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Effects of pentoxifylline and vitamin E on pregnancy rate in infertile women treated by ZIFT: a randomized clinical trial

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Abstract

Background: Reviewing the literature, reveals that pentoxifylline (PTX) plus tocopherol (vitamin E) are used mainly to promote sperm quality. However trials focusing on the effects of these drugs in female partner are limited. Combination of pentoxifylline and vitamin E appeared to improve the pregnancy rate in patients with a thin endometrium by increasing the endometrial thickness and improving ovarian function.

Objective: To determine whether combined PTX and tocopherol treatment can improve clinical pregnancy rate.

Materials and Methods: One hundred twelve infertile women undergoing standardized controlled ovarian hyperstimulation for ICSI- ZIFT entered this randomized clinical trial. Patients were randomized to equal groups of combined PTX and tocopherol therapy or none (not receiving PTX and tocopherol). These drugs were administered to the intervention group for two cycles before starting ICSI-ZIFT cycle. Main outcome measure was clinical pregnancy rate. SPSS.11 software (SPSS Inc. Chicago IL.) was used for data collection and analysis.

Results: The clinical pregnancy was higher in the intervention (combined PTX and tocopherol) group in comparison to the other group (57.14% vs 39.29%, p=0.01). However, there was no difference in the mean endometrial thickness, number of retrieved oocytes, the number of metaphase II oocytes and grade of them in both groups.

Conclusion: This study showed that PTX plus tocopherol could improve the ZIFT outcome in infertile couples. Local effects and anti oxidative characteristics of these drugs may be the cause of better results.

Key words: *Endometrium, Pentoxifylline, ZIFT, Vitamin E, Pregnancy outcome.*

Introduction

The success of modern assisted reproductive technology (ART) has completely revolutionized both the evaluation and treatment of infertility. Ultrasound assessment of the endometrium has Become a standard procedure during the diagnostic work–up and treatment of infertility. The endometrial thickness is reported to be between 7 to 12 mm. It is accepted that a minimal endometrial thickness is necessary for pregnancy (about 6 mm) by most researchers (1).

Treatment by combination of pentoxifylline and vitamin E appeared to improve the pregnancy rate in patients with a thin endometrium by increasing the endometrial thickness and improving ovarian function (1, 2). This was especially noticeable in patients who had previously received total body irradiation (2).

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A significant increase in endometrial thickness in six patients with radiation-induced thin endometrium (from 3–6 mm), after 12 months of this combination treatment was reported (2). This combined therapy has been reported to be an effective treatment for musculocutaneous radiation-induced fibrosis, both in experimental models and in humans (3). Pentoxifylline acts as a phosphodiesterase inhibitor and protects the cells from lipid peroxidation by H2O2, so it may be beneficial to reduce H2O2 induced embryo damage and improve IVF outcome (4).

Due to few available studies about the impact of pentoxifylline and vitamin E on pregnancy rate (especially in ICSI-ZIFT cases), this research was designed to evaluate ART (ZIFT) success rate (commonly expressed as occurrence of clinical pregnancy) and endometrial thickness in female patients treated with pentoxifylline and vitamin E combined therapy.

Materials and methods

This randomized clinical trial was conducted on 112 patients of Infertility Department of Shariati Hospital, affiliated to Tehran University of Medical Sciences. The study evaluated the effects of pentoxifylline and vitamin E on pregnancy outcomes of infertile women planned for ZIFT (zygote intra fallopian transfer) from April 2006 to April 2007. The project was approved by the Ethical Committee of the Infertility Department and was initiated after achieving written consents of the participants. The infertile patients were under 39 years of age without a previous history of ZIFT or IVF failure (first ICSI cycle). Exclusion criteria were hypothalamic amenorrhea, drug reactions or complications, endometriosis and fibroids. Patients were randomly divided into two equal groups. Computer generated random number table was used for randomization of the investigation. Group assignments were placed in sealed opaque sequentially numbered envelopes. For each participant a questionnaire was filled by the researchers. The questionnaire contained questions about age, duration of infertility, the type and cause of infertility. Data were collected from questionnaires, clinical, laboratory notes and ultrasound reports. A long term desensitization protocol using the GnRH agonist Buserelin (Superfact, Hoechst, Frankfort, Germany) 500 micrograms subcutaneously, started at the day 22 of the previous cycle. After complete desensitization, ovarian stimulation using HMG (Menogan, Ferring, Germany) was commenced on day 3 of the next cycle at a daily dose (150-225 IU) which determined for each patient on the basis of age and response to previous treatments. Ultrasound (Zimens, Sonoline G20) for follicular development was done on days 9, 11, 12 of the cycle. Final oocyte maturation triggered when at least 2 follicles with diameter of at least 17 mm was observed, with HCG (Pregnyl, Organon) 10000 IU, administered as a single intramuscular injection. Endometrial thickness (by Zimens, Sonoline G20) was measured on the day of the HCG prescription in both groups by one of the two attending physicians. Oocytes were collected 36-38 hours later using transvaginal guided follicle aspiration (by Zimens, Sonoline G20) under general anesthesia. After fertilization through intracytoplasmic sperm injection (ICSI), 5 sperm injected oocytes (zygotes) were transferred immediately by ZIFT (zygote intrafallopian transfer) procedure. This rapid ZIFT procedure was performed via laparoscopy under general anesthesia. Luteal phase support was started on the day of ovum pick up by administration of progesterone suppository (Cyclogest, Actovis, UK) 800 mg daily and 25 mg progesterone in oil a week later (until fetal heart rate detection). Chemical pregnancy was detected by serum beta-hCG analysis 14 days after embryo transfer and transvaginal ultrasound (Zimens, Sonoline G20) scan was scheduled 2 weeks later to confirm the diagnosis of clinical pregnancy. The intervention group received pentoxifylline (Apo, Canada) 400 mg/BD plus vit E (webber, Canada) 400 mg/BD 2 cycles before starting ZIFT cycle and the medication was continued until the beta-hCG became positive or the cycle was cancelled. The comparison group did not receive the above drugs; so the study was not blinded.

Statistical analysis

SPSS.11 software (SPSS Inc. Chicago IL.) was used for data collection and analysis. Chi square, t test and fisher exact test were used for statistical analysis. P-value less than 0.05 was considered for statistical significance.

Results

The participants had the mean age of 29.69±4.60 years (range: 20-39 years). Twenty three (20.57%) were between 20-25 years, 40 (35.71%) were between 26-30 years, 40 (35.71%) were between 31-35 years and 9 (8.04%) were between 36-39 years old. There was no statistical difference between the groups. Infertility duration was 7.33±4.49 years (range: 1-20 years). Infertility was primary in 94.64% (106) and secondary in 5.36% (6) of cases (Table I). The male factor infertility was observed in 45.54% of cases, male plus female factor in 28.57% of cases and the female factor in 28.89% of the participants. There was no statistical difference in mean age, duration and type or cause of infertility between the two groups (Table I).

The clinical pregnancy rate was higher in the pentoxifylline and vit. E group in comparison to the control group (57. 14% vs 39.29%, p=0.01). In both groups the endometrial thickness after administration of controlled ovarian hyper stimulation drugs was significantly higher than the baseline endometrial thickness (endometrial thickness before initiation of ART cycle). However, there was no difference in the mean of endometrial thickness between case and control groups (10.85mm vs 10.48mm, p=0.467). There was no difference in the mean number of retrieved oocytes, the number of metaphase 2 oocytes and grade of them in both groups (Table I). It is remembered that there was no difference in term delivery, ectopic pregnancy, abortion, premature rupture of membrane, premature delivery and multifetal pregnancy between the study groups. No cases of ovarian hyperstimulation syndrome (OHSS) were reported (Table I). During laparoscopy for ZIFT we did not find any problem adversely affecting or confounding our study. There was no patient drop out to report (all the participants were followed).

Table I. Comparison of the case and control groups.

	Case group n=56	**Control group n=56**	**p-value**
Age (years)	29.96±4.62	29.41±4.59	0.53
Infertility duration (years)	7.42±4.50	7.23±4.52	0.82
Type of infertility [No (%)]			
Primary	53(94.64%)	53(94.64%)	
Secondary	3(5.36%)	3(5.36%)	0.99
Cause of infertility [No (%)]			
Male	26(46.42%)	25(44.64%)	
Female	15(26.79%)	14(25%)	
Male + female	15(26.79%)	17(30.36%)	0.91
Total	56 (100%)	56 (100%)	
Endometrial thickness (mm)	10.85 ±1.81	10.48±1.57	0.47
Retrieved oocytes	10.3±5.4	10 ±5.6	0.78
Metaphase ц oocytes	7.42±4.6	7.36±4.62	0.95
Clinical pregnancy [No (%)]	32(57. 14%)	22(39.29%)	0.01
OHSS	0	0	1
Term delivery	19	14	0.56
Ectopic pregnancy	2	1	0.9
Abortion	6	5	0.76
preterm labor	6	3	0.16
Twin pregnancy	4	5	0.49

Mean±SD

Discussion

This study showed that PTX plus tocopherol (vitamin E) prescription in female partners improved the ZIFT outcome despite lacking significant positive effects on the endometrium. Local effects and anti oxidative characteristics of these drugs may be the cause of better results. Reviewing the literature, reveals that PTX plus tocopherol are used mainly to promote sperm quality, however our trial focuses on the effects of these drugs in female partner. Reactive oxygen species (ROS) have been implicated in the pathophysiology of infertility. High ROS level are associated with endometriosis, unexplained infertility and poor IVF outcome. Increasing levels of hydrogen peroxide (H_2O_2) are correlated with embryo arrest and increased embryo fragmentation rate. Pentoxifylline acts as a phosphodiesterase inhibitor and protects the cells from lipid peroxidation by H2O2. Pentoxifylline may be beneficial in reducing H2O2 induced embryo damage and improve IVF outcome (4). PTX, a methylxanthine derivative, is a vasodilating agent that enhances red blood cell deformability, inhibits inflammatory reactions and reduces blood viscosity by inhibiting platelet aggregation. It has therefore been used for the symptomatic treatment of various vascular disorders, including intermittent claudication, ischemic leg ulcers, and peripheral vascular diseases. Most studies of its mechanism have focused on its effect on the production and function of tumor necrosis factor (TNF) (5). PTX increases the phagocytic activity of polymorphonuclear leukocytes (PMN) and monocytes, antagonizes TNF-α production and activity and reduces *in vitro* production of many cytokines, including granulocyte-macrophage colony stimulating factor (GM-CSF) and gamma-interferon (IFN-γ). In reproduction, PTX has been reported to decrease the fetal resorption rate significantly in the CBA/JxDBA/2 murine model of spontaneous abortion. It is hypothesised that PTX works by reducing local TNF-α production (6). The establishment of pregnancy has been described as a delicate equilibrium between the Th1 and Th2 cytokines (7).

The physiological role of tocopherol (vitamin E) is to scavenge ROS at times of oxidative stress, when antioxidant enzymes such as superoxide dismutase (SOD) or catalyses are unable to limit the damaging effects of ROS, and thus cannot protect cell membranes against lipid peroxidation. Found mainly in cell membranes, vitamin E is the most important antioxidant protecting membrane phospholipids against oxidative damage. In women, the antioxidant system, like the thioredoxin system, has been reported to change during the menstrual cycle in endometrial glands and stroma: levels are highest in the early secretary phase, that is, during the implantation window (8, 9).

Some studies reveal that PTX and vit. E are effective treatment for radiation fibrosis (3, 10). Also it is mentioned that this combination for about 6 months increases endometrial thickness (1). In a case series these findings are corroborated. This regimen, with minimal risks, provides a potential avenue for endometrial insufficiency (11).

Despite the aforementioned studies, our study did not reveal any statistical difference in the mean endometrial thickness. We could not describe the cause; however the endometriums of our patients were not irradiated or damaged. This may describe the discrepancy between our results with others. Although the women were randomized but a limitation to our study is the lack of patient's blindness. All of our patients obeyed the protocol medications; so we think this factor did not have any adverse effect on our research. Also it is remembered that the surgeons conducted the operations were blinded to the patients' groups. Although the rate of pregnancy was significantly higher in PTX and vit. E group (57.14% vs 39.29%, $p=0.01$), the outcome of these pregnancies was the same in both groups. The high rate of implantation in PTX and vit. E group could be referred to other effects except for increasing endometrial thickness or oocyte aspects. Decreasing TNF-α value by PTX may cause better implantation. For instance, Zhang and his coworkers (4) showed that PTX may cause lower damage of hydrogen peroxide in fetus and make IVF outcome better. The attachment of sperm to zona pellucida could become easier by PTX (12), although we did not aim to confirm this effect, but we consume if there is any unknown positive effects for better ART outcomes.

However there is many suggested ways to improve thin endometrium, such as the administration of low dose aspirin, estrogen, vaginal sildenafil citrate, pentoxifylline, vitamin E, and gonadotropin releasing hormone agonist with an aim to increase the pregnancy and implantation rates in assisted reproductive technology cycles. These various recent modalities proposed for the treatment of thin endometrium seem to be inefficient from an evidence-based medicine point of view (13).

It is concluded that Pentoxifylline (PTX) plus Tocopherol (vitamin E) prescription in female partners improved the ZIFT outcomes despite lacking significant positive effects on the endometrium. Probably in ART failures, with some interventions in this regard, successful results could be achieved. More prospective and randomized clinical trials to determine the potential target for future treatment remains to be seen.

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