J Assist Reprod Genet (2012) 29:325–328
DOI 10.1007/s10815-012-9714-1

ASSISTED REPRODUCTION TECHNOLOGIES

Vitamin E effect on controlled ovarian stimulation of unexplained infertile women

Nedim Cicek · Ozlem Gun Eryilmaz · Esma Sarikaya · Cavidan Gulerman · Yasemin Genc

Received: 14 November 2011 / Accepted: 17 January 2012 / Published online: 1 February 2012

Abstract
Purpose To determine Vit E effect on the treatment outcomes of women with unexplained infertility undergoing controlled ovarian stimulation and intrauterine insemination (IUI).
Methods The study group (Group A, $n=53$) underwent controlled ovarian stimulation with clomiphene citrate with Vit E administration, 400 IU/day p.o. while the control group (Group B, $n=50$) underwent ovulation induction without Vit E. Treatment outcomes were compared between the groups.
Results There were no significant differences between the two groups with respect to the demographic outcomes. The difference in endometrial thickness on the day of hCG administration was significant between the two groups ($p=0.001$). The effect of receiving Vit E on the implantation and the ongoing pregnancy rates were assesed Odds Ratio (OR) and corresponding 95% Confidence Intervals (CI). Receiving Vit E was not significantly associated with the implantation and the ongoing pregnancy rates; OR = 1.22, 95% CI (0.44–3.4) and OR = 1.43, 95% CI (0.49–4.1), respectively.
Conclusion Vit E administration may improve the endometrial response in unexplained infertile women via the likely antioxidant and the anticoagulant effects. It may also modulate the antiestrogenic effect of clomiphene citrate and the problem of a thin endometrium in these cycles may be adjusted.

Keywords Vitamin E · Unexplained infertility · Endometrium

Capsule Vitamin E administration may improve the endometrial thickness in the controlled ovarian stimulation and intrauterine insemination cycles of unexplained infertile patients, but may not affect the pregnancy outcomes.

N. Cicek · O. G. Eryilmaz (✉) · E. Sarikaya · C. Gulerman
Zekai Tahir Burak Women Education and Research Hospital,
Obstetrics and Gynecology,
Devlet Mah. Subay Loj.Hızırreis Apt.No:19/11 Yenisehir,
Ankara, Turkey
e-mail: drozlemgun@yahoo.com

Y. Genc
Department of Medical Statistics, Ankara University,
Faculty of Medicine,
Ankara, Turkey

Introduction

Oxidative stress is a developing research field and is being examined in female infertility. Prooxidants, also called free radicals or reactive oxygen species (ROS), and their neutralizing agents the antioxidants are the main chemicals of the oxidation mechanism. The term oxidative stress refers to the dysequilibrium between the free radicals and the antioxidants in favor of the free radicals. In actuality, free radicals are not so frightening, since they are necessary for the adequate reproductive functions within the ovary and the endometrium [1]. Riley et al. showed the effect of reactive oxygen species (ROS) and the antioxidant enzymes in oocyte maturation, ovulation and luteal function [2]. These radicals play an important role as intercellular and intracellular messengers in the ovary and act as a mediator for the responses between the ovarian germ and stromal cells [3]. Related with the endometrium, various antioxidants were found to be associated with the cyclicity of the glands. Thioredoxin, superoxide dismutase (SOD) and tumor necrosis factor α (TNF-α)-induced manganese SOD (MnSOD) were identified in the endometrial tissues and were thought to be related to endometrial receptivity [4]. Healthy reproduction requires a balance between these radicals and their antioxidants, and any imbalance related to increased ROS or decreased antioxidants causes the oxidative stress, which may lead to

anovulation, dysfunctional oocytes, fertilization failure, implantation failure, or miscarriage.

Vitamin E (Vit E, α-tocopherol) is an exogenous, lipid-soluble antioxidant molecule. It is thought to be a direct free radical scavenger by activating the intracellular antioxidant enzymes and saving the cell membranes from lipid peroxidation, which was demonstrated on sperm membrane components [5]. Its antioxidant effect was concluded in cancer therapy, high-risk pregnancy and male infertility [6–10].

This randomized controlled trial was set up to investigate the Vit E action in idiopathic infertility, since it remains an unspecified subject. In this study, we aimed to analyze the Vit E effect on the controlled ovarian stimulation outcomes in patients with unexplained infertility. We hypothesized that clinical outcomes of controlled ovarian stimulation and intrauterine insemination (IUI) cycles may improve with Vit E administration.

Material methods

This study is a prospective, single-center, randomized controlled clinical trial assessing the impact of Vit E administration on infertile women undergoing controlled ovulation induction and IUI. The study was conducted between June 2011 and December 2011 in Zekai Tahir Burak Women's Training and Research Hospital, Reproductive Endocrinology and Infertility Department, Ankara, Turkey.

Patient selection

A total of 109 patients with the diagnosis of unexplained infertility were included into the study. Of these, two patients refused to participate in the study and were excluded before randomization. After randomization, four patients were also excluded due to incorrect dose ingestion ($n=3$) and cycle cancellation ($n=1$). The final study size was 103patients. The study patients were being followed with the diagnosis of unexplained infertility, defined according to the World Health Organization (WHO) as those couples with no known ovulatory problem, and with a normal hysterosalpingography or laparoscopy and a normal semen sample [11].

The exclusion criterias included the conditions with likely prooxidant status: Endometriosis, hypertension, diabetes mellitus, uterine myoma, ovarian cyst, chronic drug usage, excessive alcohol intake,heavy caffeine drinking, chronic illness and smoking. The local ethical committee approved the study, and written informed consent was obtained from all of the patients before randomization.

Study design

The patients were randomly assigned into two groups according to a randomization table. The study group (Group A, $n=53$) underwent controlled ovarian stimulation with Vit E administration, while the control group (Group B, $n=50$) underwent controlled ovarian stimulation without Vit E. Related to the determination of Vit E dose in the present study, an IVF research instructed with the usage of 500 IU/day [12] The maximal dosed preparation of Vit E in our markets contained 400 IU so the dose of our study was determined as 400 IU (Kocak Farma, Istanbul, Turkey). Administration of Vit E had begun from the 3rd to the 5th day of the menstrual cycle until the human chorionic gonadotropin (hCG) injection day of the controlled ovarian stimulation. The primary outcome of the study was the ongoing pregnancy rate.

Stimulation protocol

The controlled ovarian stimulation was done with clomiphene citrate (CC) (Serophene 50 mg, Merck Serano, Istanbul, Turkey) starting on Day 5 of a spontaneous menstrual cycle. CC was administered as 100 mg daily for 5 days. Transvaginal ultrasonography was performed on the 12th to 14th days of the stimulation. 250 μg recombinant hCG (r-hCG) (Ovitrelle, Merck Serono, Italy) was administered when at least one follicle had reached a diameter of 16 mm and IUI was performed 36 h later. All patients received luteal phase support as 90 mg/day of vaginally administered progesterone (Crinone gel, Merck Serono, Central Pharma, UK) starting on the day of IUI.

Biochemical pregnancy was established when serum β-hCG level was 20 IU/L or more on the 12th day after IUI, and clinical pregnancy was defined as the presence of a gestational sac on ultrasound performed at 6 weeks gestational age.

Outcome variable and statistical analysis

When the infertility prevalence of 7.4% in the general population [13] and 30% rate of unexplained infertility were taken as reference [14] with a 95% confidence, an alpha of 0.05 and 85% power, the sample size was calculated as 42 cases and 42 controls. Fifty-three cases and 50 controls were evaluated for this study.

Statistical analysis was carried out using the Statistical Package for the Social Sciences software (SPSS, version 11.5). Data were presented as either Median (Min-Max) or Mean ± SD as appropriate. All variables were tested for normal distribution with Kolmogorov-Smirnov Test, histogram and P-P plots. Variables were compared with either Independent Samples t Test or Mann–Whitney U test depending on the normality of the data. All categorical variables were compared with Pearson Chi-Square Test. The effects of receiving Vit E on pregnacy and implantation were also assesed Odds Ratio (OR) and corresponding 95% Confidence Intervals (CI). A p value<0.05 was considered as statistically significant.

Results

All of the study patients had continued Vit E supplementation without any reported side effects. The demographic data and the treatment outcomes of the women who did (Group A) and did not (Group B) receive Vit E are summarized in Table 1. There were no significant differences between the two groups with respect to mean age, body mass index (BMI), the duration of infertility, the day of hCG administration, and the number of follicles 12–16 mm on hCG administration day. The difference in endometrial thickness on the day of hCG administration was significant between the two groups ($p=0.001$). The implantation and ongoing pregnancy rates were similar ($p>0.05$).

The effect of receiving Vit E on pregnacy was assesed Odds Ratio (OR) and corresponding 95% Confidence Intervals (CI). Receiving Vit E were not significantly associated with pregnacy [OR = 1.43, 95% CI (0.49–4.1)].

The effect of receiving Vit E on implantation was also assesed Odds Ratio (OR) and corresponding 95% Confidence Intervals (CI). Receiving Vit E were not significantly associated with implantation [OR = 1.22, 95% CI (0.44–3.4)].

Discussion

In this study, we found that Vit E supplementation in unexplained infertile patients had beneficial effects in improving the endometrial thickness during controlled ovarian stimulation and IUI cycles.

Related with the endometrial problems encountered in infertility treatment, there are studies concerning the antioxidant effect of Vit E. One of these done by Lédée-Bataille et al. had studied the patients who were enrolled in an oocyte donation program because of irradiation, as a result of which all had a thin endometrium [12]. These patients were administered Vit E together with pentoxifylline, and were found to have a significantly thicker endometrium after treatment. The ovarian functions of these patients had also improved. In a case series by Letur-Konirsch et al., patients with premature ovarian failure were studied with respect to the Vit E effect on the endometrium, and they also reported the result of increased endometrial thickness [15]. They concluded that the positive changes in the subendothelial fibrous tissue might be the reason for the improved endometrial response and the decreased uterine resistance to hormonal therapy. Our study was different from the above because the endometria in this study were free of any previously diagnosed problems. The related infertility problem in the unexplained group may be in any step of the reproductive pathway. Paszkowski et al. studied selenium (Se) levels in the follicular fluid of women with unexplained infertility in in vitro fertilization (IVF) cycles, and showed that Se as a trace element of the antioxidant reactions was significantly lower in these patients [16]. They suggested that the antioxidant activity of Se-dependent enzymes might play a role in the pathomechanism of idiopathic infertility. Considering the relation of oxidative stress and unexplained infertility, we thought that the antioxidant effect obtained with Vit E may improve the infertility treatment outcomes. The increased endometrial thickness in the Vit E-administered group supported a likely oxidative stress present in the endometrium, such that the antioxidant effect may help to overcome any problems related to endometrial growth.

Related with the effect of Vit E on endometrium, the anticoagulant effect may be another reason for the improvement in the thickness [17]. This anticoagulant activity may increase the blood supply to the follicles and the proliferating granulosa cells so that may adequate the estrogen production which may be one of the pathway to have a good endometrium. On the other hand this anticoagulant effect may directly increase the endometrial blood flow that may improve the endometrial developement.

It is well known that the antiestrogenic effect of CC on the endometrium is one of the causes of a thin endometrium

Table 1 The demographic data and the treatment outcomes of the women who received (Group A) and not received (Group B) Vit E

	Received Vit E group A ($n=53$)		Not received Vit E group B ($n=50$)		p value
	Mean ± SD	Median (Min-Max)	Mean ± SD	Median (Min-Max)	
Age (years)	25.3±4.8	24.5 (18–38)	26.4±5.3	24 (20–37)	NS
BMI (kg/m^2)	23.8±2.7	23.9 (18.7–31.6)	22.7±2.9	22.4 (18–30)	NS
Infertility duration (years)	3.6±2.4	3 (1–11)	3.9±3.6	3 (1–21)	NS
Duration of stimulation(days)	11.6±2.3	11.5 (7–18)	11.5±2.0	11 (7–17)	NS
No. of follicles 12–16 mm	1.7±1.0	1 (0–6)	1.8±1.1	1 (0–4)	NS
Endometrium thickness on hCG day	9.6±2.1	9.5 (5–13)	8.2±2.0	8 (4–13)	0.001
Implantation rate (%)	10/53 (18.9%)		8/50 (16%)		NS
Ongoing pregnancy rate (%)	10/53 (18.9%)		7/50 (14%)		NS

SD Standard Deviation, *NS* Not significant

during ovulation induction and IUI cycles [18, 19]. In our study, this antiestrogenic effect seemed to be weakened. We could not explain the likely mechanism of this result, but it may have been due to the increased estrogen bioavailability within the microenvironment of the decreased oxidative stress status. Endometrial stromal and endothelial cells may answer the hormonal stimuli in a healthier manner in the presence of an antioxidant.

Another important result of this study was the higher implantation and ongoing pregnancy rates observed in the Vit E-administered group, even though the differences were not significant. These improvements again may be a result of the improving antioxidant effect of Vit E on the endometrial receptivity.

In conclusion, in cases with likely oxidative stress, such as in women with unexplained infertility, Vit E may improve the endometrial environment via its antioxidant effect. It may also modulate the antiestrogenic effect of CC, and the problem of a thin endometrium in these cycles may be adjusted. Further studies related to the measurement of the oxidants-antioxidants in the serum or the endometrial flushing fluids of the patients during an antioxidant treatment may be more diagnostic.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. Curr Opin Obstet Gynecol. 2006;18:325–32.
2. Riley RC, Behrman HR. Oxygen radicals and reactive oxygen species in reproduction. Proc Soc Exp Biol Med. 1991;198:781–91.
3. Roselli M, Keller PJ, Dubey RK. Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. Hum Reprod Update. 1998;4:3–24.
4. Sugino N, Karube-Harada A, Taketani T, et al. Withdrawl of ovarian steroids stimulates prostaglandin F2alpha production through nuclear factor-kappaB activationvia oxygen radicals in human endometrial stromal cells:potential relevance to mensturation. J Reprod Dev. 2004;50:215–25.
5. Palamanda JR, Kehrer JP. Involvement of vitamin E and protein thiols in the inhibition of microsomal lipid peroxidation by glutathione. Lipids. 1993;28(5):427–31.
6. Agarwal A, Sekhon LH. Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: ıs it justified? Indian J Urol. 2011;27(1):74–85.
7. Blot WJ, Li JY, Taylor PR, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence and disease-specific mortality in the general population. J Natl Canc Inst. 1993;85:1483–92.
8. Bouayed J, Bohn T. Exogenous antioxidants-double-edged swords in cellular redox state. Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxid Med Cell Longev. 2010;3(4):228–37.
9. Chappell LC, Seed PT, Briley AL, et al. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. Lancet. 1999;354:810–6.
10. Porter R, Whelan J. Biology of vitamin E. Ciba foundation symposium 101. Proceedings of a symposium on biology of vitamin E. London: Pitman; 1984. p. 1430–9.
11. WHO. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization; 2010.
12. Lédée-Bataille N, Olivennes F, Lefaix JL, et al. Combined treatment by pentoxifylline and tocopherol for recipient women with a thin endometrium enrolled in an oocyte donation programme. Hum Reprod. 2002;17(5):1249–453.
13. Chandra A, Martinez GM, Mosher WD, Abma JC, Jones J. Fertility, family planning and reproductive health of U.S. women: data from the 2002 National Survey of Family Growth. Vital Health Stat. 2002;23(25):1–160.
14. Hull MG, Glazener CM, Kelly NJ, et al. Population study of causes, treatment and outcomes of infertility. Br Med J (Clin Res Ed). 1985;291:1693–7.
15. Letur-Konirsch H, Delanian S. Successful pregnancies after combined pentoxifylline-tocopherol treatment in women with premature ovarian failure who are resistant to hormone replacement therapy. Fertil Steril. 2003;79(2):430–41.
16. Paszkowski T, Traub AI, Robinson SY, McMaster D. Selenium dependant gluthatione peroxidase activity in human follicular fluid. Clin Chim Acta. 1995;236:173–80.
17. Tovar A, Ameho CK, Blumberg JB, et al. Extrahepatic tissue concentrations of vitamin K are lower in rats fed a high vitamin E diet. Nutr Metab (Lond). 2006;20(3):29.
18. Bromer JG, Aldad TS, Taylor HS. Defining the proliferative phase endometrial defect. Fertil Steril. 2009;91:698–704.
19. Siebert TI, Kruger TF, Lombard C. Evaluating the equivalence of clomiphene citrate with and without metformin in ovulation induction in PCOS patients. J Assist Reprod Genet. 2009;26 (4):165–71.