Effect of estrogen priming through luteal phase and stimulation phase in poor responders in in-vitro fertilization

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Received: 19 August 2011 / Accepted: 20 November 2011 / Published online: 8 December 2011
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Abstract

Purpose To verify whether a novel protocol administering E₂ during the luteal phase of the preceding cycle and during ovarian stimulation in GnRH antagonist cycle could enhance follicular response and hence improve outcomes in poor responders.

Methods In this retrospective analysis, a total of 155 poor responder patients subjected to IVF/ICSI were analyzed. All the patients had history of more than one prior IVF cycle failure with poor response (less than 5 oocytes retrieved and/or maximal E₂ level less than 500 pg/mL) by using conventional long agonist or antagonist protocol. In luteal E₂ treatment protocol ($n=86$), oral estradiol valerate 4 mg/day was initiated on luteal day 21 and either stopped at menstrual cycle day 3 (Protocol A, $n=28$) or continued during the period of ovarian stimulation until the day of hCG injection (Protocol B, $n=58$). IVF parameters and pregnancy outcome of luteal E₂ treatments group were compared with a standard GnRH antagonist protocol ($n=69$) which the patients received no hormonal pretreatment.

Results Compared to standard GnRH antagonist protocol, cancellation rate was lower with luteal E₂ group (15.1% vs

37.7%, $p<0.01$). Moreover, patients treated with luteal estrogen resulted in an increased number of oocytes retrieved (4.5 ± 2.9 vs 3.2 ± 1.9 ; $p<0.01$). A trend toward increase in number of normally fertilized embryos (2.9 ± 2.1 vs 2.3 ± 1.9 ; $p=0.043$), and increased prevalence of good quality embryos (51.2% vs 25%; $p=0.047$) were noted. Comparing protocol A and B, there were no significant difference between embryologic data, however there were slight increase in ongoing pregnancy rate in protocol B compared to A (27.1% vs 20%, $p=0.357$), although statistical significance was not achieved.

Conclusion Estrogen priming through luteal phase and stimulation phase improved ovarian responsiveness and this may lead to an increase in pregnancy rate in poor responders with failed cycle.

Keywords Luteal estradiol supplementation · Poor responder · In vitro fertilization · Embryo morphology

Introduction

The incidence of poor response to ovarian hyperstimulation during *in vitro* fertilization (IVF) has been shown to vary from 9 to 24% [1]. Various factors, including aging and previous ovarian surgery, which mainly reflect early depletion of ovarian reserves, have been associated with a poor response; however, mechanisms other than a deficiency in ovarian function can be involved in poor responses, such as alterations in intraovarian factors or gonadotropin receptor regulation [2]. A poor response to ovulation stimulation results in high cancellation rates of up to 76% and extremely low pregnancy rates from 3.2–14% [3–8]. Various strategies

Capsule Estrogen priming through luteal phase and stimulation phase improved ovarian responsiveness and this may lead to an increase in pregnancy rate in poor responders.

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for poor responders, including flare regimens and agonist and traditional antagonist protocols have been attempted; however, at present, there is no definitive evidence that poor outcomes can be reversed by a specific protocol [6, 9–11].

Although not fully known, poor responses may partly result from a shortened follicular phase with limited ability to recruit a sizable cohort, or a potentially increased sensitivity to the sustained suppressive effects of the recent corpus luteum [12, 13]. Oral contraceptive pills and gonadotropin-releasing hormone (GnRH) agonist are commonly used to prevent corpus luteal function. However, these drugs can adversely affect ovarian responsiveness [14, 15]. Moreover, patients with diminished ovarian reserve appear especially susceptible to the suppressive effects of pituitary desensitizers on ovarian function, leading to low oocyte yield [16]. Thus, incorporating natural estradiol (E2) pretreatment to the GnRH antagonist cycle is gaining attention. Ovarian E2 exerts negative feedback within the reproductive axis that includes inhibition of GnRH secretion and suppression of GnRH responsiveness. Both actions can be executed and maintained even at the low physiological ranges of serum E2 levels [17]. Previous studies have shown that utilizing the natural negative feedback of the hypothalamus–pituitary–ovary axis induced by E2 pretreatment can effectively prevent inter-cycle increases in follicle-stimulating hormone (FSH), improve follicle synchronization, and eventually result in more coordinated follicular development, leading to the recovery of more mature oocytes [18, 19]. However, these studies were not designed to detect improvements in pregnancy outcomes, and there was important methodological bias in that patients were using their own preceding failed cycle as a control. Moreover, the appropriate time at which to start gonadotropin administration following luteal E2, and when to stop E2, remains undefined.

In this study, we evaluated the effect of E2 pretreatment in patients with poor response to ovarian hyperstimulation in IVF. Using a retrospective cohort analysis, we compared IVF parameters and pregnancy outcomes in patients who were pretreated with luteal E2 using a standard GnRH antagonist protocol in poor responders undergoing IVF. In addition, to establish the appropriate use of luteal E2, we administered two different luteal E2 protocols and compared their outcomes.

Materials and methods

Patients

In this retrospective cohort analysis, a total 155 patients with a history of poor response to controlled ovarian hyperstimulation (COH) from January 2009 and May 2010 were recruited. Patients included the study were <45 years old, with <5

oocytes retrieved and/or a maximal E2 level <500 pg/ml in a prior cycle or previous cycle cancellation due to poor follicular recruitment. Patients underwent ovarian stimulation with either conventional antagonist or luteal E2 protocols. All procedures were performed by one fertility specialist and ovarian stimulation protocols were chosen mainly based on the patient's agreement to proceed with a relatively novel protocol. The study was approved by our Institutional Review Board.

Study parameters, including days of stimulation, dose of gonadotropin administered, peak E2 level on the day of human chorionic gonadotropin (hCG) administration, number of oocytes retrieved, number of embryos, and number of good quality embryos were evaluated. Pregnancy outcomes, including implantation and clinical and ongoing pregnancy rate, were also analyzed. We defined embryos as good quality if they had a least seven cells on day 3, contained <10% fragmentation, and exhibited no apparent morphological abnormalities.

Stimulation regimens

In 86 patients, oral estradiol valerate (E2) (Progynova; Schering Korea, Seoul, Korea), 4 mg, was initiated on luteal day 21 and stopped at day 3 in the next menstrual cycle (Protocol A, $n=28$) or continued during the period of ovarian stimulation until the day of hCG injection (Protocol B, $n=58$). Recombinant FSH (Gonal-F; Merck–Serono, Seoul, Korea) was initiated on menstrual cycle day 3 at an initial dose of 300 IU/day. Serum FSH and E2 levels were measured on days 2–3 of the menstrual cycle before starting gonadotropin administration. Transvaginal ultrasound was performed and serum E2 and luteinizing hormone (LH) levels were monitored during stimulation. The gonadotropin dose was adjusted according to serum E2 levels and serial ultrasound monitoring. The GnRH antagonist (Cetrotide; Merck–Serono, Seoul, Korea) was administered at a dose of 250 µg/0.5 ml/day when the leading follicle reached 14–15 mm in maximum diameter. GnRH administration continued until the day of hCG injection. Transvaginal ultrasound was performed and serum E2 levels were measured to monitor follicular growth. When the leading follicle exceeded a mean diameter of 18 mm, a single 10,000 IU intramuscular injection of hCG (Pregnyl; Organon, Seoul, Korea) was administered. Transvaginal oocyte retrieval was performed 35 h after hCG administration, and embryo transfer (ET) was performed 3 days after oocyte retrieval. For luteal support, intravaginal progesterone (8% Crinone gel; Merck–Serono) was administered vaginally starting on the day of ET.

In 69 patients, ovarian stimulation was performed using a standard GnRH antagonist protocol that is routinely used in our center, with an initial dose of 300 IU/day recombinant FSH. GnRH antagonist administration, hCG injection,

Table 1 Comparison of patient characteristics for cycles using luteal estrogen (E₂) versus standard GnRH antagonist protocol

Variable	Luteal E ₂ group (n=86)	Standard GnRH antagonist group (n=69)	P value
Age in years (range)	39.9±4.7 (31–43)	39.3±4.5 (31–45)	.384
BMI (kg/m ²)	22.4±3.5	22.7±4.8	.566
Antral follicle count	3.9±1.5	4.2±1.7	.220
Basal FSH (mIU/mL)	12.5±8.2	12.5±6.1	.976
Basal E ₂ (pg/mL)	26.6±14.8	28.3±19.4	.559
Prior IVF attempts	3.4±1.9	2.5±1.2	.001
Duration of infertility (years)	3.2±2.1	3.5±2.0	.419

Values are presented as mean±SD

BMI body mass index, FSH follicle stimulating hormone, IVF In-vitro fertilization

transvaginal oocyte retrieval, ET, and luteal support were performed in an identical manner as for luteal E₂ cycle. None of the patients in the standard GnRH antagonist group received any hormonal pretreatment.

Pregnancy was diagnosed by a positive serum β-hCG test 12 days after ET. Clinical pregnancy was defined by observation of a fetal heartbeat using transvaginal ultrasonography at 5–6 weeks gestation.

Statistical analysis

All statistical analyses were performed using SPSS version 19.0 (Chicago, IL, USA). A *t* test was used to compare the mean values between two different stimulation protocols. Differences in outcome rates were analyzed using a χ^2 test or Fisher's exact test. *P*<0.01 was considered statistically significant.

Results

Population characteristics

The baseline characteristics for patients enrolled in the two different stimulation protocols are presented in Table 1. The groups were similar with respect to age, body mass index (BMI), duration of infertility, antral follicle count, and basal

FSH and E₂ levels. However, the number of previously failed cycles was significantly higher in the luteal E₂ protocol group as compared with the standard GnRH antagonist group (3.4±1.9 vs 2.5±1.2, *P*=0.001) (Table 1). There were no reported major side effects after E₂ administration and the entire protocol was well tolerated by all patients.

For patients in the luteal E₂ protocol, the mean day 3 FSH level after beginning E₂ administration was significantly lower as compared with the mean basal FSH level (menstrual cycle day 3) (5.3±2.6 vs 12.5±8.2 mIU/ml, *P*<0.0001), and the mean basal E₂ levels after E₂ administration was significantly higher than the mean basal E₂ (145.4±125.7 vs 26.6±14.8 pg/ml, *P*<0.0001), as expected.

Comparison of stimulation outcomes

During the standard GnRH antagonist protocol, treatment was cancelled in 26 cycles (37.7%) due to inadequate response, whereas treatment was cancelled in only 13 cycles (15.1%) during the luteal E₂ protocol (*P*=0.002). Intracytoplasmic sperm injection was performed in 45% versus 51% of cases included in the luteal E₂ and control groups, respectively. The total gonadotropin dose used was significantly higher (2356.3±824.8 vs 1980.9±714.9 IU; *P*=0.004) in the luteal E₂ protocol group as compared with

Table 2 Comparison of stimulation data and embryologic data for the luteal estradiol (E₂) and standard protocols

Variable	Luteal E ₂ group (n=86)	Standard GnRH antagonist group (n=69)	P value
Total dose of gonadotropin (IU)	2356.3±824.8	1980.9±714.9	.004
Total length of stimulation (day)	10.8±2.0	10.1±2.7	.045
Peak E ₂ level (pg/mL)	938.9±653.9	588.5±318.5	<.0001
Maximal endometrial thickness (mm)	10.2±3.6	9.8±2.3	.586
No. of oocytes retrieved	4.5±2.9	3.2±1.9	.001
No. of 2PN embryos	2.9±2.1	2.3±1.9	.043
Fertilization rate	65.6%	65.6%	1.00
Good quality embryo rate	51.2%	25.0%	.047
No. of embryos transferred	2.2±0.9	1.6±1.2	.014

Values are presented as mean±SD, unless otherwise stated.

2PN=2 pronucleus

Table 3 Clinical outcomes for the luteal estradiol (E₂) and standard GnRH antagonist protocols

Variable	Luteal E ₂ group (n=86)	Standard GnRH antagonist group (n=69)	P value
Cancellation rate	15.1%	37.7%	.002
Implantation rate	19.3%	8.7%	.020
Pregnancy rate per ET	37.0%	16.3%	.021
Clinical pregnancy rate per ET	30.1%	11.6%	.024
Ongoing pregnancy rate per ET	24.7%	9.3%	.051

ET embryo transfer

the standard GnRH antagonist protocol group. Mean length of stimulation was not significantly different (10.8 ± 2.0 vs 10.1 ± 2.7 days; $P=0.045$). Stimulation parameters and embryological data for the luteal E₂ group and the standard GnRH antagonist group are presented in Table 2. Significantly higher peak E₂ levels were achieved in the luteal E₂ group as compared with the standard GnRH antagonist group (938.9 ± 653.9 vs 588.5 ± 318.5 pg/ml, $P<0.001$). Moreover, a greater oocyte yield was observed in the luteal E₂ group (Table 2). There was a trend towards a better quality of transferred embryos in the luteal E₂ group, as indicated by the prevalence of good quality embryos (51.2% vs 25% ; $P=0.047$).

As summarized in Table 3, tendencies for a higher implantation rate (19.3% vs 8.7% ; $P=0.02$), clinical pregnancy rate (30.1% vs 11.6% ; $P=0.024$), and ongoing pregnancy rate (24.7% vs 9.3% ; $P=0.051$) were observed in the luteal E₂ protocol group as compared with the controls; however, due to the small number, statistical significance could not be reached.

When comparing luteal E₂ protocols A and B, there was no significant difference between stimulation outcomes. However, there was a trend towards a slight improvement in the ongoing pregnancy outcome in

protocol B as compared with protocol A (20.0% vs 27.1% ; $P=0.357$) (Table 4).

Discussion

The greatest challenge of assisted reproductive technology is the effective treatment of patients with a poor response to controlled ovarian stimulation. With adjuvant use of luteal E₂ to an antagonist protocol, we demonstrated a significantly decreased cancellation rate and increased yield of oocytes. No significant improvement in pregnancy outcome was noted with the luteal E₂ protocol. However, there was a trend towards an increase in implantation rate, clinical pregnancy rate, and ongoing pregnancy rate in the luteal E₂ protocol as compared with the standard antagonist protocol.

The concept of a luteal E₂ protocol was first suggested by Fanchin et al. based on the assumption that reducing the size, and improving the homogeneity, of early antral follicles would optimize the ovarian response and improve cycle outcomes [18, 19]. Follicular discrepancy, which results from a dissimilar sensitivity of each follicle to FSH and the gradual elevation of FSH after the demise of the corpus luteum, has been shown to be counter-reproductive, and adjuvant GnRH

Table 4 Comparison of the outcomes between protocol A and B

Variable	Protocol A (n=28)	Protocol B (n=58)	P value
Total dose of gonadotropin (IU)	2299.1±817.2	2470.5±842.9	.372
Total length of stimulation (day)	10.6±2.0	11.4±1.8	.063
Peak E ₂ level (pg/mL)	870.6±632.7	1080.5±685.9	.164
Maximal endometrial thickness (mm)	10.3±2.6	10.9±3.5	.857
No. of oocytes retrieved	4.2±2.7	5.1±3.2	.219
No. of 2PN embryos	2.9±2.1	3.2±2.2	.559
Fertilization rate	62.8%	66.7%	.609
Good quality embryo rate	42.5%	52.0%	.444
No. of embryos transferred	1.8±1.1	2.0±1.0	.525
Cancellation rate	17.2%	10.7%	.434
Implantation rate	17.4%	22.9%	.473
Pregnancy rate per ET	36.0%	37.5%	.900
Clinical pregnancy rate per ET	28.0%	31.3%	.774
Ongoing pregnancy rate per ET	20.0%	27.1%	.357

agonists and oral contraceptive (OC) pills are commonly used to offset this discrepancy [2]. However, the use of these two adjuvants for poor responders has been disappointing, owing to overly suppressed pituitary function and the extrapituitary actions of the GnRH agonist, which mainly repress the function of the reproductive tract, including the ovaries [20–22]. Moreover, combined OC pills are associated with lengthy treatment courses and menstrual bleeding postponement.

Incorporating both luteal E2 patches and GnRH antagonists in the preceding luteal phase or using oral luteal E2 starting on luteal day 21 suggests that poor responders would indeed benefit from this protocol [23–25]. In the present study, we administered two different luteal E2 protocols and compared the outcomes. In an attempt to synchronize follicular growth, both protocols started using E2 from day 21 of the preceding cycle. However, the day of E2 discontinuation differed. For protocol A, similar to most previous studies, we administered E2 from day 21 of the preceding IVF cycle until day 3 of the following cycle. We expected a significant rebound action of FSH, leading to further promotion of multi-follicular development. For protocol B, instead of discontinuing estrogen before starting ovarian stimulation, we continued its use during stimulation. The first reason for this extended use was to prevent the rebound of endogenous gonadotropins and subsequent FSH receptor downregulation after E2 discontinuation [26, 27]. Second, we hypothesized that extended use of estrogen through ovarian stimulation would enhance the stimulatory effect of FSH on granulosa cell FSH receptors and improve embryo quality. This supposition was based on the well-known fact that follicular growth and granulosa cell proliferation are enhanced by estrogens and FSH [28]. Estrogen alone is known to induce FSH receptor proliferation in granulosa cells, and concurrent injection of E2 and FSH synergistically increases FSH receptors in granulosa cells and luteal hormone/hCG receptors in granulosa and theca interstitial tissues [29]. Although the two different luteal E2 protocols showed similar stimulation outcomes, there were slightly improved embryologic and pregnancy outcomes with extended use of E2, although this difference did not reach statistical significance. A power analysis was performed, which determined a need for 331 patients in each group to confirm a statistically significant difference in ongoing pregnancy rates between the two protocols.

Our results showed that the luteal E2 protocol gave a better ovarian response as compared with the standard antagonist protocol. Gonadotropin dose was increased in the luteal E2 group and this may reflect a slower and more coordinated stimulation process, resulting from improved homogeneity of antral follicles. Cancellation rates were significantly lower using the luteal E2 protocol and the number of retrieved oocytes was significantly increased

following E2 pretreatment. As a result, a trend towards better pregnancy outcomes was noted in the luteal E2 protocol group. Additionally, although no supernumerary blastocysts were available for freezing in the standard cycle, a mean of 1.7 blastocysts per cycle were cryopreserved in seven patients after luteal E2 pretreatment. Although statistical significance was not achieved due to the small number of patients, considering that the luteal E2 group had more prior cycle failures, treatment in this group of patients may lead to improvements in pregnancy outcomes. The observed improved IVF outcome may have resulted from both synchronization of follicular growth and the E2 stimulatory effects on FSH receptors.

Our review included 155 poor responder patients with a history of IVF failure who received the standard GnRH antagonist or luteal E2 protocol. The main limitation of this study was its retrospective design and small sample size. However, the poor responder population represents 5–10% of patients in most assisted reproduction clinics, which creates logistic problems when performing a prospective study with sufficient power. Although the patients were not randomized, the two populations had similar baseline characteristics, which made it possible to compare IVF outcomes between the groups. By using a prospectively recorded electronic database, bias was minimized.

The results of our study show that luteal estrogen use not only increased the number of oocytes retrieved, but also the possibility of improvement in pregnancy outcome. A coordinated slower response resulting in even growth with restoration of ovarian responsiveness due to the stimulatory effects of E2 on granulosa cell FSH receptors may have been the cause of these promising results. This technique represents a simple ovulation induction protocol for poor responders.

Acknowledgements This study was supported by a grant (no. A084923) from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea.

These findings are presented at the 27th Annual Meeting of the European Society of Human Reproduction and Embryology, Stockholm, Sweden on 3–6 July, 2011.

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