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Effects of estradiol injection on outcome of in-vitro fertilization: a randomized, double-blind, placebo controlled trial

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Summary

Purpose: To evaluate the effects of estradiol (E2) supplementation on pregnancy outcome in patients with unexplained infertility undergoing in vitro fertilization (IVF). *Materials and Methods:* A total of 100 women with unexplained infertility and candidates for IVF, were included in this study and were randomly assigned to receive E2 supplementation or placebo during the luteal phase. The E2 serum levels in the hCG administration day and third and seventh day after ovum retrieval were measured in control group. The rate of pregnancy was also quantified and compared between the two study groups. *Results:* There was no significant difference between two study groups regarding baseline characteristics. E2 level decreased significantly in third (1765.34 ± 680.09; $p < 0.001$) and seventh (1459.66 ± 593.80; $p < 0.001$) days after ovum retrieval (2411.16 ± 713.52). The authors found that the serum level of E2 was significantly lower in those who received E2 supplementation at day 3 ($p < 0.001$) and 7 ($p<0.001$). However the pregnancy rate was not significantly different between two study groups ($p = 0.849$). In the same way, there was no significant difference between two study groups regarding the number of retrieved oocytes ($p = 0.563$) and number of MII oocytes ($p = 0.103$). *Conclusions:* E2 supplementation during the luteal phase in patients with unexplained infertility undergoing IVF, is associated with decreased serum levels of E2 after hCG injection. However the fertility outcome was not affected by E2 supplementation.

Key words: Estradiol (E2); Infertility; In vitro fertilization (IVF); Luteal phase support; Pregnancy rate.

Introduction

Insufficiency of estradiol (E2) and progesterone after ovulation is referred to luteal phase deficiency, which is a common etiology of failure of assisted reproduction techniques [1]. Increased secretion of the steroid in early luteal phase results in luteinizing hormone (LH) inhibition which in turn decreases the secretion of E2 and progesterone resulting in luteal phase deficiency [2]. In assisted reproduction techniques, hormonal support is necessary for preventing luteal phase deficiency. Implantation is influenced by both the endometrium and the embryo itself. Endometrial capacity for implantation can be decreased due to decreased hormone production of ovaries and corpus luteum during luteal phase of induced cycles [3]. Without hormonal support, the decreased serum levels of E2 and progesterone result in decrease in the implantation and pregnancy rates [4-7]. Thus it is believed that luteal phase hormonal support increases the success rate of the assisted reproduction techniques.

Although the luteal phase hormonal support for alleviating the assisted reproduction techniques is proved, there is however controversy regarding the agents to be used for luteal phase support in stimulated cycles [8-10]. Previous reports have clearly shown that supplementation of progesterone and human chorionic gonadotropin (hCG) increase the implantation and pregnancy rates [11-14]. However there is still no consensus regarding the use of E2 for luteal phase support, as results of the studies is conflicting. Although some studies have shown favorable results with administration of E2 as luteal phase support in induced cycles [6, 15], some authors barely reported beneficial effects [16, 17]. The latter two studies indicated that their sample size population was not appropriate to draw any conclusions, thus their results are interpreted as pilot studies. The present authors therefore designed this randomized clinical trial in order to determine the effects of E2 supplementation on implantation and pregnancy rates in induced cycles for in vitro fertilization (IVF).

Materials and Methods

Study population

This was a randomized, double-blind, placebo controlled clinical trial performed in Qadir Mother and Child Hospital, a tertiary healthcare center affiliated with Shiraz University of Medical Sciences during a 12-month period from July 2012 to March 2013. The study protocol was approved by institutional review board (IRB) and medical ethics committee of Shiraz University of Med-

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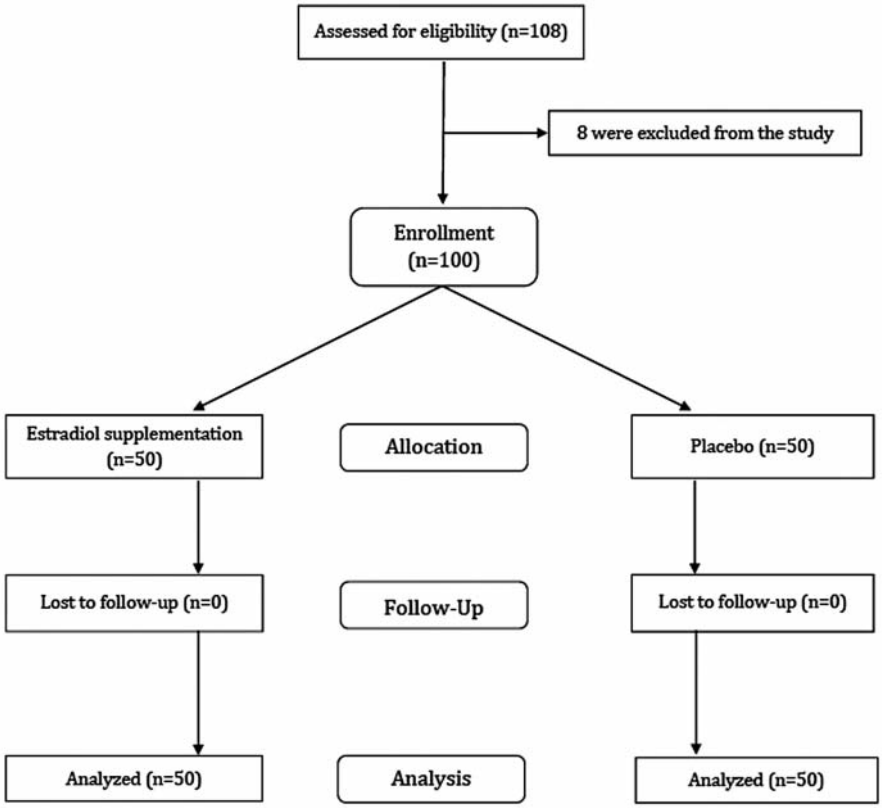

Figure 1. — CONSORT flow diagram of the study.

ical Sciences. All the patients provided their informed written consents before inclusion in the study. The authors included a total number of 100 patients with male factor infertility referred to out center for IVF or intracytoplasmic sperm injection (ICSI). They included patients younger than 35 years who were candidates for IVF/ICSI due to unexplained infertility or subfertile male factor (≥ five million total progressive motile spermatozoa per milliliter). They excluded women with diminished ovarian reserve (FSH > 12 IU/ml), an hCG day E2 level above 3,000 pg/ml (because of ovarian hyperstimulation syndrome [OHSS] risk), endometriosis, severe male factor (< five million motile spermatozoa per milliliter requiring testicular sperm extraction, frozen-thawed cycles, endocrine disorders, and polycystic ovary syndrome.

Unexplained infertility was diagnosed after exclusion of all the known infertility etiologies, such as hormonal disorders, infections, genetic anomalies, immunologic problems, and abnormal anatomic structures. For this purpose all the patients had normal parental chromosome analyses (father and mother), normal hysterosalpingogram, negative lupus anticoagulant and anticardiolipin antibodies, and normal serum testosterone levels. All the included women had an infertility duration of two to six years, had received no drugs for infertility at least for the past three months, and had a body mass index (calculated as weight in kilograms divided by the height squared in meters) of 18–25 kg/m^2, anti-Müllerian hormone (AMH) more than one µg/L, follicle stimulating hormone (FSH) levels of less than ten mIU/ml on the third day of the cycle, and at least 10–12 follicles in antral follicle count (AFC). None of the participants were smokers and none of them had history of alcohol consumption. Participants who had previous exposure to anesthetic gases, perchlorethylene (a dry-cleaning solvent), heavy metals (mercury, lead), and isotretinoin (accutane) were excluded from the study.

Intervention

A total number of 100 patients fulfilled the study criteria and were further included in the trial. The patients were randomly assigned to two study groups using a computerized random digit generator based on their registration number in order of referral. Those assigned to E2 group received oral E2 of four mg/d for two weeks after receiving IVF. Those assigned to control group received placebo with the same frequency.

Study protocol

All the patients underwent a complete history evaluation and physical examination by the attending gynecologist who was blinded to the study. The patients were scheduled for IVF/ICSI and E2 level was measured before oocyte pick up. All the patients underwent controlled ovarian hyperstimulation (COH) with standard long protocol. Primary sampling was done during COH cycle with hCG administration. After that, a second sampling was done at the third day after ovum retrieval - the day in which the patient admitted for embryo transfer to uterus. The third sampling was done seven days after ovum retrieval by going to the patient's residence. Then the samples were transferred to the laboratory of Mother and Child Ghadir Hospital regarding the sample handling principles pointed out in protocols of lab kits of E2 level measurement, and the E2 level was quantified in these samples by use of the mentioned kits. All the measurements were performed using ELISA technique.

Follow-up and outcome measurement

All the patients were followed and the pregnancy test was requested two weeks after the ET. Pregnancy was documented by transvaginal sonography, at three weeks of gestation after obtaining a positive pregnancy test. Main outcome measurements were

Table 1. — *Baseline characteristics of 100 patients with infertility undergoing IVF with (n=50) or without (n=50) estradiol supplementation.*

	Estradiol group (n=50)	Placebo group (n=50)	*p*-value
Age (years)	26.4 ± 2.3	26.1 ± 2.8	0.356
Infertility duration (years)	5.54 ± 2.7	5.35 ± 1.9	0.125
Weight (kg)	63.1 ± 11.4	67.1 ± 7.8	0.022
Height (cm)	161.2 ± 4.5	161.7 ± 2.3	0.395
BMI (kg/m^2)	24.3 ± 4.4	25.6 ± 3.2	0.047
Day 3 LH (mIU/ml)	7.4 ± 6.5	7.3 ± 5.6	0.473
Day 3 FSH (mIU/ml)	7.2 ± 3.8	6.8 ± 2.5	0.185
TSH (µg/dl)	3.6 ± 1.8	3.9 ± 2.1	0.215
Prolactin (mg/dl)	15.7 ± 6.7	15.4 ± 5.8	0.306
Basal E2 level (pg/ml)	40.8 ± 11.6	41.9 ± 9.6	0.652

BMI: body mass index; LH: luteinizing hormone;
FSH: follicular stimulating hormone; TSH: thyroid stimulating hormone.

Table 2. — *The study outcome in 100 patients with infertility undergoing IVF with (n=50) or without (n=50) estradiol supplementation.*

	Estradiol group (n=50)	Placebo group (n=50)	*p*-value
Estradiol level (pg/ml)			
On hCG injection day	2411.1 ± 713.5	2532.6 ± 957.1	0.251
On day 3	1765.3 ± 680.9	2371.5 ± 567.9	<0.001
On day 7	1459.6 ± 598.8	2311.8 ± 485.3	<0.001
Duration of stimulation (days)	9.6 ± 1.3	9.7 ± 1.5	0.998
Number of oocytes retrieved	10.6 ± 2.3	9.3 ± 1.1	0.563
Number of MII oocytes	8.1 ± 0.8	6.9 ± 0.7	0.103
Pregnancy rate (%)	12 (24.0%)	13 (26.0%)	0.849

implantation rate (detected by positive β-hCG) and pregnancy rate (detected by positive β-hCG and sonography). The authors also recorded the abortion, multiple pregnancy, and ongoing pregnancy rates (calculated by subtracting abortion from pregnancy rate). Clinical pregnancy was defined as the observation of gestation sac with fetal echoes and pulsations on transvaginal sonography. Multiple gestational sacs were counted as one clinical pregnancy. Implantation was defined by a rising β-hCG level in serum without the detection of a gestational sac. The abortion rate was defined as the loss of pregnancy before 20 weeks of gestation. Loss of pregnancy after 20 weeks of gestation was defined as stillbirth.

Statistical analysis

Based on 85% power and with α coefficient 0.05 to detect significant differences between corresponding variables ($p = 0.05$, two-sided), 43 patients were required for each study group. For compensating for non-evaluable patients, the authors included 50 patients in each study group. The statistical software package SPSS, version 16.0 was used for data analysis. The paired *t*-test was used to compare results within groups, the independent *t*-test to compare results between the groups, and the χ^2 test to compare proportions. Data were reported as mean ± SD. A *p*-value less than 0.05 was considered significant.

Results

Overall 108 patients were screened for eligibility out of whom 100 fulfilled the study criteria and were randomly assigned to two study groups (each containing 50 patients). None of the patients were lost to follow-up and all of them finished the study. Thus the final number of patients that were included in the final analysis was 100 (Figure 1). The baseline characteristics of the patients were comparable between two study groups. The baseline characteristics are summarized in Table 1. There was no significant difference between two study groups regarding the baseline characteristics.

Basal E2 levels were comparable between two study groups ($p = 0.652$). In the same way, the day of stimulation was not significantly different between two study groups (Table 2). The authors also found that the E2 level at day of hCG injection was comparable between two study groups ($p = 0.251$). The serum level of E2 decreased significantly in study group on day 3 (1765.3 ± 680.9; $p < 0.001$) and 7 (1459.6 ± 598.8; $p < 0.001$), when compared to baseline. However the changes on day 3 (2371.5 ± 567.9; $p = 0.288$) and 7 (2311.8 ± 485.3; $p = 0.196$) were not significant in control group. The authors found that the serum level of E2 was significantly lower in those who received E2 supplementation on day 3 ($p < 0.001$) and 7 ($p < 0.001$). However the pregnancy rate was not significantly different between two study groups ($p = 0.849$). In the same way, there was no significant difference between two study groups regarding the number of retrieved oocytes ($p = 0.563$) and number of MII oocytes ($p = 0.103$).

Discussion

Estrogen administration in follicular phase can improve endometrium preparation, but its role in the luteal phase is still controversial [18-20]. In unsuccessful cycles, the late luteal E2 levels decline which may compromise peri-implantation endometrial developments [21]. Vlahose *et al.* found that the addition of estrogen to progesterone in the luteal phase can increase L-selectin ligands –adhesion molecule in the endometrium during implantation [22]. All the aforementioned data raise the speculation about a potential positive correlation between luteal phase E2 levels and pregnancy outcomes. In this study, in order to evaluate the relationship between serum E2 levels and clinical pregnancy rates after IVF cycles, E2 serum levels were measured in three distinct periods of time which consisted of the hCG administration day, the third and the seventh day of ovum retrieval of patients undergoing IVF. The authors found that the E2 supplementation was associated with sig-

nificant decrease in E2 levels after hCG injection when compared to placebo. However the clinical pregnancy rate and number of retrieved oocytes were not affected by the E2 supplementation, which is consistent with previous studies [23, 24].

The results showed that E2 serum levels in hCG administration day and the third and seventh day after ovum retrieval in these patients were noticeably decreased. There are many studies that reported a decline in E2 levels on the hCG administration day and on the sixth day after ovum retrieval (12 to 16), much like what was observed in this study. In order to evaluate the effects of E2 serum levels on clinical pregnancy rates in patients with infertility due to unknown etiologies or polycystic ovarian syndrome (PCOS), the decline in E2 levels in studied times was compensated by administering E2 to the patients in E2 group (n=50); then pregnancy rate was compared between control group and E2 group. The results showed that the increase in serum E2 levels did not have any effects on pregnancy rates and outcome of IVF/ICSI treatments. These results are in accordance with former studies and meta-analyses; including the studies performed by Gelbaya *et al.* and Papageorgiou *et al.* that assessed the relation between hCG administration day E2 levels and success of IVF cycles in producing pregnancy, but similar to the present study, could not find any positive relations between E2 levels and pregnancy rates [5, 25].

Histological data from endometrial biopsies of patients undergoing IVF on days 21 and 25 of cycles has shown that omitting the E2 injections from treatment cycles does not have any effects on unity of uterine endometrium, estrogen receptor numbers, and hormone profile of these patients. In these studies, it has been suggested that perhaps the high physiologic levels of progesterone compensates for loss of E2 during the midluteal phase and masks its effects on unity of endometrium, embryo implantation, and as a result the outcome of IVF [24]. In addition, it has been suggested that since the optimal dose of E2 administration during the luteal phase is still not determined and is of question, perhaps the reason for controversial results on this subject is due to ineffectiveness of E2 supporting doses during the luteal phase [5]. In a properly designed clinical study, different doses of E2 (0, 2 or 6 milligrams daily) were administered to patients already receiving six milligrams of progesterone daily. In this study, the patients who received low doses of E2 had significantly more implantation and pregnancy rates in comparison with those who received high dose or no E2. Furthermore, different studies use different methods to add E2 to the regimen of their patients. These methods include oral, transdermal, and vaginal administration of E2. That is why there is no general agreement on effective and optimal dose of E2 and the period of its consumption in each of these methods; this interferes with evaluation of the real effects of E2 on clinical pregnancy rates in IVF cycles [26, 27].

In other studies, evaluation of effects of E2 administration during the midluteal phase of IVF cycles, only in patients with very low serum E2 levels has been performed; but even in these studies, since determination of decreased E2 levels required multiple samplings, the time needed for E2 administration period and compensation of its loss in these patients was missed and subsequent evaluation of compensatory effects of E2 on outcome of IVF cycles was inaccurate [5].

Finally, it should be considered that although in this study the administration of E2 during the mid-luteal phase showed no significant effects in improvement of embryo implantation and pregnancy rate outcome during IVF cycles, further frequent and accurate studies are required in this field.

Conclusion

In conclusion, E2 supplementation during the luteal phase in women with unexplained infertility undergoing IVF, is associated with decreased serum levels of E2 after hCG injection. However the fertility outcome was not affected by E2 supplementation.

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