RESEARCH ARTICLE

Changes in vitamin E levels as a marker of female infertility

Mussarat Ashraf,[1] Fatima Mustansir,[2] Syed Mujtaba Baqir,[3] Faiza Alam,[4] Rehana Rehman[5]

Abstract

Objective: To study the impact of Vitamin E (VE) levels of follicular fluid (FF) on oocyte competence, embryo development and pregnancy outcome in patients after intra cytoplasmic sperm injection (ICSI).

Methods: It was a cross-sectional study conducted in Islamabad Clinic Serving Infertile Couples in which follicular fluid of 137 females booked for ICSI, was obtained during oocyte retrieval, centrifuged and stored for analysis. VE levels in FF were analyzed by enzyme linked immune sorbent assay. Receiver Operating Curve (ROC)was used to demarcate VE levels required for acquiring pregnancy. Generalized linear model using log binomial regression was applied to see the effect of VE on pregnancy, the effect of VE on oocyte and embryo parameters was assessed by linear regression; all p-values less than 0.05 were considered statistically significant.

Results: ROC suggested 5.49 (unit) as the cutoff value of VE in the pregnancy group, with 72.9% area under the curve. Ninety-one females comprised Group I with VE > 5.49, whereas forty six females formed Group II with VE < 5.49. Follicular fluid VE levels were significantly high in 39 (28.5%) females who compromised pregnancy group. Chances of pregnancy increased to 4% with an increase in VE levels (p-value 0.01). VE gave significant positive relationship with all oocyte (retrieved, mature and fertilized) parameters, cleavage of embryo till its differentiation to blastocysts ($p<0.01$).

Conclusion: Adequate amount of VE in follicular fluid enhances the possibility of maturation of oocytes which resulted in better reproductive outcome after ICSI.

Keywords: Vitamin E, Follicular fluid, Intracytoplasmic sperm injection, Oxidative stress. (JPMA 70: 1762; 2020)

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Introduction

Lipid soluble vitamin E (VE) also called tocopherol plays a vital role in fertility, owing to its antioxidant and anti-inflammatory effects on pregnancy, birth and reproductive outcomes.[1] VE is a generic descriptor for all tocopherols and tocotrienols that exhibit alpha-tocopherol activity. It collectively signifies 8 different structurally-related tocopherols and tocotrienols, all possessing antioxidant activity. Due to its property of being a peroxyl free radical scavenger, it functions as a chain breaking antioxidant by preventing the proliferation of free radicals in membranes and plasma lipoproteins. As a lipid-soluble antioxidant, it protects polyunsaturated fatty acids (PUFAs) from lipid peroxidation (LPO). For this reason, it is hypothesized that VE is probably required during embryonic development to protect PUFAs that are essential for development.[2]

VE has been shown to regulate ROS (reactive oxygen species) production probably because it can readily pass through the placenta. The production of (ROS) by macrophages, neutrophils and granulosa cells surrounding the oocyte, is a normal process that occurs in the cellular mitochondrial respiratory chain.[3] Under normal to moderate circumstances, the antioxidants remove ROS actively but when the production of ROS exceeds the rate at which they can be countered by antioxidants, this is called oxidative stress (OS) and this produces a great impact on the cellular function.[4] OS has been proven to harm oocytes during maturation and cleavage, leading to segregation of chromosomes during meiosis, impaired fertilization, blockade of embryos in the two-cell stage and low pregnancy outcomes. ROS mediated damage also contributes to derangements in microtubule assembly and cytoskeletal alterations in oocytes.[5]

Antioxidants can be detected in follicular fluid (FF) which is easily available during oocyte pick-up and theoretically represents an optimal source on non-invasive biochemical predictors of oocyte quality.[6] Some studies aimed to find a good molecular predictor of oocyte quality in FF, were unable to identify substances that could be used as reliable markers of oocyte competence to fertilization, embryo development and pregnancy.[7,8] Therefore, this study aimed to evaluate the effects of follicular VE levels on oocyte competence to fertilization, embryo development and pregnancy outcome after intracytoplasmic sperm injection (ICSI).

[1,5]Department of Biological and Biomedical Sciences, [2,3]Graduate Student 2019, [4]Department of Physiology, University of Karachi, Karachi, Pakistan.
Correspondence: Rehana Rehman. Email: drrehana7@gmail.com

Methodology

A cross sectional study was carried out from August 2014 till May 2016 recruiting one hundred and thirty-seven patients booked for ICSI treatment, after obtaining ethical approval from Islamabad Clinic serving Infertile Couples. A written informed consent was obtained from all participants. Sample size was estimated using Open Epi sample size calculator version 3.01, after inserting the mean difference in VE levels of 2 groups[9] at 95 % confidence interval and 95% power we got n = 14 for both the groups in this study, (http://openepi.com/menu/openepi menu.htm). We recruited all the females who reported to infertility clinic during the study period in order to prevent the drop-outs, and finally selected 137 females between the age of 20 and 40 years in their first fresh ICSI- ET cycle. They were included on the basis of infertility due to female factors or unexplained causes. Endometriosis, hypertension, diabetes mellitus, uterine myoma, ovarian cyst, chronic drug usage, heavy caffeine drinking, chronic illness smoking and infertile females with male cause of infertility were excluded from the study.

Treatment Protocol: Long agonist protocol was followed by down regulation of ovaries by sub cutaneous administration of 1 mg of subcutaneous BuserelinAcetate (Suprefact, Sanofi Aventis, Pakistan) in mid luteal phase of previous cycle. Accomplishment of procedure was established by thin endometrial lining and quiet ovaries confirmed by trans-vaginal scan (TVS). The treatment protocol was followed by controlled ovarian hyper stimulation (COS) as described in another study.[10] The initiation dose of COS was calculated on the basis of ovarian reserve (age of the subject, basal serum FSH and estradiol concentrations, AFC) and body mass index (BMI). It was carried out by using subcutaneous administration of recombinant FSH (r FSH) 50 IU preparation (Puregon registered; NV Organon, Oss, The Netherlands). Dose was later adjusted by follicular tracking with TVS (7.5 MHz probe Aloka 500, Tokyo Japan) from the fifth day of COS on alternate days. The pre-ovulatory follicle count (PFC) and endometrial thickness was measured by TVS on the day of ovulation induction (OI). On this day, human chorionic gonadotropin (hCG) injection was administered after confirmation of maturity of follicles. Oocytes were retrieved 36 ± 1 hours after OI on 14th, 15th or 16th day of COS by vaginal ultrasound probe with 16 G adapter and double lumen oocyte aspiration needle (Cook Australia; Queens land, Australia) under short general anaesthesia. During oocyte retrieval, FF was obtained from a dominant follicle (≥18 mm) from each ovary and aspirated into 10 mL tubes. The needle was retracted and the content was emptied without addition of culture medium in the collection tubes. FF was collected, with efforts to minimize blood and culture contamination, then centrifuged at 300 rpm for 7 minutes, stored at -20°C for 7 minutes to remove cellular components, and finally the clear supernatant was divided into aliquots and stored at -20°C for further analysis.[11] Maturity of oocytes was assessed by the presence of oocyte zona pellucida, nuclear maturity, presence or the absence of the germinal vesicle or the first polar body. The cytoplasm was further examined for vacuoles or any other abnormalities in the texture of the ooplasm. Only normally fertilized (with two Pronuclei and two polar bodies) were considered for eventual embryo transfer. Cleavage of embryos was confirmed after another 24 hours. Cleavage rate was assessed by counting the number of cells in the embryo on day three. Normal cleavage was considered by presence of six to eight cells, slow cleavage by five cells or fewer and accelerated cleavage with nine or more cells.

Estimation of Vitamin E: VE was analyzed by commercially prepared ELISA kit (cat # MBS016543, MyBioSource), which was validated to be used for determination of VE in human body fluids. The assay had high sensitivity i.e. 1.0 umol/L and excellent specificity. No significant cross reactivity or interference between human VE analogues was observed. Both Intra-assay CV(%) and Inter-assay CV(%) was less than 15%. Clinical pregnancy was defined on the basis of β human chorionic gonadotropin (hCG) concentrations and TVS performed 2 and 6 weeks after embryo transfer respectively.

Data Analysis: Data was stored and analyzed using SPSS version 16.0. Receiver Operating Curve (ROC) was used to demarcate VE levels required for acquiring pregnancy. Mean and standard deviation were reported for age, BMI, PFC, number of cleaved embryos and other parameters among both groups. Two group means were compared statistically using independent sample t-test. ROC analysis was performed to identify the cut-off value of VE in pregnancy and then binary logistic regression was used to estimate the odds ratio and 95% confidence interval to see the effect of VE and other covariates in pregnancy; all p-values less than 0.05 were considered statistically significant.

Results

All females (266) booked for ICSI-ET cycle during the study period were approached. The total number of females agreed to participate in the study were 222, however 137 were recruited for the study after fulfilment of inclusion criteria. The participation rate was 61.7%. The excluded females were endometriosis 14, hypertension 15, Diabetes Mellitus 25, Ovarian pathologies 4, history of

Table-1: Comparison of study characteristics in comparison groups on the basis of Vitamin E levels.

Characteristics	Group I (n=91) Vitamin E < 5.49		Group II (n=46) Vitamin E > 5.49		p-value
	Mean	S.D	Mean	S.D	
Age (years)	32.03	4.55	32.59	5.07	0.52
BMI kg/m^2	24.49	3.63	23.06	3.34	0.034*
Pre Ovulatory follicle count	7.41	1.7	8.36	1.83	<0.01*
No of cleaved embryos	5.7	1.28	6.47	1.1	<0.01*
Embryo-Grade - I	1.03	0.48	3.72	1.08	<0.01*
Embryo-Grade - II	3.21	1.02	1.7	0.47	<0.01*
Number of Oocytes /patient	7.29	1.51	8.26	1.64	<0.01*
Number of Oocytes Metaphase II	6.92	1.65	7.95	1.51	<0.01*
Number of Oocytes fertilized	5.76	1.28	6.59	1.15	<0.01*
Blastocyst formed	1.94	0.38	2.36	0.54	<0.01*
Endometrial thickness (mm)	6.84	2.9	11.26	2.07	<0.01*
Antral Follicle Count	15.27	2.56	13.21	2.69	<0.01*

Table-2: Association of Vitamin E with pregnancy and other parameters using binary logistic regression.

Parameters	Odds Ratio	95% Confidence Interval	p-value
Pregnant	0.343	(0.159 - 0.742)	0.01*
Age (Years)	0.984	(0.912 - 1.062)	0.69
Metaphase	0.165	(0.091 - 0.298)	<0.01*
Pro nuclei	0.157	(0.081 - 0.306)	<0.01*
Blastocysts formed	0.2	(0.077 - 0.52)	0.01*
Endometrial thickness	0.989	(0.889 - 1.101)	0.85
Sacs	0.442	(0.27 - 0.725)	0.01*
Implantation rate	0.991	(0.982 - 0.999)	0.04*
Antral follicle count	0.86	(0.749 - 0.987)	0.04*
Body Mass Index kg/m^2	1.002	(0.907 - 1.106)	0.98
Preovulatory follicle count	0.068	(0.025 - 0.186)	<0.01*
Cleaved Embryo	0.19	(0.103 - 0.35)	<0.01*
Embryo- Grade-I	0.623	(0.479 - 0.811)	<0.01*
Embryo- Grade-II	0.685	(0.495 - 0.947)	0.03*

*p<0.05 was considered significant.

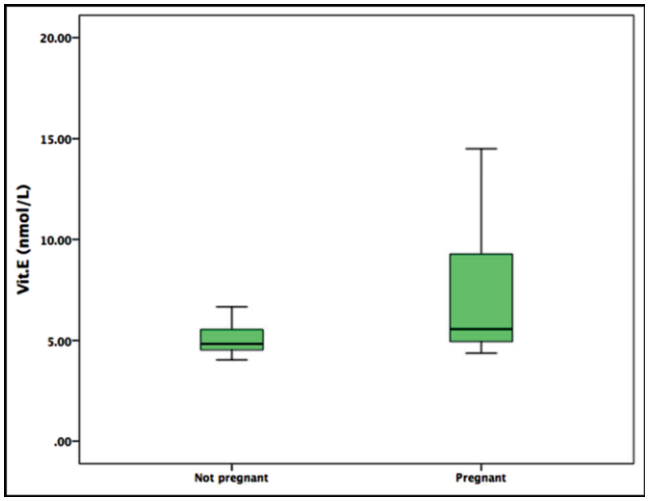

Figure-1: Comparison of VE levels in pregnant and non-pregnant females.

drug usage 7, addiction with caffeine 5, other chronic illnesses 5 and history of smoking 10. In the recruited females, 39 clinical pregnancies occurred whereas 98 females were not able to conceive pregnancy. ROC suggested 5.49 (unit) as the cut off value of VE in the pregnancy group, with 72.9% area under the curve. Present study showed that the mean age of VE in normal woman was 31.02±4.76 years while in deficient woman it was 32.43±4.65 years. Table-1 gives descriptive statistics of study population categorized in Group I with VE > 5.49 and in Group II with VE < 5.49; 91 and 46 females respectively. The mean values of PFC, number of oocytes, metaphase, fertilized oocytes, number of cleaved, grade-I-II embryos, Blastocysts formed, were significantly low among VE deficient woman as compared to vitamin E normal woman.

Results in Table-2 showed that, with the increase in VE, chances of increase in maturity of oocyte (Metaphase), cleavage of embryo and formation of blastocyst increased (p-value 0.01). VE levels were found significantly higher in those females who conceived; pregnancy group as compared to non-pregnant women (Figure-1).

Discussion

The protective role of VE in treatment of infertile couples can be emphasized by researches that observed better oocyte morphology and embryo quality with higher VE concentrations.[12] The failure of antioxidant defense mechanisms to counter OS thus is accompanied by poor oocyte fertilization, decreased IVF success rates and

reproductive outcomes.[13,14] Increased VE levels in pregnant females in our study is well in accordance with a greater likelihood of finding normal oocytes and more pregnancies through IVF by reduction in ROS.[12] No association of VE with age and BMI was observed in our study, which is also supported by Saffari et al.[15]

The mean number of good quality retrieved oocytes has also been linked to improvement with micro nutrient supplementation.[12] In our study, we observed that higher VE levels in the follicular fluid helped in oocyte maturity. This is in accordance with its antioxidant functions role to protect oocytes from ROS and prevent LPO by reacting with peroxyl and alkoxyl free radicals.[2] We observed that females with higher VE levels in FF had increased number of mature oocytes (metaphase II) that got fertilized and developed into blastocysts. Our findings are supported by a study in which α-tocopherol supplementation of culture media significantly increased the proportion of oocytes that reached metaphase II and blastocyst rates.[16] The increase in oocyte maturity is further supported by the study that documented increase in developmental potential of porcine denuded oocytesf upon VE supplementation to culture media.[17] The association of increase in oocyte maturity with increase in chances of conception in patients of ART has been noticed.[18]

Studies on elevated reactive oxygen species (ROS) levels in granulosa cells (GC) and its subsequent effect on fertilization are limited.[19] The greater fertilization of oocytes in our study can however be explained by Oyawoye et al., who demonstrated that higher levels of total antioxidant capacity are related to a greater fertilization potential in women undergoing IVF.[20]

As far as embryo development is concerned, balance between ROS levels and antioxidants within invitro maturation media helps in the development to the blastocyst stage.[21] We also observed that VE played a positive role in development potential of embryos from cleavage till differentiation into good quality embryo (grade I). Tao et al observed that although α-tocopherol did not increase the in vitro maturation rates, yet improved the blastocyst rate.[17]

The success of ICSI depends on the quality of embryo as well as uterine receptivity, the latter is the characteristic of endometrium which permits blastocyst to attach, infiltrate, and cause decidualization.[22] VE exerts an anticoagulant effect because of increase in endometrial blood flow and increase in estrogen production. Females with high VE levels acquired optimal endometrial thickness in addition to oocyte competence and good quality embryos, which helped in becoming pregnant. In a supportive study, VE administration improved the endometrial response in unexplained infertile women via the likely antioxidant and the anticoagulant effects.[23]

Our focus can be extended to improve pregnancy outcomes by looking into the administration of VE with other substances, such as its supplementation with Selenium during pregnancy has minimized the harmful effects of aluminum induced nephrotoxicity. At the same time, administration of combination of VE/progesterone had maintained first pregnancy with a better reproductive performance in the second parity with respect to live birth, conception rate, abortion rate, gestational length and many others.[24]

It has been documented that maturity of oocytes and embryo quality could be related to certain levels of vitamin E in the FF. The supplementation with micronutrients before initiation of ART cycle, therefore, has also been recommended to protect follicular microenvironment from OS and hence, increasing good quality oocytes for pick up.[12] To the best of authors' knowledge, this is the first study in Pakistan, results of which can be considered useful to choose proper dose of VE in interventional studies for health and reproductivity.

Limitation

This study did not include the dietary intake of VE and other antioxidants like Vitamin A, C and folate. The bioavailability of VE is influenced by several factors like age, competing nutrients, extent of intestinal absorption, hepatic metabolism, vascular transport and genetic constitution, which was not taken into account. Moreover, total antioxidant capacity (TAC) levels of follicular fluid and variations in levels of VE in different follicles were not considered. Research has illustrated the difference in biological activities of both isoforms of VE on lung function tests,[25] however, we studied only the α-tocopherol isomer which exerts an anti-inflammatory activity.

Conclusion

Adequate amount of VE in follicular fluid enhances the possibility of maturation of oocytes on account of the anti-oxidant potential, which, resulted in better reproductive outcome after ICSI. Due to the limited availability of literature regarding mechanistic functionality of VE in human reproduction, further detailed human studies are required to elucidate its role in pregnancy.

Disclaimer: None to declare.

Conflict of Interest: None to declare.

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