Luteal transdermal estrogen supplementation in patients with polycystic ovarian syndrome undergoing in vitro fertilization treatment: A prospective randomized controlled study

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Abstract: The aim of this study is to investigate the effectiveness of transdermal E2 supplementation in the luteal phase of the patients with polycystic ovarian syndrome (PCOS) undergoing IVF treatment. The present study was a prospective, single-centre, randomized, controlled, group-comparative clinical trial assessing E2 supplementation during luteal phase in PCOS women undergoing ICSI-ET. Patients were randomized into the study group (n=45) received E2 supplementation in the form of transdermal patches (100 μg/day) twice per week starting the following night after oocyte retrieval. Patients were randomized to the control group (n=46) received no E2 supplementation. Luteal phase support was in the form of 50 mg IM progesterone. There were no significant differences in the implantation (21.5% vs. 30%), clinical pregnancy (19/45 [42.2%] vs. 24/46 [52.1%]; p=0.43), and ongoing pregnancy rates (16/45 [35.5%] vs. 21/46 [45.6%]; p=0.29) between the study and control groups, respectively. Early pregnancy loss and multiple pregnancy rates were (3/19 [15.7%] vs. 3/24 [12.5%]; p=0.82) (4/19 [21%] vs. 5/24 [20.8%]; p=0.39) in study and control groups, respectively. The addition of transdermal E2 supplementation to the luteal phase P support of IVF cycles does not improve cycle outcomes in regard to implantation and pregnancy rates in PCOS patients.

Key Words: Luteal phase support, Estradiol supplementation, Polycystic ovarian syndrome, Pregnancy rate.

The success of IVF treatments is still something to be improved and it depends on several factors including oocyte quality and endometrial receptivity [1]. The technology of IVF up to embryo transfer is very effective. The last stage, implantation phase is still a problem, and pregnancy rates are largely unpredictable. Uterine receptivity depends on the hormonal changes of the endometrium at the time of implantation. The role of progesterone supplementation in the luteal phase of down-regulated cycles is well established. Standard doses and routes of administration are routinely used worldwide [2]. However, there are conflicting reports regarding the the value of estradiol (E2) supplementation in the luteal phase on pregnancy rates, and still under evaluation.

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility with a prevalence rate of approximately 20% in the infertile population [3,4]. It is a well known fact that PCOS patients have oligo-/anovulation. The ovulation induction in PCOS patients is a still controversial issue. The clomiphene citrate is the first agent in ovulation induction of PCOS patients [5]. However, ovulation or pregnancy may not be obtained in 20-30% patients [6]. Those who do not conceive are candidate for gonadotrophin treatment or assisted reproductive techniques. There has been a long and heated debate over the outcome of assisted reproduction in women with PCOS. High estradiol concentration and multifollicular development, so com-
monly associated with ovarian stimulation in these patients, have been regarded as detrimental factors affecting implantation and pregnancy rates. Therefore, PCOS patients undergoing assisted reproductive techniques represent a great challenge for the fertility specialist [7]. Luteal phase support in these patients is another controversial issue. In the literature, there are limited data on luteal phase support with P and E2 in PCOS patients undergoing ICSI procedure. In this prospective study, we aimed to evaluate the efficacy of E2 supplementation during luteal phase in PCOS patients treated by means of long stimulation protocol IVF cycles.

**Materials and Methods**

The present study was a prospective, single-centre, randomized, controlled, group-comparative clinical trial assessing E2 supplementation during luteal phase in PCOS women undergoing ICSI-ET. A total of 108 consecutive women who underwent ICSI-ET cycles at the Assisted Reproductive Unit of Meram Medical Faculty from February 2008 to April 2009 were included in this study. Randomized assignment of the two procedures was performed by a computer-based program. The sequence of allocation to the two groups was not concealed and the study was not blind. Patients could participate in the study only once. The study was approved by the Ethics Committee of Meram Medical Faculty and informed consent was obtained from all patients before entry into the study.

Patients were enrolled into the study if they fulfilled the following criteria: diagnosed as PCOS according to Rotterdam Criteria (K), age 20-35 years at the time of screening, early serum follicular phase serum FSH concentration of < 12 IU/L, undergoing their first cycle of ICSI-ET, body-mass index (BMI) 25-35 kg/m2, and having a peak serum E2 level on hCG administration day > 2000 pg/mL. Women thought to be at risk for the development of ovarian hyperstimulation syndrome and having an underlying medical disorder such as diabetes mellitus and hypothyroidism were excluded from the study.

All the women in the study underwent GnRH agonist suppression with 1 mg/day Leuprolide acetate (Lucrin, Abbot, France) started on the 21st day prior to menstruation for pituitary desensitization. All patients used recombinant FSH (r-FSH, Gonaf P, Serono, Italy) alone or in combination with purified urinary FSH (Menogenon, Ferring, Sweden) for ovarian stimulation. The standard daily initial dose of gonadotrophins was 75-100 IU depending on the age, BMI and basal serum FSH levels on the second day of menstruation for 6 days. The dose of gonadotrophins was adjusted individually according to the response of the ovaries and estradiol concentrations. When the leading follicle reached 18 mm in diameter or at least two follicles were >17mm in diameter, a total of 10,000 units of HCG (Pregnyl, Organon, Turkey) were administered intramuscularly. Oocyte retrieval was performed 35-37 h later. Embryos were cultured for 2-3 days and were transferred transvaginally on day 2-3.

All patients received 50 mg of P in oil daily IM for luteal support starting the evening after oocyte retrieval and continued until negative pregnancy test or positive fetal heart beat was documented by transvaginal ultrasonography. In addition, patients randomized to the study (group 1) were administered 100 microgram/day of transdermal E2 patches (Estraderm TTS 100, Novartis, Basel, Sweden). Treatment was started from the day of oocyte retrieval and continued until 12th day of ET. Control group (group 2) received no E2 supplementation during the luteal phase.

Main outcome measure was the clinical pregnancy rate. Secondary measures of interest were implantation rate, ongoing pregnancy rate, and miscarriage rate. Clinical pregnancy was defined as a positive serum β-hCG test result with ultrasonography with a gestational sac and fetal heart. The implantation rate was defined as the number of gestational sacs containing fetal hearts on ultrasonography, divided by the number of embryos transferred. Ongoing pregnancy was defined as pregnancy progressing beyond 12 weeks' of gestation. Early pregnancy loss was defined as miscarriage after ultrasound evidence of a gestational sac with or without a fetal pole.

Considering that reaching enough sample size for an adequately powered investigation is not feasible for a single-center study in a special group of patients (PCOS). As reported in a previous study
[8], we arbitrarily chose a set of patients to provide data that clinically useful, and that could be incorporated at a later stage into a further analysis.

Values were expressed as mean±SD for continuous variables and number and percentage for categorical variables. The $\chi^2$ test and Student's t test were used to compare categorical data and the nonparametric Mann-Whitney U test to check for differences between numeric variables between the 2 groups. All P values were 2-tailed and P<0.05 was considered statistically significant.

**Results**

A total of 108 patients were eligible for recruitment, but 4 refused to participate and 5 patients did not fulfill criteria for randomization, did not undergo IVF cycle. Ninety-nine patients were therefore randomized on the day of oocyte retrieval into the study group (n=49) and the control group (n=50). In the study group, 45 patients received full allocation, but two patients did not continue the treatment because of failure in fertilization and embryo development, two patients were started vaginal estrace by their primary physicians. In the control group, two patients discontinued due to failed fertilization and two patients due to failed embryo development; therefore 46 patients completed the full allocated intervention.

Baseline characteristics and outcome of ovarian stimulation are shown in Table 1. There were no differences between two groups in the mean age, duration of infertility, body mass index, or baseline serum FSH levels. The groups were similar in terms of duration of ovarian stimulation and the total dose of gonadotrophins required for ovarian stimulation. There were no differences between the study and control group in the number of oocytes retrieved, proportion of mature oocytes (MII) retrieved, fertilization rates, number of embryos transferred. The serum E2 and progesteron concentrations 9 days after oocyte retrieval were similar between the two groups (Table 1).

The outcome of the treatment cycle is displayed in Table 2. There was a trend toward a lower clinical pregnancy rate in the study group compared with control group (19/45 [42.2%] vs. 24/46 [52.1%]; p=0.43), although the difference was not statistically significant. There was also no difference in the ongoing pregnancy rate between the two groups (16/45 [35.5%] vs. 21/46 [45.6%]; p=0.29). Similarly the early pregnancy loss was comparable between the groups, and miscarriage rates were not different between the study and the control groups (3/19 [15.7%] vs. 3/24 [12.5%]; p=0.82). Implantation rate was higher in control group (30%) than study group (21.5%), but statistical significant difference was not obtained (p=0.36).

**Table I.** Demographic characteristics of patients in the randomized trial

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Mean age/year</td>
<td>30.4 ± 4.3</td>
<td>29.2 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Infertility duration</td>
<td>8.4 ± 2.3</td>
<td>7.3 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>24.8 ± 3.8</td>
<td>25.2 ± 6.07</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline FSH (IU)</td>
<td>6.09 ± 1.8</td>
<td>6.1 ± 1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>10.3 ± 1.54</td>
<td>10.4 ± 1.38</td>
<td>NS</td>
</tr>
<tr>
<td>Total gonadotrophin doses (IU)</td>
<td>2588 ± 912</td>
<td>2507 ± 623</td>
<td>NS</td>
</tr>
<tr>
<td>No. of oocytes</td>
<td>12.6 ± 3.92</td>
<td>13 ± 2.73</td>
<td>NS</td>
</tr>
<tr>
<td>No. of MII oocytes</td>
<td>10.2 ± 2.9</td>
<td>11.3 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>2.93 ± 0.69</td>
<td>3.0</td>
<td>NS</td>
</tr>
<tr>
<td>E2 on day 7 of ET (pg/ml)</td>
<td>672 ± 558</td>
<td>512 ± 468</td>
<td>NS</td>
</tr>
<tr>
<td>P on day 7 of ET (ng/ml)</td>
<td>41.8 ± 11.4</td>
<td>44.5 ± 18.7</td>
<td>NS</td>
</tr>
</tbody>
</table>
Multiple pregnancy rate was also compared between two groups, and there was no significant difference in regard to multiple pregnancy rate (4/19 [21%] vs 5/24 [20.8%]; p=0.39).

Discussion

Presence of anovulation, the delay in conception and the high prevalence of miscarriage usually occur in PCOS patients [9]. PCOS patients undergoing assisted reproductive techniques represent a great challenge for the fertility specialist [7]. In these patients, progesterone supplementation in IVF cycles is highly recommended for achieving a successful pregnancy [10]. The addition of estrogens for luteal phase support is still a matter of debate, and different results were obtained in various studies. In the present study, we evaluated the efficacy of transdermal E2 applied after oocyte retrieval to cycle outcome in PCOS patients, and found no difference between the patients having luteal phase support with or without E2.

Table II. The outcomes of the treatment cycles

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical pregnancy</td>
<td>19/45 (42.2%)</td>
<td>24/46 (52.1%)</td>
<td>0.43</td>
</tr>
<tr>
<td>Ongoing pregnancy</td>
<td>16/45 (35.5%)</td>
<td>21/46 (45.6%)</td>
<td>0.29</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>21.3% (28/131)</td>
<td>30%</td>
<td>0.44</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>3/19 (15.7%)</td>
<td>3/24 (12.5%)</td>
<td>0.82</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>4/19 (21%)</td>
<td>5/24 (20.8%)</td>
<td>0.39</td>
</tr>
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</table>

It is known that an increment in LH level is a typical finding in PCOS patients [11]. This unsuitable elevation of LH is suspected to adversely influence follicular development and ovulation. The possibility to normalize LH levels in PCOS patients with progesterone administration has been found effective [12,13]. It has been shown that the high estrogen level during the early luteal phase of an IVF cycle induces a strong negative feedback on the pituitary, decreasing LH secretion to very low levels [14,15]. However, it was demonstrated that early luteal phase administration of high-dose estrogen did not induce premature luteolysis in regularly cycling women [16]. The role of estrogens in human implantation has been extensively investigated, and the data from oocyte donation cycles are very good clinical model to explore its role. There appears to be a transition from the non-receptive to receptive state in response to estrogen and progesteron. The molecular basis of this receptive state when endometrium is conductive to blastocyst acceptance and implantation remains poorly understood. Using embryo transfers and progesterone-delayed-implantation model in mice were able to demonstrate that concentration of estrogen within very narrow range determine the duration of window of uterine receptivity [17].

In a prospective randomized study, Smitz et al. [18] evaluated the possible benefit of adding E2 valerate to the vaginal micronized progesterone given as luteal phase support in women treated with GnRH agonist and hMG for IVF. They showed no difference in clinical pregnancy rate between E2 co-treatment and and progesterone-only treatment. Fatemi and colleagues [19] evaluated the effects of oral micronized E2 supplementation in patients using a protocol of GnRH antagonist and showed no differences in pregnancy rates. A recent study by Engmann et al [1] determined no significant differences in the implantation, clinical pregnancy and ongoing pregnancy rate in the patients with and without receiving luteal phase vaginal E2 supplementation. These findings are in agreement with the findings of the present study, which also showed no significant differences in clinical pregnancy rate in our study population. Differently, our study groups were all PCOS women, and they received a protocol of long GnRH agonist and luteal phase support with transdermal E2.

Some studies have also indicated that such a beneficial effect of luteal phase E2 supplementation may depend on the protocol used for IVF. Farhi et al [20], in a prospective, randomized study, evaluated the effect of adding E2 to progestin supplementation during luteal phase on the day after oocyte retrieval in patients undergoing IVF. They showed that for those patients who had
been treated with long GnRH agonist protocol, the addition of E2 to the progesterin support regimen had a beneficial effect on pregnancy and implantation rates. However, in this study any beneficial effect of luteal phase E2 supplementation was not shown after the use of short GnRH agonist protocol [20]. In a previous study from our clinic, Görkemli et al. [21] compared the pregnancy outcomes of progesterone or progesterone + E2 for luteal phase support, and they showed improved pregnancy rates with E2 supplementation after using long GnRH agonist protocol. In the present study, we administered a protocol of long GnRH agonist and did not also find any difference between PCOS patients with and without receiving additional E2 supplementation to progesteron.

One possible reason for the differences in the effects of E2 supplementation reported in previous studies may depend on the use of oral micronized E2 support that have variable effect on the endometrium because of the rapid hepatic inactivation to estrone and other metabolites [22]. Alternate routes of administration, such as vaginal or transdermal E2, have been described to avoid first pass effect through the liver and to facilitate treatment compliance. We also used transdermal E2 to avoid hepatic metabolism, which eliminates the majority the oral dose from the circulation [23]. This approach has been investigated by a recent report showing no beneficial effect of transdermal E2 supplementation during the luteal phase on implantation and pregnancy rates [8]. A vaginal route seems to more physiologic because of first uterine pass where E2 is absorbed from the vaginal mucosa directly into the endometrium [24]. Engermann et al. [1] administered vaginal E2 as luteal phase support, but they found no difference in implantation, clinical pregnancy and ongoing pregnancy rates between the groups.

High estrogen concentration associated with ovarian stimulation particularly in PCOS patients may be detrimental to embryonic implantation. Valbuena et al. [25] showed that supraphysiological levels of E2 potentially had an adverse effect on endometrial maturation and implantation at the cleavage stage. In the present study, there was a trend toward a lower clinical pregnancy rate in patients receiving E2 + progesterone when compared with only progesterone, despite no statistical significant difference. It may be partly due to the deleterious effect of E2 supplementation to embryo adhesion, because we administered E2 support after oocyte retrieval.

In conclusion, the findings of the present study do not suggest the additional usage of luteal phase E2 supplementation to progesteron via the transdermal route during IVF cycles of PCOS patients. Further studies are needed to investigate the adverse effects of transdermal E2 support in PCOS women after using long GnRH agonist protocol.

References