Estradiol supplementation during the luteal phase in poor responder patients undergoing in vitro fertilization: a randomized clinical trial

Marzieh Aghahosseini · Ashraf Aleyassin · Sepideh Khodaverdi · Fatemeh Esfahani · Robabeh Mohammadbeigi · Shohreh Movahedi · Ali Kord Valeshabad · Atossa Mahdavi · Parvin Fallahi · Parisa Shabani · Zahra Rezaeian · Maryam Khodaverdi

Received: 29 April 2011 / Accepted: 31 May 2011 / Published online: 1 July 2011
© Springer Science+Business Media, LLC 2011

Abstract

Purpose This study was designed to evaluate the effects of adding Estradiol (E2) supplementation to progesterone (P) on improvement of pregnancy outcomes in poor responder patients who underwent in vitro fertilization (IVF).

Capsule For poor responder women who underwent IVF, addition of E2 to P supplementation could not significantly improve pregnancy outcomes. Treatment outcomes were not significantly different between two groups.

M. Aghahosseini · A. Aleyassin · S. Khodaverdi (✉) · S. Movahedi · A. Mahdavi · P. Fallahi · P. Shabani · Z. Rezaeian
Department of Infertility,
Zanjan University of Medical Sciences,
North Karegar Street,
Zanjan 1411713135, Iran
e-mail: sepidehkhodaverdi@yahoo.com

F. Esfahani
Research Development Center, Shariati Hospital,
Tehran University of Medical Sciences,
Tehran, Iran

A. Kord Valeshabad
Students' Scientific Research Center (SSRC),
Tehran University of Medical Sciences (TUMS),
North Karegar Street,
Tehran 1439957181, Iran

R. Mohammadbeigi
Kurdistan University of Medical Sciences,
Sanandaj, Iran

M. Khodaverdi
Research Development Center,
Zanjan University of Medical Sciences,
Zanjan, Iran

A. Kord Valeshabad
Farzan Clinical Research Institute,
Tehran, Iran

Methods In a prospective randomized clinical trial, 118 poor responder patients, older than 38 years without contraindications of estradiol consumption from Infertility clinic of a university hospital were randomly divided (by computerized software) into two groups. Control group (59 patients) received only P and intervention group (59 patients) received P and E2 (4 mg/d). Supplementation was done with 4 mg E2 in the luteal phase. Fertilization rate, implantation rate, biochemical and clinical pregnancy rates, abortion rate, ongoing pregnancy, multiple pregnancy and ectopic pregnancy rates were documented for those who completed the study protocol in each group (per protocol analysis) and compared between groups.

Result Fifty five patients in control group and 53 patients in intervention group successfully completed the study protocol. Treatment outcomes were not significantly different between two groups.

Conclusion For poor responder women who underwent IVF, addition of E2 to P supplementation could not significantly improve pregnancy outcomes.

Keywords E₂ supplementation · Luteal phase support · Implantation rate · Poor responder patients · IVF

Introduction

In recent decades, rising the age of marriage and subsequent increased probability of infertility have been become a large concern for health services in various nations. Nowadays, assisted reproductive technology (ART) is so popular in treatment of infertility, but the main problem in these methods is treatment of poor responder patients [1]. The rates of poor ovarian responses to the stimulation have

been reported from 9% to 24% [2, 3] leading to a low pregnancy rate in these patients (about 2%–4%) [4–7].

There are a variety of definitions for poor ovarian response in different studies and this makes it difficult to reach a unique assessment and to compare proposed treatments for these patients. Factors such as mature follicular number less than 3–5 in sonography imaging, increasing serum follicle stimulating hormone (FSH) level to at least 15 mmol/ml in the early follicular phase, number of picked-up follicles from the ovary, optimum Estradiol (E2) level toward previous cycle or the least cumulative dosage or required gonadotropins stimulation days in the previous cycle have been used for the definition of poor response to the stimulation [1, 8].

Several treatment protocols have been presented for improvement of ART outcomes in poor responders, but they are still in accompanying with high treatment failure rates [9–11]. The role of progesterone (P) supplementation in the luteal phase of in vitro fertilization (IVF) cycles is well

established worldwide [12], but the influence of the luteal phase E2 level on implantation is not clearly defined [13–15]. First Stewart et al. revealed a significant difference in E2 levels between fertile and infertile cycles of fertile women who underwent donor insemination [16]. There are some other studies which believe that decline in the E2 levels in the human luteal phase does not appear to adversely affect the developmental capacity of the endometrium and pregnancy rate [1, 17].

The low rate of implantation is the main issue of ART cycles in poor responders [18]. Besides progesterone, estrogen and other steroid hormones are made by luteal body. This produced this question for researchers whether adding E2 to P supplementation could improve implantation rate or not [19]. Furthermore, most of the previous studies which reached positive effects of luteal phase E2 administration were designed for patients with a good ovarian response [7, 18, 20]. This controlled randomized clinical trial is designed to

CONSORT Flow Diagram

Fig. 1 Participant CONSORT flow diagram

Table 1 Inclusion and exclusion criteria of the study

Inclusion criteria	Exclusion criteria
Age ≥ 38 years	Age >38 with good ovarian response
FSH ≥ 12 mIU/ml in the early follicular phase	History of DVT with OCP
Number of oocytes at the pickup time 5 or less	Submucosal myoma
Estradiol level more than 70 pg/dl in the early follicular phase	Severe male factor infertility
	Extended endometriosis (stage 3 or more)
	All contraindications for estradiol

FSH follicle stimulating hormone, DVT deep venous thrombosis, OCP Oral contraceptives pills

investigate the effects of adding Estradiol supplementation to progesterone on improvement of pregnancy outcomes in poor responder patients who underwent IVF.

Materials and methods

Study design, inclusion and exclusion criteria and randomization

In this randomized clinical trial between March 2008 and October 2009, all consecutive patients who presented for the purpose of ART to the infertility ward of Shariati hospital, Tehran, Iran, were enrolled the study. Among these patients, those who fitted inclusion criteria were selected for the treatment protocol and those with any of exclusion criteria

Table 2 Demographic characteristics and clinical data of intervention and control groups

Variables	Intervention group (n=53)	Control group (n=55)	P value
Age (year)	36.0 $\pm$ 5.4	34.9 $\pm$ 6.3	0.303
Body mass index	27.8 $\pm$ 4.4	26.2 $\pm$ 4.2	0.059
Marriage duration (year)	11.5 $\pm$ 7.3	9.4 $\pm$ 5.9	0.096
Infertility duration (year)	9.7 $\pm$ 7.3	8.4 $\pm$ 5.9	0.334
Primary infertility (%)	88.7	83.6	0.449
Causes of infertility (%)			
Male factor (%)	37.7	20.0	0.046
Female factor (%)	37.7	54.5	
Both (%)	24.6	20.0	
Not identified (%)	0.0	5.5	
History of ART (%)	43.4	43.6	0.980
History of week response (%)	58.5	60.0	0.873

Data was shown as Mean $\pm$ standard deviation or percentage

were taken apart (Fig. 1). The study protocol was approved by ethics committee of Tehran University of Medical Sciences and each patient gave informed consent before enrollment in the study. Those without an informed consent were excluded from the study. Table 1 shows inclusion and exclusion criteria of the study. These selected patients were divided randomly into two groups (intervention or control) using computerized software in a 1:1 fashion. The sequence

Fig. 2 Outcomes of the treatments in each stage of the protocol for each group (Intervention and control)

of allocation to the two groups was not concealed and thus it was possible for the treating physicians ($n=2$) to be aware of the next treatment to be allocated.

Sample size calculation

The primary outcome measure was implantation rate. In our situation, in average, the implantation rate is about 8% in poor responder, and the mean embryo transfer per subject is 2. Our hypothesis was that E2 would increase the implantation rate to 20%. Assuming significant level of 0.05 and power of 0.8, 130 embryo transfers (or 65 patients) in each group, are required to detect the expected difference.

Ovarian stimulation and Estradiol supplementation

Based on the study protocol and inclusion criteria, 59 patients (age 38–45 years) were considered as intervention group and 59 others (age 38–46 years) were classified as control group and were assigned to received related protocol. Demographic characteristics and clinical data of each group are shown in Table 2.

Infertile patients with ART indications, received GnRH-a (Super fact, Hoechst AG, Germany) 500 mg/sc/d since day 21 of their previous menstrual cycles. In the day 3 of the cycle based on the age, FSH level and response rate in the previous cycle (if existed), Gonal-f (Serono, Switzerland) and HMG (Menogan, Ferring, Germany) were started as a long protocol. Sonography imaging was performed 7 days after stimulation and when there were at least two follicles with the size of

>17 mm, HCG 10000 Iu/Im (Organon, pregng 5000) was administrated. Oocyte pickup (OPU) was performed 35–36 h later. Oocytes in metaphase II underwent microinjection and 4–8 cells embryos were transferred 2–3 days after OPU.

Patients who had 5 oocytes or less, Estradiol level ≥ 70 pg/dl, age ≥ 38 years or FSH ≥ 12 mIU/ml during OPU were considered as poor responder. Control group received Cyclogest 400 mg (2 suppository daily). For the intervention group, besides Cyclogest with the same treatment program, estradiol 4 mg/d (Aburaihan pharmaceutical, Iran) was added. Two weeks later, β HCG test was performed and when the test result was positive, sonography imaging applied for confirmation. Clinical pregnancy was approved when fetal heart rate (FHR) was present in the imaging and in these cases luteal phase support was continued up to week 12 of the pregnancy. For those without confirmed clinical pregnancy by imaging, luteal phase support was discontinued.

The primary outcome of the study was implantation rate. Secondary outcomes were rates of fertilization, biochemical pregnancy rate per transfer, clinical pregnancy, spontaneous abortion, ongoing pregnancy, multiple pregnancy and ectopic pregnancy.

Statistical analysis

Fifty nine patients in each group were initially received the related treatment, but only those who completed the protocol were considered for the final analysis (per protocol analysis). Results were reported as mean $\pm$ standard deviation (SD) or median (25% quartile–75% quartile) for quantitative variables

Table 3 Laboratory results and final outcomes of the treatment in intervention ($n=53$) and control ($n=55$) groups

Variables	Intervention group	Control group	<i>P</i> value
FSH (mIU/ml)	13 (5.1–17.8)	12.1 (4.8–15.9)	0.436
Estradiol (pg/ml)	73.0 (34.5–110)	66.3 (37.2–84.3)	0.230
Picked up follicles	4 (3–5)	5 (3–5)	0.385
Picked up oocytes	3 (2–4)	3 (2–4)	0.360
Metaphase II	2 (1–3)	2 (2–3)	0.466
Injected ovules	2 (1–3)	3 (2–3)	0.230
Embryo grade (%)			
I	21.2	18.8	0.027
II	66.0	81.2	
III	12.8	0.0	
Embryos	2 (1–2)	2 (1–3)	0.253
Transferred Embryos per cycle	2 (1–2)	2 (1–3)	0.212
Cycle cancelation (%)	15.1	12.7	0.722
Fertilization rate	79.2	82.0	0.689
Implantation rate	9.5	8.2	1.00
Chemical pregnancy (%)	22.6	20.0	0.737
Clinical pregnancy (%)	13.2	10.9	0.714
Ongoing pregnancy (%)	7.5	10.9	0.742
Miscarriage (%)	13.2	7.3	0.308

When it is not mentioned in the table data are shown as median (25% quartile–75% quartile)

and percentages for categorical variables. The groups were compared using the Student's *t*-test for continuous variables and the chi-square test (or Fisher's exact test if required) for categorical variables. *P* values of 0.05 or less were considered statistically significant. All the statistical analyses were performed using SPSS version 13 (SPSS Inc, Chicago, IL, USA) for Windows.

Results

Treatment outcomes were not significantly different between two groups. Figure 2 shows the outcomes of the treatments in each stage of the protocol for each group. Laboratory results and final quantitative and qualitative outcomes of the treatment in each group are summarized in Table 3. All intervention and control patients were followed for 4 months since reaching the outcomes of the study. No complication was detected during treatment and follow up periods among the patients.

Discussion

Estrogen administration in follicular phase can improve endometrium preparation, but its role in the luteal phase is still under doubt [21–23]. In unsuccessful cycles, the late luteal E2 levels decline which may compromise peri-implantation endometrial developments [24]. Vlahose et al. found that the addition of Estrogen to P in the luteal phase can increase L-selectin ligands –adhesion molecule in the endometrium during implantation [25]. All above data raise the speculation about a potential positive correlation between luteal phase E2 levels and pregnancy outcomes.

In this study, treatment outcomes-except embryo grad-were not significantly different between two groups. In the same protocol to our study, Engmann et al. revealed that addition of vaginal E2 supplementation (4 mg/d) to the routine P supplementation as luteal support does not improve pregnancy rates in IVF treatment [11]. In a systematic review article in 2008, Gelbaya et al. reached no beneficial effects for IVF/ICSI patients who received E2 besides P [26]. Other studies confirmed the same results [9, 27, 28]. In contrary, there are a variety of evidences which support the effectiveness of E2 supplementation in order to improve number of successful pregnancies [29–31].

The main reason of this variety in the results is still under question. Some studies demonstrated that adding E2 supplementation in luteal phase could not significantly affect patients' hormone profile [10], histological pattern of the endometrium [1] or estrogen receptors [3]. It seems that the optimal E2 level is not the same in different patients. In a well-conducted randomized trial, E2 supplementation was

added in different doses (0, 2 or 6 mg/d) to P regimen (6 mg/d). Significantly higher implantation and pregnancy rates were recorded in those who received low-dose E2 supplementation compared with who did not [32]. Various studies used different routes for E2 administration (orally, transdermal or vaginal) and there is no confirmed consensus about optimal E2 dosage and its duration.

Furthermore, most of the studies who reached positive effects of luteal phase E2 administration were designed for patients with a good ovarian response [2, 5, 17]. In poor responder patients, because of damages to the granulosa cells and few numbers of follicles, higher doses of E2 may be required for reaching the same results. These make it more ambiguous to judge about the best E2 supplementation regimen. Although the necessity of luteal phase E2 supplementation in all IVF patients is arguable, in some selected patients it may increase clinical pregnancy rates [33].

Study limitations

In this study we missed 38/146 patients (26.02%) among enrolled ones because of not fitting the inclusion or exclusion criteria and ten patients were loosed during follow up due to immigration and incomppliance and these might affect the study outcomes. Only those who complete the protocol were considered for the final analysis (per protocol analysis), however practically intention-to-treat analysis is recommended. In order to obtain a clearer view, further large, multicenter RCTs are required to clarify the optimal E2 supplementation regimen.

Conclusion

In summary, our data demonstrated that adding E2 supplementation to P in poor responder patients who underwent IVF could not significantly improve pregnancy outcomes.

Acknowledgment The authors wish to express their sincere gratitude to Tehran University of Medical Sciences for financial support of this study. Also we would like to appreciate assistance of Dr Shohreh Movahedi and Sahar Abroshan in the study.

Conflicts of interest Authors report no conflicts of interests.

Financial support None.

References

1. Surry ES, Schoolcraft WB. Evaluating strategies for improving ovarian response of the poor responder undergoing assisted reproductive technique. *Fertil Steril*. 2000;73:667–76.

2. Jenkis JM, Davies DW, Devonport H, Anthony FW, Gadd SC, Watson RH, et al. Comparison of “poor” responders with “good” responders using a standard buserelin/human menopausal gonadotropin regime for in vitro fertilization. *Hum Reprod*. 1991;6:918–21.
3. Ben-Rafael Z, Bider D, Dan U, Zolti M, Levran D, Mashiach S. Combined gonadotropin releasing hormone agonist/human menopausal gonadotropin therapy (GnRH-a/hMG) in normal, high, and poor responders to hMG. *J In Vitro Fertil Embryo Transf*. 1991;8:33–6.
4. Schoolcraft W, Schlenker T, Gee M, Stevens J, Wagley L. Improved controlled hyperstimulation in poor responder in vitro fertilization patients with a microdose follicle-stimulating hormone flare, growth hormone protocol. *Fertil Steril*. 1997;67:93–7.
5. Ulug U, Ben-Shlomo I, Turan E, Erden HF, Akman MA, Bahceci M. Conception rates following assisted reproductive in poor responder patients: a retrospective study in 300 consecutive cycles. *Reprod Biomed Online*. 2003;6:439–43.
6. Mohamed KA, Davies WA, Allsopp J, Lashen H. Agonist “flare-up” versus antagonist in the management of poor responders undergoing in vitro fertilization treatment. *Fertil Steril*. 2005;83:331–5.
7. Schmidt DW, Bremner T, Orris JJ, Maier DB, Benadiva CA, Nulsen JC. A randomized prospective study of microdose leuprolide versus ganirelix in in vitro fertilization cycles for poor responders. *Fertil Steril*. 2005;83:1568–71.
8. Hellberg D, Waldenstrom U, Nilsson S. Definition a poor responder in in vitro fertilization. *Fertil Steril*. 2004;82:488–90.
9. Serna J, Cholquevilque JL, Villasante A, Oriol B, Requena A, García-Velasco J. Adding estradiol patches to the luteal phase of IVI/ICSI cycles did not improve pregnancy nor miscarriage rates. *Fertil Steril*. 2006;86(Supplement 3):S73.
10. Fatemi HM, Camus M, Kolibianakis EM, Tournaye H, Papanikolaou EG, Donoso P, et al. The luteal phase of recombinant follicle—stimulating hormone/gonadotropin-releasing hormone antagonist in vitro fertilization cycles during supplementation with progesterone or progesterone and estradiol. *Fertil Steril*. 2007;87:504–8.
11. Engmann L, DiLuigi A, Schmidt D, Benadiva C, Maier D, Nulsen J. The effect of luteal phase vaginal estradiol supplementation on the success of in vitro fertilization treatment: a prospective randomized study. *Fertil Steril*. 2008;89:554–61.
12. Tarek AG, Kyrgiou M, Tsoumpou L, Nardo LG. The use of estradiol for luteal phase support in in vitro fertilization/ intracytoplasmic sperm injection cycles: a systematic review and meta-analysis. *Fertil Steril*. 2008;90:2116–25.
13. Finn CA. The implantation reaction. In: Wynn RM, editor. *Biology of the uterus*. New York: Plenum; 1977. p. 245–308.
14. Younis JS, Ezra Y, Sherman Y, Simon A, Schenker JG, Laufer N. The effect of estradiol depletion during the luteal phase on endometrium development. *Fertil Steril*. 1994;62:103–7.
15. Edgar DH. Estrogen and human implantation. *Hum Reprod*. 1995;10:2–4.
16. Steward DR, Overstreet JW, Nakjama ST, Lasley BL. Enhanced ovarian steroid secretion before implantation in early human pregnancy. *J Clin Endocrinol Metab*. 1993;76:1470–6.
17. Hung Yu Ng E, Shu Biu Yeung W, Yee Lan Lau E, Wai Ki So W, Chung Ho P. A rapid decline in serum estradiol concentrations around the mid-luteal phase had no adverse effect on outcome in 763 assisted reproduction cycles. *Hum Reprod*. 2000;15:1903–8.
18. Dragisic KG, Davis OK, Fasoulitis SJ, Rosenwaks Z. Use of luteal estradiol patch and gonadotropin—releasing hormone antagonist suppression protocol before gonadotropin stimulation for in vitro fertilization in poor responder. *Fertil Steril*. 2005;84:1023–6.
19. Kyrrou D, Kolibianakis EM, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. *Fertil Steril*. 2009;91:749–66.
20. Tarlatzis BC, Zepiridis L, Grimbasis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: a systematic review. *Hum Reprod Updat*. 2003;9:61–76.
21. Morris JM, Van Wagenen G, McCann T, Jacob D. Compounds interfering with ovum implantation and development: II. Synthetic estrogens and antiestrogens. *Fertil Steril*. 1967;18:18–34.
22. Bill CH, Keyes PL. 17 beta-estradiol maintains normal function of corpora lutea throughout pseudopregnancy in hypophysectomized rabbits. *Biol Reprod*. 1983;28:608–17.
23. Shetty G, Krishnamurthy H, Krishnamurthy HN, Bhatnagar AS, Moudgal RN. Effect of estrogen deprivation on the reproductive physiology of male and female primates. *J Steroid Biochem Mol Biol*. 1997;61:157–66.
24. Smits J, Devroey P, Camus M, Deschacht J, Khan I, Staessen C, et al. The luteal phase and early pregnancy after combined GnRH agonist/HMG treatment for superovulation in IVF or GIFT. *Hum Reprod*. 1988;3:585–90.
25. Vlahos NF, Lipari CW, Bankowski B, Lai TH, King JA, Shih IeM, et al. Effect of luteal-phase support on endometrial L-selectin ligand expression after recombinant follicle-stimulating hormone and ganirelix acetate for in vitro fertilization. *J Clin Endocrinol Metab*. 2006;91:4043–9.
26. Gelbaya TA, Kyrgiou M, Tsoumpou I, Nardo LG. The use of estradiol for luteal phase support in in vitro fertilization/ intracytoplasmic sperm injection cycles: a systematic review and meta-analysis. *Fertil Steril*. 2008;90:2116–25.
27. Dunham S, Krey LC, Noyes N. Luteal serum estradiol levels during in vitro fertilization-embryo transfer (IVF-ET) cycles do not suggest a role for estradiol supplementation. *Fertil Steril*. 2004;82:s258–9.
28. Karlikaya G, Karagozoglu H, Kumbak B, Lacin S, Guney A, Kahraman S. Adding estradiol to progesterone for luteal phase support does not improve the clinical outcome in assisted reproductive technologies (ART) cycles. *Fertil Steril*. 2006;86:s112.
29. Atmaca S, Erdem M, Erdem A, Guler I. The impact of luteal phase support on pregnancy rates in intrauterine insemination cycles; a prospective randomized study. *Fertil Steril*. 2007;88:s163.
30. Frattarelli JL, Hill MJ, McWilliams GD, Miller KA, Bergh PA, Scott Jr RT. A luteal estradiol protocol for expected poor-responders improves embryo number and quality. *Fertil Steril*. 2008;82:s118–22.
31. Hill MJ, McWilliams GD, Miller KA, Scott Jr RT, Frattarelli JL. A luteal estradiol protocol for anticipated poor-responder patients may improve delivery rates. *Fertil Steril*. 2009;91:739–43.
32. Lukaszuk K, Liss J, Lukaszuk M, Maj B. Optimization of estradiol supplementation during the luteal phase improves the pregnancy rate in women undergoing in vitro fertilization-embryo transfer cycles. *Fertil Steril*. 2005;83:1372–6.
33. Leondires MP, Ezcurra D, Chi L, Mangieri R, Moore M, Richlin S. Estradiol supplementation during the luteal phase in patients undergoing controlled ovarian stimulation for IVF improves clinical pregnancy rates. *Fertil Steril*. 2006;86(Supplement 3):S346.