatives) to a vial containing a reagent composed of butter crystals 0.37, pH 8.2, salicylaldoxime 12.5, dimethylglyoxime 1.25 mm, and preservatives. A sample from the patient was placed in a gel-type test tube, and 1 mL of the working reagent was added. The mixture was cleaned with 50 μ L of a cleaning solution. Light from the light source passes through the slit and falls on a filter, which is set to 560 nm as selected from the device's screen. The wavelength formed then exits through a slit to the cuvette. The resulting colored spectrum is detected and converted into a digital reading, which is transmitted to the device screen.

Validation of Colorimetric Assays for Serum and Follicular Zinc

Despite poor efficiency compared with flame atomic absorption spectrometry, it is simple, has a short analysis time, and has similar mean serum and follicular zinc concentrations as found by Monge et al (14).

Assessment of Oocyte and Embryo

The number, quality, and maturity of the oocytes retrieved were assessed according to the ESHRE grading system (14). Oocyte injection by sperm was performed within 4 hours after oocyte retrieval (38–40 hours after the HCG trigger) to ensure the maturation of the mitotic spindle and increase the chances of successful fertilization during ICSI.

At 16–18 hours post-insemination, the fertilization rate was assessed by determining the percentage of fertilized oocytes out of the total number of oocytes injected. After

that, the embryo quality was assessed during development on day 3 before embryo transfer, according to Gardner and Schoolcraft in 1999 (15). 1–3 embryos were transferred approximately 48 hours (6–8 cell stages) after fertilization according to ASRUM embryo transfer guidelines according to the patient's age and embryo quality (16).

Follow-up of the Study Groups

Luteal phase support with 400 mg progesterone vaginal suppositories, inserted twice per day for 2 weeks (Merck Actives, England), was started on the oocyte retrieval day and continued until a pregnancy test yielded positive findings for up to 12 weeks of pregnancy. A pregnancy test was conducted 2 weeks after embryo transfer (biochemical pregnancy). Clinical pregnancy was defined as having one or more gestational sacs with cardiac activity as detected on transvaginal ultrasound at 5–6 weeks of gestation.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) for Windows (version 28 released in 2021, IBM Corp., Armonk, USA) was used for the data analysis. The data were presented as standard deviations and means. The variance was analyzed to evaluate the relationship between the variables. *P* values of less than 0.05 were used to assess whether the associations between the variables were statistically significant. Pearson's correlation coefficient (*r*) was used to determine the strength of the relationship between the continuous variables.

Results

The 120 cases were classified into 3 groups according to the Bologna criteria based on patients' COS response to ovulatory stimulation. Three groups of different responding patients were found to be comparable in age, with a mean age of 28.5 in poor responders, 28.1 for normal responders, and 28.5 for hyper-responders, as shown in Figure 1.

There was a positive direct relationship between the serum and follicular zinc levels, as shown in Figure 2.

According to the numbers of oocytes retrieved after ovum pick up, if oocytes were retrieved, 4 were categorized as poor responders: ($n = 40$); 4–15 retrieved oocytes were

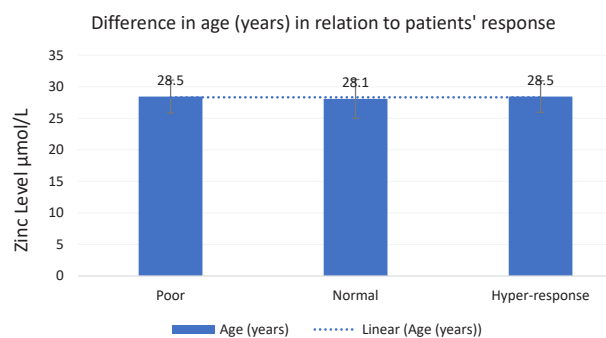


Figure 1. Differences in the Age Groups in the Three Groups of Patients.

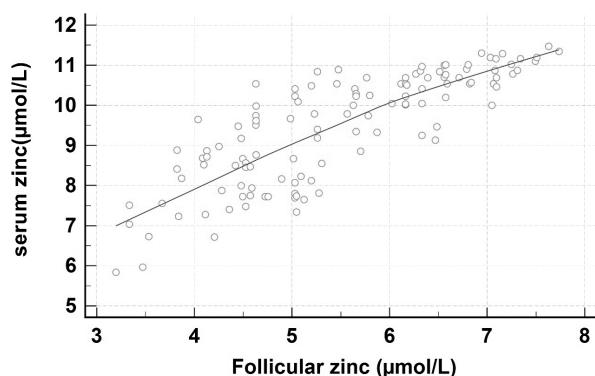


Figure 2. Correlation Between the Serum and Follicular Zinc Levels.

categorized as normal responders: (n = 40); and hyper-responders: >15 retrieved oocytes (n = 40).

The three groups were compared in serum zinc on cycle day 2 and follicular zinc levels at the time of ovum pick-up. According to the Bologna criteria, there was a correlation between serum zinc and follicular zinc levels and the response to ovulatory stimulation.

The poor responders had significantly lower serum and follicular zinc levels than the others. As shown in Figure 3, there was a significant difference between the three groups, with a *P* value of 0.0001.

Table 1 analyzes the correlation between serum and follicular zinc levels and other factors in all three subgroups. There was a moderately negative correlation between the serum and follicular zinc levels and the total gonadotropin dose. Conversely, there was a moderately positive correlation between the serum and follicular zinc concentration and the stimulation response in the ICSI cycle regarding the number of oocytes, FOI, OSI, and FORT. There was a strong positive correlation between the serum and follicular zinc levels and both the fertilization rate and the number of MII oocytes, while there was a weak positive correlation with the number of MI oocytes. All correlations were significant.

Curves of the receiver's operating characteristics were

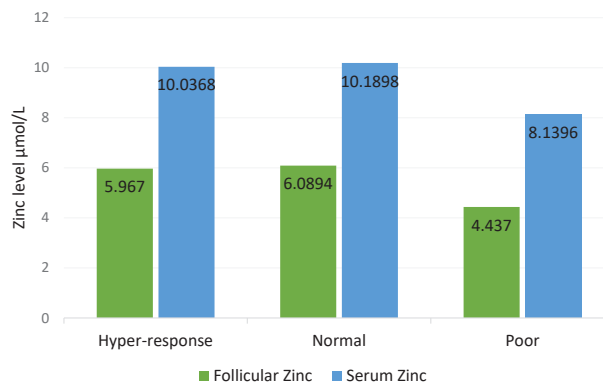


Figure 3. Differences in the Serum and Follicular Zinc Levels According to Patient Response.

applied to assess the validity of the serum zinc level as a predictor for clinical pregnancy. At a width of 0.697 cm, the sensitivity was 47.1%, while the specificity was 96.5%. Further, the area under the curve (AUC) was 0.727 (Figure 4).

Receiver operating characteristic curves were also applied to evaluate the validity of the follicular zinc level as a predictor for clinical pregnancy. At a width of 0.697 cm, the sensitivity was 47.1%, the specificity was 86.0%, and the AUC was 0.663, as shown in Figure 5.

Discussion

This original article studies the effect of a serum zinc deficiency in patients prepared to enter the ICSI program and the association of zinc levels in serum and follicular fluid at the time of ovum pickup.

There was a positive correlation between the serum zinc and follicular zinc levels, suggesting that the zinc in serum and follicular fluid correlated directly; this is consistent with what was found by Schmalbrock et al (17). The clinical implications are that the serum zinc reflects the amount of zinc in the follicular fluid level.

Poor responders to ovulatory stimulations in the ICSI program had lower serum and follicular zinc levels than

Table 1. Correlation of the Serum and Follicular Zinc Levels With the Ovarian Response Parameters

Variable	Serum Zinc Level		Follicular Zinc Level	
	<i>r</i>	<i>P</i> (t-test)	<i>r</i>	<i>P</i> (t-test)
Total dose of gonadotropin	-0.401	0.000*	-0.439	0.000*
No. of oocytes	0.522	0.000*	0.497	0.000*
No. of MII	0.804	0.000*	0.837	0.000*
No. of MI	0.260	0.004*	0.232	0.011*
Rate of fertilization	0.791	0.000*	0.668	0.000*
FOI	0.422	0.000*	0.385	0.000*
OSI	0.452	0.000*	0.456	0.000*
FORT	0.485	0.000*	0.434	0.000*

**P* < 0.05, statistically significant. Linear correlation interpretation: >0.6 = strong correlation, 0.3–0.6 = moderate correlation, 0–0.3 = weak correlation, positive value = directly proportional, negative value = inversely proportional.

M (metaphase); FORT (follicular output rate); OSI (ovarian sensitivity index); FOI (follicle-to-oocyte index).

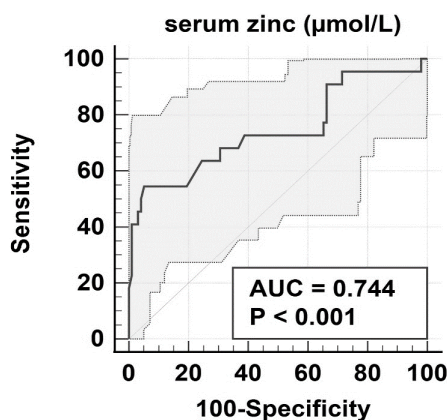


Figure 4. Receiver Operating Characteristic Curve for the Validity of the Serum Zinc Level for the Prediction of Clinical Pregnancy.

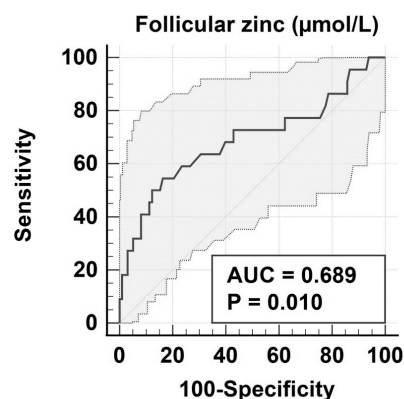


Figure 5. Receiver Operating Characteristic Curve for the Validity of the Follicular Zinc Level for the Prediction of Clinical Pregnancy.

the normal and hyper-responders, suggesting that zinc level plays a role in the response to ovarian stimulation, as zinc is involved in several biological processes, including ovulation, folliculogenesis, and luteal function (18). This may be attributed to zinc's crucial role in regulating hormones, germ cell growth, oocyte quality, fertilization, division, proliferation in a preimplantation embryo, and immunological responses (19). Its antioxidant properties help to reduce oxidative stress and inflammation, which can contribute to decreased ovarian function through DNA damage and mitochondrial ER (endoplasmic reticulum) function disturbance due to mitochondria and stress (18). Influences the activity of many antioxidant enzymes, synergistic with other antioxidants (e.g., vitamin E) (20).

Serum zinc deficiency causes reduced synthesis and secretion of FSH and LH and, by this mechanism, affects the response to ovulatory stimulation, folliculogenesis, oocyte maturation, and oocyte grade (2) (21). Zinc is involved in synthesizing Vitamin A reductase; when there is zinc deficiency, serum Vitamin A decreases, leading to ovulation failures (22) (6). Serum zinc deficiency affects the serum level and metabolism of insulin-like growth factors and is found to increase cytokine-induced apoptosis and oxidative damage (23). decreased ovarian function and poor response to stimulation protocols may be because of IGF-I deficiency, which affects follicular stimulation development, E2 production, and oocyte maturation by affecting receptors in ovarian membranes (24). Conversely, adequate zinc levels are associated with a normal response and a hyper-response to stimulation. This finding suggests that adequate zinc levels may play a role in a normal response to stimulation with fertility treatments (25).

Zinc levels in serum and follicular fluid are associated with the success of the ICSI program, including responses to stimulations shown by oocyte number and quality, embryo numbers and quality, and pregnancy rate. This study evaluated the associations between zinc levels in

serum and follicular fluid and ovarian response indicators of assisted reproductive technology such as the FORT, FOI, and OSI. No previous article has discussed these aspects of the association, and the study controlled for other factors that may influence the success of an ICSI cycle.

The numbers of mature MII oocytes and fertilization rate showed a strong positive correlation with serum zinc level before starting the stimulations and follicular zinc level at the time of pick-up, and this is like that found by Tolunay et al, who showed a positively correlated level of follicular zinc with the rate of fertilization and the number of MII oocytes retrieved (26). Sun et al showed a positive association with the fertilization rate (6) as the effect of zinc in promoting oocyte and early embryonic development. The follicular zinc level was associated with more MII oocytes; this is consistent with the findings by Janati et al (2). Sun et al showed no correlation between the numbers of MI and MII and the levels of follicular zinc (6); this may be attributed to the use of different protocols (GnRH-agonist long protocol) and selection criteria in their study (6). Abbood et al (27) found a non-significant correlation with the MI count, probably because they did not exclude women taking zinc supplements in the previous 3 months from their study (27). Zinc is required to complete meiosis I, and zinc deficiency negatively impacts the quality of oocytes (28). Sun et al (6) observed a positive relation between follicular fluid zinc level and the oocyte number retrieved in patients undergoing ICSI. They stated that a lower quality and quantity of oocytes were associated with decreased zinc levels, which is consistent with the present results.

The current study also revealed that serum and follicular zinc levels correlated with clinical pregnancy rates and even found serum and follicular zinc as predictors of clinical pregnancy. This finding contrasts with the report by Abbood et al (27) that the pregnant and non-pregnant groups show no significant difference between follicular or serum zinc levels. This may be because low zinc levels affect the quality of oocytes and embryos, as it acts as an

antioxidant. Its deficiency leads to cytokines-induced apoptosis of cumulus cell proliferation and poor growth of it with disturbed synthesizing and transfer of glutathione to the oocytes from cumulus cells, which affects cytoplasmic maturation, growth, and quality of oocytes and decreases the number of good-quality embryos (2). Fertilization rate and embryo growth are affected by low zinc levels caused by disturbed methylation of DNA and tone protein in oocytes, leading to deficiencies in epigenetic programming (29).

Limitations of the study

Despite the highly selective criteria for patients involved in the study, the sample size should be larger to strengthen the results and make them more dependable. Additionally, more sensitive tests should be used to measure serum zinc levels before enrolling participants in the ICSI program.

Conclusions

The serum zinc level on stimulation day was reflected in the follicular fluid zinc levels after stimulation and at the time of ovum pick-up. Both of them predict the success of an ICSI cycle, including the response to stimulation and pregnancy rate, and even serum zinc levels can predict cases that can develop clinical pregnancy. Measurement of the serum zinc levels in women planning to enroll in ICSI is essential and postponed starting until the serum zinc level is optimized as the serum zinc predicted response for stimulation and success of the ICSI cycle. Encouraging adequate zinc intake prevents the potential impact of altered zinc levels on these women's response to stimulation and success rate.

Recommendation

Further studies are needed to assess the optimal level of oral zinc consumed by patients undergoing ICSI. Also, future studies should determine the exact cut-off level of serum zinc found to give a better response to ovulatory stimulations. Advising on nutritious foods that are rich in zinc may be beneficial for women undergoing ICSI. The level of serum zinc should be optimized before starting the stimulation cycle and postponed if it is low.

Authors' Contribution

Conceptualization: Zainab Abdul Ameer Jaafar.

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Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

This study was approved by the Ethics Committee of the Scientific Council of the Arab Board in Iraq (No: 44 in January 2022). Before their involvement, all patients were informed of the purpose of the research and the method applied. Informed written consent was obtained from the patients who participated in the study. Patients could withdraw at any time.

Financial Support

The authors funded the research.

Acknowledgments

The authors would like to thank all women who participated in this study.

References

1. Ebisch IM, Thomas CM, Peters WH, et al. The importance of folate, zinc, and antioxidants in the pathogenesis and prevention of subfertility. *Hum Reprod Update*. 2007;13(2):163-174. doi:10.1093/humupd/dml054
2. Janati S, Behmanesh MA, Najafzadehvarzi H, et al. Follicular fluid zinc level, oocyte maturity, and embryo quality in women with polycystic ovary syndrome. *Int J Fertil Steril*. 2021;15(3):197-201. doi:10.22074/IJFS.2021.135426.1006
3. Mirnamniha M, Faroughi F, Tahmasbpour E, et al. An overview of the role of some trace elements in human reproductive health, sperm function, and fertilization process. *Rev Environ Health*. 2019;34:339-348. doi:10.1515/reveh-2019-0008
4. Nasiadek M, Stragierowicz J, Klimczak M, et al. The role of zinc in selected female reproductive system disorders. *Nutrients*. 2020;12:2464. doi:10.3390/nu12082464
5. Udechukwu MC, Collins SA, Udenigwe C. Prospects of enhancing dietary zinc bioavailability with food-derived zinc-chelating peptides. *Food Funct*. 2016;12;7(10):4137-4144. doi:10.1039/c6fo00706f
6. Sun Y, Lin Y, Niu M, et al. Follicular fluid concentrations of zinc and copper are positively associated with in vitro fertilization outcomes. *Int J Clin Exp Med* 2017;10(2):3547-3553. www.ijcem.com /ISSN:1940-5901/IJCEM0045056
7. Revelli A, Gennarelli G, BIASONI V, et al. The Ovarian Sensitivity Index (OSI) Significantly Correlates with Ovarian Reserve Biomarkers, Is More Predictive of Clinical Pregnancy than the Total Number of Oocytes and Is Consistent in Consecutive IVF Cycles. *J Clin Med*. 2020;9:1914. doi:10.3390/jcm9061914
8. BIASONI V, Patriarca A, Dalmasso P, et al. Ovarian sensitivity index is strongly related to circulating AMH and may be used to predict ovarian response to exogenous gonadotropins in IVF. *Reprod. Biol. Endocrinol*. 2011; 9:112–119. doi: 10.1186/1477-7827-9-112. Doi: 10.1186/1477-7827-9-112.
9. Alviggi C, Conforti A, Esteves SC, et al. Understanding ovarian hypo-response to exogenous gonadotropin in ovarian stimulation and its new proposed marker-the follicle-to-oocyte (FOI) index. *Front Endocrinol (Lausanne)*. 2018;9:589. doi:10.3389/fendo.2018.00589
10. Gallot V, Berwanger da Silva AL, Genro V, et al. Antral follicle responsiveness to follicle-stimulating hormone administration assessed by the Follicular Output RaTe (FORT) may predict in vitro fertilization-embryo transfer outcome. *Hum Reprod*. 2012;27:1066-72. doi:10.1093/humrep/der479
11. Genro VK, Grynberg M, Scheffer JB, Roux I, Frydman R, Fanchin R. Serum anti-Mullerian hormone levels are negatively related

- to Follicular Output RaTe (FORT) in normo-cycling women undergoing controlled ovarian hyperstimulation. *Hum Reprod.* 2011;26:671–7. doi:10.1093/humrep/deq361.
12. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod.* 2011;26:1616–24. doi: 10.1093/humrep/der092.
 13. Barman N, Salwa M, Ghosh D. Reference Value for Serum Zinc Level of Adult Population in Bangladesh. *EJIFCC.* 2020;31(2):117–124.
 14. Monge E, Vicente A, et al. Comparison study between colorimetric method and flame atomic absorption spectrophotometry in serum zinc status. *Nutr Clin Diet Hosp.* 2018; 38(2):133-138. doi:10.12873/382escobedo.
 15. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Hum Reprod.* 2011;26(6):1270-1283. doi:10.1093/humrep/der037
 16. Practice Committee of the American Society for Reproductive Medicine and the Practice Committee for the Society for Assisted Reproductive Technologies. Guidance on the limits to the number of embryos to transfer: a committee opinion. *Fertil Steril.* 2021;116(3):651-654. doi:10.1016/j.fertnstert.2021.06.050.
 17. Schmalbrock LJ, Weiss G, Eddy R, et al. Pronounced Trace Element Variation in Follicular Fluids of Subfertile Women Undergoing Assisted Reproduction Nutrients. *Nutrients.* 2021;13(11):4134. doi:10.3390/nu13114134.
 18. Biasoni V, Patriarca A, Dalmaso P, et al. Ovarian sensitivity index is strongly related to circulating AMH and may be used to predict ovarian response to exogenous gonadotropins in IVF. *Reprod Biol Endocrinol.* 2011;9:112–119. doi:10.1186/1477-7827-9-112.
 19. Garner TB, Hester JM, Carothers A, Diaz FJ. Role of zinc in female reproduction. *Biol Reprod.* 2021;104(5):976-994. doi:10.1093/biolre/ioab023
 20. Ebisch IM, Thomas CM, Peters WH, Braat DD, Steegers-Theunissen RP. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. *Hum Reprod Update.* 2007;13(2):163-174. doi:10.1093/humupd/dml054
 21. Özer A, Bakacak M, Kiran H, et al. Increased oxidative stress is associated with insulin resistance and infertility in polycystic ovary syndrome. *Ginekologia Polska.* 2016;87(11):733-738. doi:10.5603/GP.2016.0079.
 22. Özkaya MO, Nazýrođlu M. Multivitamin and mineral supplementation modulates oxidative stress and antioxidant vitamin levels in serum and follicular fluid of women undergoing in vitro fertilization. *Fertil S teril.* 2010;94(6): 2465-2466.
 23. Jafaar ZA, Obeid RZ, Salman DA. Serum Zinc: A Noninvasive Biomarker for the Prediction of Invasive Placentation. *Int J Womens Health Reprod Sci.* 2021;9(1):35–41. doi:10.15296/ijwhr.2021.07.
 24. Ipsa E, Cruzat VF, Kagize JN, Yovich JL, Keane KN. Growth Hormone and Insulin-Like Growth Factor Action in Reproductive Tissues. *Front Endocrinol (Lausanne).* 2019;10:777. Published 2019 Nov 12. doi:10.3389/fendo.2019.00777
 25. Cummings JE, Kovacic JP. The ubiquitous role of zinc in health and disease. *J Vet Emerg Crit Care (San Antonio).* 2009;19(3):215-240. doi:10.1111/j.1476-4431.2009.00418.x
 26. Tolunay HE, Pükür YE, Ozkavukcu S, et al. Heavy metal and trace element concentrations in blood and follicular fluid affect ART outcome. *Eur J Obstet Gynecol Reprod Biol.* 2016;198:73-77. doi:10.1016/j.ejogrb.2016.01.001
 27. Abbood MS, Burhan SA and Al-Ani NK. Measurement of zinc concentration in serum and follicular fluid to assess its relation with oocyte and embryo quality in women undergoing intra cytoplasmic sperm injection. *Int J Adv Res.* 2017;5(11):1333-1337. doi:10.21474/IJAR01/5903
 28. Huang X, Hong L, Wu Y, et al. Raman Spectrum of Follicular Fluid: A Potential Biomarker for Oocyte Developmental Competence in Polycystic Ovary Syndrome. *Front Cell Dev Biol.* 2021;9:777224. Published 2021 Nov 11. doi:10.3389/fcell.2021.777224
 29. Jeon Y, Yoon JD, Cai L, et al. Zinc deficiency during in vitro maturation of porcine oocytes causes meiotic block and developmental failure. *Mol Med Rep.* 2015;12(4):5973-5982.

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