# Impact of Prolonged Versus OCP Plus Long Protocol on IVF-ET Outcomes in Patients With Grade III-IV Endometriosis: A Randomized Clinical Trial

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**Abstract**- The purpose of the current study was to investigate the impact of a 2-months implementation of GnRH-agonist (prolonged) versus the OCP+long protocol before in-vitro fertilization-embryo transfer (IVF-ET) on IVF outcomes in infertile patients with Grade III-IV endometriosis. A total of 70 infertile patients with endometriosis participated in this randomized clinical trial and randomly received either the prolonged GnRH-agonist protocol (38 patients) as control or the OCP+long protocol (32 patients) as the case group. This was followed by standard controlled ovarian hyperstimulation (COH) in all subjects. The fertilization rate, the implantation rate, and the clinical pregnancy rate were measured and compared between the two groups. A statistically significant trend toward better embryo quality was observed in the control group ($P$=0.01). In addition, clinical pregnancy rate, implantation rate and fertilization rate did not differ significantly between two groups ($P$=0.43, $P$=0.54, $P$=0.1 respectively). GnRH agonist treatment for 2 months before ART in women with high-grade endometriosis was associated with better embryo quality compared to three weeks of treatment with OCP. OCP before assisted reproductive technology (ART) was as effective as GnRH agonist treatment regarding clinical pregnancy and implantation rates in infertile patients with severe endometriosis.



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## Introduction

Endometriosis is a common disorder characterized by implantation of endometrial tissue outside of the uterine cavity which is observed in up to 50% of patients suspected with subfertility (1). The exact relationship between endometriosis and infertility is not completely determined but several probable mechanisms have been proposed to explain infertility observed in women with endometriosis. Possible mechanisms consist of reduced quality of oocyte and/or embryo as well as low fertilization and implantation rates. These conditions can be explained mainly by an observed increase in inflammatory cytokines and vasoactive peptides in the peritoneal fluid (2-4). It has been estimated that more than one-third of women undergoing in-vitro fertilization (IVF) have endometriosis. It has been suggested that the severity of endometriosis has a negative impact on IVF outcome success (5,6). There is strong body of evidence supporting pre-IVF suppressive therapy with Gonadotropin-releasing hormone (GnRH) agonists demonstrating benefits in advanced disease even though the ideal duration and type of suppression are yet to be determined (7). Sallam *et al*., in their systematic review, demonstrated that according to the currently available evidence in the literature, pre-IVF administration of GnRH agonists for a duration of 3 to 6 months significantly increases both rates of clinical pregnancy and live births (8). In another study, De Ziegler and colleges showed that administration of the oral contraceptive pill (OCP) for 6-8 weeks before IVF could result in higher pregnancy rates compared to controls in endometriosis patients. Moreover, the authors suggested that 6 to 8 weeks of treatment with OCP could improve oocyte quality and clinical pregnancy rates comparable to 3 to 6 months of treatment with GnRH agonists and result in lower adverse effects (9). There is concern that use of GnRH agonists for long periods of time may cause a greater degree of pituitary suppression and requires higher doses

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of gonadotropin for standard controlled ovarian hyperstimulation (COH). In one study, Zhang *et al*., reported that treatment with the long protocol before in-vitro fertilization-embryo transfer (IVF-ET) in patients with endometriosis resulted in a better ovarian response and higher clinical pregnancy rates compared to the prolonged protocol (10).

Limited number of studies which evaluated effects of these two protocols before IVF in women with endometriosis exists. Hence, we designed this randomized clinical trial to evaluate the effects of administering two months of long-acting GnRH agonists in comparison with 3 weeks of OCP plus long protocol on ART outcomes in candidates of IVF affected by endometriosis.

In previous studies, the prolong protocol implemented 3-6 months before IVF had a similar outcome as 2 months of treatment. All together the results were in consistent in previous studies. Therefore, we considered 2 months of treatment with GnRH agonists as control. For intervention, we investigated 3 weeks of OCP treatment plus standard long protocol before assisted reproductive technology (ART) to evaluate if this pre-ART treatment could be as effective as suppressing ovarian function with GnRH agonists for 2 months in order to optimize ART outcomes in endometriosis grade III-IV. Noticeably, ovarian suppression could improve ART outcome in severe endometriosis but not in the early stage of disorder.

## Materials and Methods

### Trial design and setting

This randomized, parallel group trial was performed at Arash hospital affiliated with Tehran University of Medical Sciences (TUMS) between October 2014 and December 2016. The institutional review board (IRB) of TUMS approved the trial and the study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions. After a detailed explanation of the aims and the study protocol, all cases were asked to complete an informed consent form before their entry into the study. Patients were free to enter the study or leave the study at any time without any impact on the medical care they would receive. The trial was registered at the Iranian registry of clinical trials (www.irct.ir; registration number: IRCT201409301556N64).

### Participants

Infertile women aged less than 38 years with the diagnosis of endometriosis were included. Diagnosis of endometriosis had been previously made through laparoscopic surgery or having an endometrioma more than three cm on ultrasonography. Inclusion criteria were endometriosis grade III or IV, body mass index (BMI) between 20-27 $kg/m^2$, serum FSH levels of less than 10mIU/ml, and presence of two functional ovaries.

Exclusion criteria were poor responders, endocrinological disorders, uterine anomaly, more than two previous IVF cycles, infertility caused by polycystic ovary syndrome (PCOS), or severe male factor.

### Intervention and outcome

Eligible participants were randomly assigned to receive either a prolonged GnRH agonist or OCP plus long protocol. The first group received a long-acting GnRH agonist (3.75 mg triptorelin acetate via intramuscular injection, (Beaufour-Ibsen-Pharma, Boulogne France) monthly for 2 months during the previous early follicular phase. The second group (OCP+long protocol) received a 0.03 mg OCP preparation containing ethinyle E2 (EE) and 0.15 mg of levonogestrol (Ovocept-LD iranfarma Iran) starting on day 2 of the menstrual cycle. Pre-ART use of OCP was continued for 3 weeks in group 2 patients, then triptorelin (0.1 mg daily, Decapeptyl; Beaufour-Ipsen-Pharma, Boulogne France) was initiated 3 days before the scheduled termination of the OCP treatment.

After pituitary downregulation was achieved, FSH (75 IU/d,Gonal-f , Merck, Serono) and/or hMG (75 IU, Menogon, Ibsa, Italy) was started on day 2 or 3 of the menstrual cycle at a dosage of 150-300 IU/d according to patient's age, body mass index (BMI), antral follicular count in the third day of the cycle, and ovarian responsiveness in the previous cycle. Ovarian response was monitored by transvaginal ultrasonography.

In the prolonged GnRH group, FSH/HMG was started 7-10 days after the second injection of the long-acting GnRH agonist. When at least 2 follicles reached>17 mm, 10,000 IU of urinary human chorionic gonadotropin (HCG) was injected and oocyte retrieval was performed within 35-36 hours. No more than 3 embryos were transferred in the two or three days after oocyte retrieval. Vaginal progesterone (Cyclogest, 400 mg) was used in order to support the luteal phase. Patients received one dose of Cyclogest (400 mg) on the day of oocyte retrieval followed by daily administration of two Cyclogest doses (400 mg per dose) until the tenth week of pregnancy. Embryos were graded on day 2 or 3 based on cell symmetry, fragmentation, and blastomere number. Grade A

embryo was defined as blastomeres of equal size with no fragmentation, grade B embryo has minimal fragmentation (≤20% fragmentation) and grade C is considered as embryo with blastomeres of unequal size or major fragmentation (20%-50% fragmentation). Serum β-hCG was checked 14 days after embryo transfer and a β-hCG level of >10 IU/L was considered as positive. Additionally, transvaginal ultrasound was performed at 5 and 7 weeks of gestation to identify the gestational sac and fetal cardiac activity, respectively. Number of oocytes, number of oocytes in metaphase II, quality of embryo, implantation rate, fertilization rate and clinical pregnancy rate and ovarian hyperstimulation syndrome were compared between the two groups.

Our primary outcome was clinical pregnancy rate. The secondary outcomes were the number of oocytes, number of oocytes in metaphase II, quality of embryo, implantation rate, fertilization rate and ovarian hyperstimulation syndrome.

**Randomization**

A computerized random number generator was used for randomizing participants to receive either GnRH agonist or OCP plus long-term pituitary down-regulation by an independent party who was not involved anywhere else in the study (the permuted randomization block, allocation ratio 1:1, blocks of four). Sequentially numbered, sealed, opaque and stapled envelopes were used to conceal treatment allocations.

**Statistical analysis**

The IBM SPSS software package for windows version 20 was used for data analysis. Mean±SD of continuous variables and frequency (%) of categorical variables are presented. The independent t-test was used to compare continuous variables and the categorical variable was compared between groups using the chi-square or Fisher's exact test as appropriated. All analyses were performed two-sided and a *P* of less than 0.05 was considered statistically significant.

## Results

One hundred patients were screened for enrollment in the current study of whom 80 participants fulfilled the eligibility criteria and were randomized to receive prolonged GnRH agonist (group 1) or OCP plus the long protocol (group 2) (n=40 in each group). Two patients from group 1 and eight patients from group 2 withdrew from the study before beginning the protocol. A total number of 70 patients were enrolled in the final analysis (38 patients in group 1 and 32 patient in group 2, figure 1). The mean age and BMI of participants were 31.5±3.1 years and 24.36 kg/m$^2$, respectively. Fifty-six patients (80%) had primary infertility and the remaining 14 patients (20%) had secondary infertility. The patients in the two groups were comparable in terms of age, BMI, duration of infertility, and percentage of cases with primary infertility. Table 1 compares patient characteristics between the two groups.

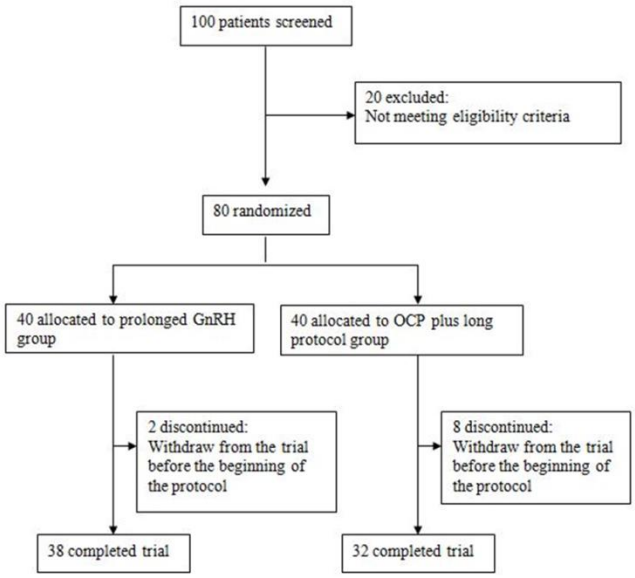


**Figure 1.** Study flow diagram

**Table 1. Demographic characteristics of patients according to treatment groups. Group 1 received prolonged protocol and group 2 received OCP+long protocol**

| Treatment group | Prolonged GnRH agonist (n=38) | OCP+long protocol (n=32) | *P* |
|---|---|---|---|
| **Age, years, mean±SD** | 31.42±3.13 | 31.78±3.16 | 0.63 |
| **Primary infertility** | 31 (81.6%) | 25 (78.1%) | 0.47 |
| **Body mass index (BMI), Kg/m$^2$, mean±SD** | 24.09±2.61 | 24.68±2.61 | 0.35 |
| **Number of FSH ampoule used, mean±SD** | 36.08±10.82 | 35.47±12.56 | 0.82 |
| **Duration of stimulation, days, mean±SD** | 10.31±1.57 | 9.89±1.55 | 0.27 |

**Table 2. Laboratory and embryological data and pregnancy outcome in prolonged GnRH agonist group *vs.* OCP plus long protocol**

| | | prolonged GnRH agonist group (n=38) | OCP plus long protocol (n=32) | *P* |
|---|---|---|---|---|
| **Serum FSH level (mIU/mL), mean±SD** | | 6.60±2.17 | 6.31±2.35 | 0.59 |
| **Length of cycle (days), mean±SD** | | 9.89±1.55 | 10.31±1.57 | 0.27 |
| **Endometrial thickness (mm), mean±SD** | | 10.38±1.03 | 10.29±1.06 | 0.71 |
| **Number of oocytes retrieved, mean±SD** | | 10.68±6.04 | 8.81±5.57 | 0.18 |
| **Number of oocytes in metaphase II, mean±SD** | | 7.11±4.41 | 6.81±4.68 | 0.78 |
| **Embryo quality** | **Grade A** | 16 (42.1%) | 4 (12.5%) | |
| | **Grade B** | 12 (31.6%) | 20 (62.5%) | 0.01 |
| | **Grade C** | 10 (26.3%) | 8 (25%) | |
| **Number of embryo transferred, mean±SD** | | 1.87±0.66 | 2.19±0.73 | 0.06 |
| **Implantation rate** | | 0.49±0.08 | 0.55±0.25 | 0.54 |
| **Pregnancy rate** | | 10 (26.3%) | 12 (37.5%) | 0.43 |
| **Fertilization rate** | | 0.49 | 0.59 | 0.1 |
| **Abortion rate** | | 0 | 3.1 | 0.45 |

## Discussion

The results of the current study showed that prolonged GnRH agonists and OCP plus long protocol before IVF in infertile women with endometriosis grade III-IV have similar outcomes regarding pregnancy and implantation rates. Present data indicate that the prolonged GnRH protocol before ART was associated with better embryo quality than 3 weeks of continuous OCP plus long protocol without diminished ovarian response to COH.

Pretreatment with GnRH agonists has been shown to improve ART outcome in women with endometriosis but the type and the optimal time of suppression are unknown (11). There is controversy on over effect of different down-regulation protocols on outcome of IVF in endometriosis patients. There is great concern among physicians that long-term pituitary down-regulation may decrease ovarian response to ovarian stimulation in addition to other side effects of this protocol such as hot flushes (10).

Previous studies showed that 3-6 months of GnRH agonist therapy before ART cycles in women with endometriosis improves implantation and pregnancy rates (8,11). Surrey *et al*., reported that the long-acting GnRH regimen for 3 months before IVF-ET could result in increase in implantation and pregnancy rates compared to standard controlled ovarian hyperstimulation (implantation rate: 42% vs. 30%, and pregnancy rate: 80% vs. 53%, respectively) (11). In a systematic review conducted by Sallam *et al*., the authors concluded that administration of GnRH agonists for 3-6 months before IVF or ICSI cycles in women with endometriosis increase pregnancy rate up to fourfold (8). If ovarian function suppression is responsible for this beneficial effect, one may conclude that OCP can also achieve the same result with fewer side effects and lower treatment cost. In line with this hypothesis, de Ziegler *et al*., showed that oral contraceptive treatment 6-8 weeks prior to ART cycles could increase pregnancy rates in women with endometriosis (9).

Our results demonstrate that implementation of both the OCP+long protocol and prolonged GnRH agonist protocol before ART cycles were associated with the same pregnancy and implantation rates in women with endometriosis. The pregnancy rate was 26.3% and 37.5% in the prolonged GnRH agonist group and in the OCP long protocol, respectively. Although our results showed significantly greater embryo quality in the

prolonged GnRH agonist protocol group, this may be explained by the fact that other factors like endometrial receptivity are important in pregnancy and implantation rates. Since the basic characteristics of the two groups were similar, personal differences between subjects cannot explain this finding.

Even though the exact mechanisms responsible for the promising effects of pre-ART treatment with either GnRH agonist or OCP are still unknown. A probable explanation is their beneficial effect on oocyte quality and endometrial receptivity. In patients with endometriosis, eutopic endometrium has significant functional and structural abnormalities (8). This includes aberrant integrin αvβ3 expression (12), altered molecular markers of the endometrium such as HOXA10 and HOXA11 (13), resistance to progesterone (14), and cytokine-mediated prostaglandin (PG)-E2 production (15). Poor embryo and oocyte quality can be caused by inflammation and oxidative stress in the pelvic cavity of these patients (16). Tumor necrosis factor (TNF)-α, which is produced by macrophages and Natural killer (NK) cells, may also have cytotoxic effects on the oocyte and the embryo (17). The results of a study by Tamura et al., showed that the concentration of TNF-α and 8-OHdG, which are cytotoxic cytokines as well as oxidative stress markers, were significantly lower in follicular fluids of patients with endometriosis who were treated with GnRH agonists before IVF (18). These findings could indicate that GnRH agonist treatment before ART could result in reduction of inflammatory markers in the peritoneal environment or implantation environment (18). On the other hand, GnRH agonists increase melatonin concentration in the follicular fluid which is considered an antioxidant (19). There is strong body of evidence supporting the role of melatonin in reduction of oxidative stress and in improvement of implantation and pregnancy rates (20).

Subsequently, ovarian function suppression will be possible by means of GnRH agonists and OCP. Hence, these agents could correct endometrial alterations. It was reported that GnRH agonist treatment can reduce cytokine levels in the peritoneal cavity in patients with endometriosis (21).

As our results show, these two treatment routes have the same effects on implantation and pregnancy rates. There is concern that ovarian suppression using prolonged GnRH agonist may decrease ovarian responses to COH, but our data suggest that using GnRH agonist for 2 months before ART does not reduce the COH response.

In conclusion, prolonged GnRH agonist before ART in women with high-grade endometriosis was associated with greater embryo quality compared to three weeks of treatment with OCP and long protocol. In addition, the low cost of OCP+long protocol should be considered in developing countries. Pregnancy and implantation rates were the same between the two groups. Further trials are needed for comparison of values obtained for ovarian suppression before IVF in the current study in order to determine the optimal treatment modality.

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