Effect of Luteal Estrogen Priming in Women with Poor Ovarian Response Ungergoing IVF Using Antagonist Protocol

Noura El-Nassery[1] MD
Amal Shohayeb[1] MD
Mostafa Gamea[1] MD
Ahmed M Maged[1] MD
Mahmoud Soliman [1] MD
[1] Obstetrics and Gynecology Department, Cairo University, Cairo, Egypt

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Abstract

Objective : to explore the benefits of the E2 priming during the luteal phase in women with poor ovarian response (POR) undergoing IVF cycles using the GnRH antagonist protocol.

Methods : This randomized controlled study conducted on 100 POR underwent ICSI trial using GnRH antagonist protocol assigned to 1 group of 2. Group I included 50 women received oral E2 valerate 4 mg/day started on luteal day 21 and continued up to the 1st day of the cycle group II included 50 control women who did not receive estrogen priming. The main outcome parameter was the number of MII oocytes. Other outcomes included implantation, chemical pregnancy and clinical pregnancy rates.

Results: No statistically significant difference between women in the estrogen priming group and other women regarding the retrieved oocytes number and quality, implantation, clinical pregnancy, or chemical pregnancy rates.

Conclusion: estrogen priming did not increase the number of MII retrieved ova in POR.

INTRODUCTION

Infertility affects 25% of couples in developing countries (more than 186 million women) (1). In vitro fertilization accounts for 2-3% in low-income countries (2).

Controlled ovarian hyperstimulation (COH) is the first phase in IVF. Poor ovarian response (POR) is not uncommon event during COH as it represents about 5 to 35 % of women with infertility (3).

POR is diagnosed by cancellation of one or more IVF cycles or failure of the long agonist protocol (4).

Corresponding author:
Noura El-Nassery
Tel +201227763517
noura_elnassery2004@hotmail.com

POR women produce inadequate number of oocytes that results in cycle cancellation (76%) (5) or insufficient numbers of embryos for transfer with the resultant lower probability of pregnancy (3.2-14%) (6).

Several approaches were tried to optimize the outcome of IVF in POR women. These include the use of different protocols as antagonist (7), agonist stop (8), short flare up or microdose flare up protocols (9), Pretreatment with different drugs as letrozole, hCG, or AndroGel (10), adjuvant therapy with aromatase inhibitors (10), clomiphene citrate or luteinizing hormone (LH) (11), maximize the starting Gn dose (12) or luteal phase support with FSH (13).

A suggested mechanism for POR is the short follicular phase with the resultant lowered ability of adequate oocyte cohorts or the increased sensitivity to corpus luteum suppressive agents (14).

Adding estrogen to GnRH antagonist during the mid luteal phase of the IVF preceding cycle was suggested to improve IVF outcomes and lower cancellation in POR (15).

E2 pretreatment enhances the negative feedback of natural estrogen on the hypothalamus–pituitary–ovary axis and results in prevention of intercycle increases in FSH and improving oocytes synchronization (14).

However, the studies evaluating the E2 pretreatment studies were not designed to detect IVF outcomes and used the same participants in their previous failed cycle as a control. Moreover, these studies failed to define the appropriate time of Gn induction after luteal E2 or the time of stop of E2 therapy.

The aim of the present study is to explore the benefits of the luteal phase E2 priming in POR undergoing ICSI cycles using the GnRH antagonist protocol.

Material and Methods

This open label randomized controlled trial included 100 women candidates for IVF/ICSI in IVF unit, Obstetrics & Gynecology Department, Cairo University between December 2019 and May 2020. The study protocol was approved by Kasr Alainy ethical committee. Signing of informed consents was done by all participants after full explanation of POR condition and possible beneficial and hazardous effects of E2; we also mentioned the lack of such studies on poor responders.

All the participants were diagnosed as POR according to Bologna criteria. The criteria require the presence of 2 or more of the following criteria: female age ≥40 years or other risk factors for poor ovarian reserve, poor ovarian response to COH with a conventional stimulation protocol (produced 3 or less oocytes) and low ovarian reserve test (AFC 5-7 or AMH 0.5 – 1.1 ng/ml) (3).

Exclusion criteria included severe male factor, uterine abnormalities (as fibroid, polyps, congenital anomalies or intrauterine synechia (evaluated by hysteroscopy), ovarian cysts, ovarian or pelvic endometriosis, hydrosalpinx, endocrinological disorders as thyroid, adrenal or hyperprolactinemia), uncontrolled metabolic or medical disorders.

All included participants were subjected to complete history, general, abdominal and pelvic examinations and ultrasonographic evaluation.

The participants were randomized using computer generated random numbers, each patient chosen a sealed envelope containing the randomized assignment to either the study or the control group. They were assigned equally to one of 2 groups. E2 priming group who received oral E2 valerate 4 mg/day (Cycloprogynova white tablets, Bayer Pharma™) started on luteal day 21 and continued up to the 1st day of the cycle (14). Control group did not receive such E2 therapy.

All participants were subjected to stimulation using GnRH antagonist conventional protocol. Starting from day 2 of the cycle, each woman received 150 U urinary Gn

(Menogon; Ferring, Switzerland) in addition to 300 U recombinant FSH

(Gonal-f; Merck Serono, Germany) then the dose was adjusted according to monitored ovarian response. Monitoring was done through transvaginal ultrasound done on alternate days using (Mindray trans-vaginal 4-7.5 MHz V6) for follicular count, size, endometrial thickness and pattern. Subcutaneous daily injection of 0.25 mg Cetrolix (Cetrotide, MerckSerono, Germany) was started when the leading follicle reached 12 mm till the day of triggering. When at least 3 follicles reached more than 14 mm, triggering was done using 10,000 IU hCG intramuscularly (Choriomon, IBSA™) (4).

Under general anesthesia transvaginal ultrasound guided oocytes retrieval was performed 34-35 hours after the hCG dose. ICSI procedure was performed in all

cases. Assessment of fertilization was done 16 - 18 hours after oocyte injection then evaluation of embryos was done 48 – 72 hours after ICSI,

Collection of Oocytes and culturing of embryos were done in ISM1 culture medium (Origiomedicult media, Denmark).

Day 2-5 embryo transfer was done using Labotect semirigid catheter; labotect GmbH, Germany).

The main outcome was the number of MII oocytes. Other outcomes included clinical pregnancy rate (one or more gestational sacs detected by transvaginal ultrasound with possible fetal pulsations), implantation rate (the number of gestational sacs divided by the number of embryos transferred) and the number of ET.

Sample size calculation was calculated by comparing the number of metaphase II (MII) oocytes in POR ICSI cycles treated with E2 priming throughout the luteal phase and those un treated matched women. As reported in previous publication (14), the mean ± standard deviation (SD) of number of retrieved oocytes in E2 primed group was approximately 4.5±2.9 oocytes, while in un-treated group it was approximately 3.2±1.9 oocytes. As a result, we determined that using the Student's t test for independent samples, 35 participants in each group were the bare minimum necessary to reject the null hypothesis with 80% power at the 0.05 level. Utilizing Stats Direct statistical software for MS Windows, version 2.7.2, Stats Direct Ltd., Cheshire, UK, calculated the sample size.

Data were statistically described using the mean, standard deviation (SD), median, and range, or, when appropriate, frequencies (number of occurrences), and percentages. Using a Student t test for independent samples, the study groups' numerical variables were compared. An analysis using the Chi-square (2) test was done to compare categorical data. When the anticipated frequency is less than 5, an exact test was used in its place. It was deemed statistically significant when the two-sided p value was less than 0.05. IBM SPSS (Statistical Package for the Social Science; IBM Corp., Armonk, NY, USA) release 22 for Microsoft Windows was used to perform all statistical calculations.

Results

There were no significant differences between E2 priming group women and controls regarding female age, BMI, number of previous IVF failures, AFC or AMH (table 1).

There were no significant differences between women in the 2 study groups regarding all outcome parameters named Gn dose, number and quality of oocytes, number and day of ET, chemical pregnancy, clinical pregnancy or implantation rates (table 2).

There was no correlation between estrogen priming and baseline characteristics or outcome parameters (table 3).

Discussion

Our study found that estrogen priming did not increase the number of MII follicles in women with POR.

The theory of estrogen therapy of PORs is to minimize the levels of FSH during the late luteal and early follicular phases to allow better recruitment of higher number of follicles for growth and maturation (16).

Addition of E2 to GnRH antagonist started during the mid luteal phase of the IVF preceding cycle was suggested to lower cancellation and improve IVF outcomes in POR as it suppresses early follicular recruitment that occurs in the perimenopausal women during the late luteal phase and enhances synchronized follicular development (15).

Although both combined estrogen and progesterone in combined oral contraceptive pills and GnRH agonist can be used for the same purpose, they can adversely affect ovarian responsiveness (17).

The results of the present study are consistent with Di Luigi and his co-workers (18). Using a micro dose leuprolide acetate (LPA) regimen or a GnRH antagonist treatment that included a luteal phase E2 patch and GnRH antagonist in the prior menstrual cycle, they conducted a randomized experiment to examine the IVF outcomes in 54 poor responder patients. In comparison to the control group, the results showed that E2 priming in GnRH antagonist cycles had no appreciable impact on the success rates of IVF in terms of oocyte retrieval, clinical pregnancy rates, and ongoing pregnancy rates.

The luteal phase synchronization of follicular growth was another method Elassar and his team (19) proposed for increasing ovarian responsiveness in underperformers. In low responders, they contrasted luteal E2 alone (n=57) with luteal E2 in addition to antagonist (n=256). When poor responders are used for IVF, the addition of GnRH antagonist to luteal E2 for luteal suppression prior to ovarian stimulation does not increase the success of the procedure. showed that E2 priming during a GnRH antagonist cycle had no discernible effect on the success of IVF in patients who had poor responses.

Stands with our findings, the McGill Reproductive Center's application of the Ata study on 75 women undergoing IVF after stimulation with luteal E2 patch (LPA)-GnRH antagonist and micro dose (MD) flare-up protocols in anticipated poor responders. Despite the fact that the clinical pregnancy rate of 38.9% and the embryo implantation rate of 16.7% in the LPA group were both nearly 50% higher than the corresponding rates of 10.3% and 22.2% in the MD group, respectively, the differences were not statistically significant (p values > 0.05 for all comparisons). This might be because patients in this trial were matched for age and markers of ovarian reserve, whereas in our investigation, age was used as an inclusion criterion with no upper limit.

Although the results do not support our findings that the E2 priming effect is not related to oocyte yield or follicular synchronization with the LPA protocol, Ata suggested that the observed trend towards higher embryo implantation and clinical pregnancy rates needs further study.

The study by Fanchin and his colleagues who studied prospectively 90 IVF-ET candidates (16), showed that by utilizing the hypothalamic-pituitary E2 priming's natural negative feedback, it was possible to successfully suppress the rise in FSH during intervals between cycles. This method may be helpful in synchronizing follicular growth during controlled ovarian hyperstimulation. Although this study's results indicate that luteal E2 administration reduces the size and enhances the homogeneity of early antral follicles on day 3, its correlation to finally bring about better growth of mature follicles with final net effect on IVF results on poor

responders is earlier to be concluded and need some more studies especially because of the variable factors implement this process.

A similar study was carried out by Ghasemzadeh and her associates (21), involving two groups of patients who were receiving gonadotropin and a GnRH antagonist for inadequate response. There were 53 patients in each group, and the oocyte producing results varied slightly. The average number of large follicles (2.9 against 2.3), M2 oocytes (3.6 against 2.8), and type II and type III embryo quality (1.3 against 0.9 and 0.7 against 0.3) were all significantly higher in the intervention group compared to the control group (P values were 0.05, 0.05, 0.05, and 0.01) on average. However, the success rate of pregnancies was 8.3%: 6.7%. which supported our findings of pregnancy rate (16: 18%) but was not statistically significant (P = 0.50), Endocrine differences due to racial disparities across different populations could be the explanation.

Yucel and his colleagues (22), compared E2 to progesterone priming in POR in micro dose flare-up and GnRH antagonist combined letrozole protocols during ICSI. They concluded that these protocols did not significantly improved the outcomes.

Our finding that E2 priming has no significant effect on the picked ovum count agrees with study by Elassar et al., (19). They compared E2 priming to non-priming in antagonist cycles and found a significantly lower total dose of Gn and E2 levels in E2 primed women with similar number and quality of retrieved oocytes, cancellation and pregnancy rates.

Contrary to our findings, the study by Frattarelli (23) used 60 patients who had a poor response rate and 120 IVF cycles, with the main outcome measure(s) being the number of embryos with 7 cells on day 3 of development. The patients themselves were used as controls, and the previous failed cycles belonging to these patients were used as the control. The number of ovum and embryos produced by the usual IVF treatment would dramatically rise as compared to the method without E2 priming, according to the results. This study did not assess and monitor the therapeutic efficacy of E2 therapies, which may account for the discrepancy between our findings despite its minimal effect on pregnancy rate.

In the retrospective investigation carried out by Hwajeong Lee and colleagues (24), 64 more poor responders were enrolled as the control group and completed standard protocols without pretreatment, while 65 poor responders underwent the E2 priming regimen. Two groups' clinical outcomes were contrasted. However, in this study, fertilized oocytes had significance of (2.251.74 vs. 1.321.26; P=0.001), good embryos (1.620.91 vs. 1.140.90, P=0.021) and number of retrieved oocytes (3.582.24 vs. 1.701.45; P=0.000) compared to mature oocytes (1.651.23; P=0.000). Additionally, the clinical pregnancy rate was significantly higher in the E2 priming group than in the control group (26.2% vs. 12.5%; P=0.048), but the variation in racial factors suggests that more multi-centric studies be conducted.

Reynolds and his coworkers (25), in a meta-analysis research, looked at the impact of E2 priming during the luteal phase. It was discovered that this approach enhanced the rate of clinical pregnancy while decreasing the risk of cycle cancellation. The original search turned up 2249 publications in all, while the bibliographies, abstracts, and other sources produced 11 more. Duplications were eliminated, leaving 1227 research, of which 8 eventually matched the requirements for inclusion. Women exposed to LE priming (n = 468) had a lower risk of cycle cancellation compared to women undergoing non-E2 primed protocols (n = 621) and an improved chance of becoming clinically pregnant (RR: 1.33, 95% CI: 1.02-1.72), but the curious thing was that there was no discernible improvement in the quantity of mature oocytes or zygotes obtained per cycle.

Finally, they reported despite its limitations,

this meta-analysis supports the use of E2 priming prior to COH in poor responders as a promising hope, this hope despite non-significant differences is the principle and the motive that promotes more trials with more priming or adjuvant agents.

The main strength of our study is its randomized nature with proper sample size calculation while its main limitation was the absence of long term follow up to calculate the live birth rate (the most important outcome in IVF cycles).

Based on a quantitative and qualitative analysis of responses, it can be concluded that no significant relation between the luteal E2 priming in the antagonist protocol in poor responders and a better ICSI outcome, neither in the number and quality of oocytes retrieved nor the pregnancy rate as well as the embryo implantation rate. Further studies are needed on a larger scale with bigger multi-centric samples to obtain more data about the effect of the luteal E2 priming on poor responders ICSI outcome. While this study limits the generalizability of the results, this approach provides new insight into other protocols and medications to be introduced as a pretreatment for poor responders.

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