# Comparison of Controlled Ovarian Stimulation Protocols on IVF Outcome in Normal and Poor Responders

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**OBJECTIVE:** Aim of this study is determining the influence of luteal long GnRH agonist and GnRH antagonist protocols on IVF/ICSI cycle outcome in group of patients considered “normal responder” and influence of luteal long GnRH agonist, microdose flare-up agonist and GnRH antagonist protocols on IVF/ICSI cycle outcome in a group of patients considered “poor responder”.

**STUDY DESIGN:** This was a retrospective analysis performed in the Hacettepe University School of Medicine IVF Center, Ankara, from January 2005 to December 2007. Normal responders (first arm) were stimulated either with luteal long GnRH analogues, (193 patients and 300 cycles) or with GnRH antagonists (215 patients and 300 cycles). Poor responders (second arm) were stimulated either with luteal long GnRH analogues, (20 patients and 32 cycles), with GnRH antagonists (21 patients and 45 cycles) or microdose flare-up protocol (27 patients and 74 cycles).

**RESULTS:** In the first arm; the clinical pregnancy, implantation and multiple pregnancy rates were comparable between the two groups in the first arm. In the second arm; clinical pregnancy, implantation and multiple pregnancy rates were comparable between three groups.

**CONCLUSION:** There is insufficient evidence to recommend GnRH agonist or GnRH antagonist protocols for patients considered “normal’ and ‘poor responder’.

**Key Words:** GnRH antagonist, GnRH agonist, Microdose flare-up, IVF

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## Introduction

Multifollicular development induced by controlled ovarian stimulation (COH) is an integral part of IVF. GnRH agonists were introduced in ovarian stimulation for IVF to inhibit the premature surge of LH with dramatic effect substantially increasing clinical pregnancy rates and reducing the number of cancelled cycles.[1] Different treatment regimens utilizing GnRH agonists in COH have been developed. In the long protocol, GnRH agonist commences in the follicular or luteal phase of the preceding cycle and continues until pituitary and ovarian suppression is achieved before stimulation with gonadotropins is started. The ‘microdose flare-up’ protocol combines GnRH agonist therapy starting after oral contraceptive pills on cycle day 24 with gonadotropins initiated two day later. The ultra-short protocol consists of daily subcutaneous administration of GnRH agonist during the first three days of ovarian stimulation). Comparing ultra-short, short and long IVF protocols; Cochrane meta-analysis showed a higher number of oocytes retrieved and higher clinical pregnancy rates with the long protocol.[2]

A decade later GnRH antagonists were introduced into clinical practice. GnRH antagonists are producing a much simpler, shorter and patient friendly approach. Although the usage of these agents is very common, there is still a considerable debate concerning their efficacy to achieve similar reproductive outcomes when compared with the most commonly used GnRH agonists. A Cochrane analysis evaluating the use of long GnRH agonist versus antagonist protocol in COH for IVF/ICSI showed no differences in live birth rates or ongoing pregnancy rates in unselected population of women and also the GnRH antagonist protocol lowers the risk of OHSS.[3]

At present the ideal stimulation regimen for normal and poor responders is not known. There is no protocol that fits all and hence treatment should be individualized to take into account of women's age, ovarian reserve, endocrine status and other associated conditions such as endometriosis, polycystic ovary syndrome (PCOS) and ovarian cysts.[4]

Aim of this study is to determine the influence of luteal long GnRH agonist and GnRH antagonist protocols on IVF/

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ICSI cycle outcome in a group of patients considered "normal responder" and influence of luteal long GnRH agonist, microdose flare agonist and GnRH antagonist protocols on IVF/ICSI cycle outcome in a group of patients considered "poor responder".

## Material and Method

This was a retrospective analysis performed in the Hacettepe University School of Medicine IVF Center, Ankara, from January 2005 to December 2007, concerning women in two groups characterized as normal and poor responders. Antral follicle count (AFC) was used as the primary tool to assess the ovarian reserve. A total of six antral follicles in both ovaries in the early follicular phase were considered as the threshold for normalcy. A normal responder was defined as patients under age 38 and had 6 or more bilateral antral follicles. A poor responder was defined as patients over age 38 and had less than 6 bilateral antral follicles. Exclusion criteria were previous ovarian surgery, previous oophorectomy and surgically retrieved sperm.

Normal responders were stimulated either with GnRH analogues, following the long standard protocol and exploiting the consequent pituitary receptors down regulation (193 patients and 300 cycles) or with GnRH antagonists (215 patients and 300 cycles).

Poor responders were stimulated either with GnRH analogues, following the long standard protocol and exploiting the consequent pituitary receptors down regulation (20 patients and 32 cycles), with GnRH antagonists (21 patients and 45 cycles) or microdose flare-up protocol (27 patients and 74 cycles).

Luteal long agonist protocol is consisting of luteal-long leuprolide acetate (Lucrin; Abbott Cedex, Istanbul, Turkey) with oral contraceptive (OCP) pretreatment (Lo-ovral; Wyeth, Istanbul, Turkey) and recombinant FSH (Gonal-F; Serono, Istanbul, Turkey) using the step-down protocol. The starting dose of gonadotropin was determined based on female age, antral follicle count at baseline trans-vaginal ultrasonography, day 3 FSH and $E_2$ levels, BMI, and previous ovarian response, if available.

GnRH antagonist protocol was started with OCP (Lo-ovral; Wyeth, Istanbul, Turkey) on day 1-2 of the menses of the previous cycle and took it for 14 to 28 days. After a washout period of 1-4 days, they were treated with a starting dose determined based on female age, antral follicle count at baseline transvaginal ultrasonography, day 3 FSH and $E_2$ levels, BMI, and previous ovarian response, if available. GnRH antagonist cetrorelix (0.25 mg/day; Cetrotide; Merck-Serono, İstanbul, Turkey) or ganirelix (0.25 mg/day; Orgalutran, Organon) GnRH antagonist was started when at least one of the following criteria were met: presence of a follicle with mean diameter 13-14 mm or serum $E_2$ level >600 pg/mL.

Microdose flare-up protocol consisted of a 21-day course of OCP (Lo-ovral; Wyeth, Istanbul, Turkey) and leuprolide acetate 40 mg SC twice daily was commenced 3 days after the last pill and continued until the day of hCG administration. Two days after initiation of leuprolide acetate, 300 IU of recombinant FSH (Gonal-F; Serono, Istanbul, Turkey) and 150 IU of hMG (Menogon; Ferring, Istanbul, Turkey) daily were commenced.

Ovarian response was monitored with frequent serum estradiol ($E_2$) measurements and transvaginal ultrasound. The criterion for hCG (Profasi; Serono, Istanbul, Turkey or Pregnyl Organon İstanbul Turkey) administration was presence of one or more follicles exceeding 17 mm in diameter and was the same for all protocols.

Oocyte retrieval was carried out under local anesthesia using vaginal ultrasound-guided puncture of follicles 36 h after hCG administration. Standard procedures were carried out for gamete-embryo handling, and embryo transfer (ET) was performed on day 3 in all cases using soft catheter. The luteal phase was supported by daily vaginal progesterone jell (Crinone; Serono) starting 1day after oocyte pick-up.

The statistical analyses were performed using the Statistics Package for Social Sciences (ver. 13.0; SPSS Inc., Chicago). The $\chi$2-test and Fisher's exact test were used to analyze nominal variables in the form of frequency tables. Normally distributed parametric variables were tested by Kolmogorov-Smirnov test and Shapiro-Wilk test. Normally distributed (Kolmogorov-Smirnov test) parametric variables were tested by the analysis of variance (ANOVA) using the Bonferroni test for post hoc analysis.

## Results

Four hundred seventy-six patients were included in the study. The patients were divided into two arms: in the first one 408 women (600 cycles) were considered to be normal responder while the 68 women (151 cycles) of the second one were considered to be poor responder.

All patient characteristics of the first arm with two different stimulation protocols were comparable in terms of the baseline characteristics, including female age, BMI, number of canceled cycles, and duration of infertility (Table 1). Despite a shorter duration of stimulation, total dose of FSH used, E2 level on the day of hCG administration and endometrial thickness at hCG administration were comparable among long agonist and antagonist group in normal responders (Table 2).

The number of oocytes and metaphase II oocytes and the number of 2 pronucleated oocytes were significantly higher in antagonist compared with the luteal long agonist protocol in the first arm (Table 3). Number of embryos transferred was higher luteal long agonist compared with antagonist protocol. However the clinical pregnancy, implantation and multiple pregnancy rates were comparable between the two groups in the first arm.

The baseline characteristics of the patients in the second arm with three different protocols are given in table 4. The duration of stimulation is shorter in antagonist group compared to microdose flare-up group. Total dose of FSH used were significantly higher in microdose flare-up group compared with antagonist and luteal long agonist groups (Table 5). However the mean number of oocyte-cumulus complexes, metaphase II oocytes, two-pronucleated oocytes and number of embryos transferred were similar among three groups. Also clinical pregnancy, implantation and multiple pregnancy rates were comparable between the three groups in the second arm (Table 6).

*Table 1: The baseline characteristics of the luteal long agonist and antagonist protocol of normal responders*

| | Luteal Long Agonist Protocol | Antagonist Protocol | P value |
|---|---|---|---|
| No. of patients | 193 | 215 | |
| No. of cycles | 300 | 300 | |
| No. of canceled cycles (n, %) | 19 (6.3) | 19 (6.3) | >0.05 |
| Female age (y) | 30.2 ±3.7 | 29.7±4.0 | >0.05 |
| Body mass index (kg/m$^2$) | 24.8±3.9 | 25.3±4.3 | >0.05 |
| Duration of infertility (m) | 86.9±52.6 | 85.1±52.4 | >0.05 |

*Table 2: The controlled ovarian hyper stimulation response of normal responders in agonist and antagonist protocol*

| | Luteal Long Agonist Protocol | Antagonist Protocol | P value |
|---|---|---|---|
| Duration of stimulation (d) | 9.6±1.6 | 8.9±1.5 | <0.01 |
| Total dose of FSH used (IU) | 2496.8±989.6 | 2533±651.4 | >0.05 |
| E2 level on the day of hCG administration (pg/mL) | 2668.6±1522.9 | 2602.9±1606.8 | >0.05 |
| Endometrial thickness at hCG administration (mm) | 10.8±2.4 | 10.8±2.3 | >0.05 |

*Table 3: The embryological data and pregnancy outcome of normal responders in agonist and antagonist protocol*

| | Luteal Long Agonist Protocol | Antagonist Protocol | P value |
|---|---|---|---|
| No. of oocyte-cumulus complexes | 14.7±7.9 | 17.2±9.9 | <0.01 |
| No. of metaphase II oocytes | 12.1±6.5 | 13.7±8.3 | <0.05 |
| No. of 2 pronucleated oocytes | 8.9±5.2 | 10.6±6.9 | <0.01 |
| No. of transferred grade 1 embryos | 0.26±0.03 | 0.17±0.03 | <0.05 |
| No. of transferred grade 2 embryos | 2.62±0.05 | 2.63±0.04 | >0.05 |
| No. of embryos transferred | 2.95±0.73 | 2.84±0.45 | <0.01 |
| Clinical pregnancy/embryo transfer (%) | 45.7 | 44.4 | >0.05 |
| Implantation rate (%) | 22.1 | 19.2 | >0.05 |
| Multiple pregnancy rate (%) | 32 | 30 | >0.05 |

*Table 4: The baseline characteristics of the luteal long agonist and antagonist protocol of poor responders.*

| | Microdose Flare-up Protocol | Luteal Long Agonist Protocol | Antagonist Protocol | P value |
|---|---|---|---|---|
| No. of patients | 27 | 20 | 21 | |
| No. of cycles | 74 | 32 | 45 | |
| No. of canceled cycles (n, %) | 9 (12.2) | 2 (6.2) | 9 (20) | >0.05 |
| Female age (y) | 40.3±2.1 | 39.3±1.8 | 40.4±2.5 | >0.05 |
| Body mass index (kg/m$^2$) | 27.4±3.7 | 26.9±4.3 | 26.9±4.5 | >0.05 |
| Duration of infertility (m) | 157.7±102.4 | 154.2±79.5 | 149.8±90.9 | >0.05 |

*Tablo 5: The controlled ovarian hyperstimulation response of poor responders in microdose flare-up, luteal long agonist and antagonist protocol*

| | Microdose Flare-up Protocol | Luteal Long Agonist Protocol | Antagonist Protocol | P value |
|---|---|---|---|---|
| Duration of stimulation (d) | 11.3±2.2(c) | 10.6±2.2 | 9.8±2.6(a) | <0.05 |
| Total dose of FSH used (IU) | 6281.7±1783.9 (b,c) | 4933.6±1549.1 | 4506.6±1456.2 | <0.05 |
| E2 level on the day of hCG administration (pg/mL) | 1277.8±818.4 | 1227.1±893.7 | 1158.4±803.4 | >0.05 |
| Endometrial thickness at hCG administration (mm) | 10.5±2 | 11.1±2.4 | 10.1±2.3 | >0.05 |

*a,c: Microdose flare up protocol is statistically different from antagonist protocol.*
*b,c: Microdose flare up protocol is statistically different from antagonist and luteal long agonist*

*Table 6: The embryological data and pregnancy outcome of normal responders in microdose flare-up, luteal long agonist and antagonist protocol*

| | Microdose Flare-up Protocol | Luteal Long Agonist Protocol | Antagonist Protocol | P value |
|---|---|---|---|---|
| No. of oocyte-cumulus complexes | 5.0±3.3 | 6.1±3.5 | 5.1±3.3 | >0.05 |
| No. of metaphase II oocytes | 4.2±2.8 | 5.0±3.2 | 4.1±2.8 | >0.05 |
| No. of 2 pronucleated oocytes | 2.8±2.0 | 3.8±2.4 | 3.5±2.5 | >0.05 |
| No. of transferred grade 1 embryos | 0.16±0.04 | 0.20±0.07 | 0.08±0.05 | >0.05 |
| No. of transferred grade 2 embryos | 2.1±1.2 | 2.4±1.1 | 2.4±1.2 | >0.05 |
| No. of embryos transferred | 2.3±1.1 | 2.5±1.2 | 2.5±1.1 | >0.05 |
| Clinical pregnancy/embryo transfer (%) | 10 | 23.3 | 21.2 | >0.05 |
| Implantation rate (%) | 11.1 | 9.2 | 8.1 | >0.05 |
| Multiple pregnancy rate (%) | 23 | 22 | 18 | >0.05 |

## Discussion

The best protocol for ovarian stimulation in IVF patients has been much debated in recent years. It has been suggested that more follicles at the time of hCG with agonists allow larger numbers of oocytes and a better selection of embryos to be transferred in the uterus.[5] Marci et al evaluated and compared the efficacy of GnRH antagonists with the GnRH agonist long protocol in "normal responder" patients. Significantly shorter stimulation duration was found in the antagonist group and the amount of drugs needed was noticeably lower. The $E_2$ level dosed on the day of hCG administration and the number of oocytes retrieved per cycle, the percentage of metaphase II oocytes and the fertilization rate were similar in both groups. The same number of embryos was transferred under the two stimulation regimens. The implantation rate was found higher, but not statistically significant, in the agonist group.[6] There are some differences between our findings and above studies. Although shorter stimulation duration was found in antagonist group, the number of oocytes and metaphase II oocytes and the number of 2 pronucleated oocyte were significantly higher in antagonist protocol with normal responders. The antagonist protocol for many years has been considered to be safer for OHSS and this consideration makes clinicians more prone to use antagonists which patients have more antral follicle count or which patients have a history of OHSS. In 2006, a meta-analysis reported a significantly lower clinical and ongoing pregnancy rate with the antagonists.[7] In 2011, Cochrane database reported no evidence of a statistically significant difference in rates of live-births or ongoing pregnancy rates in antagonists.[3] This difference was thought to be probably due to the learning curve needed to optimize their administration. In our study the clinical pregnancy, implantations rates are were comparable between luteal long agonist and antagonist.

The best treatment for IVF cycles in poor responders is still controversial. Several stimulation protocols have been proposed to improve implantation and pregnancy rates for this group of patients. There are two prospective, randomized trials comparing the microdose flare-up protocol to the GnRH antagonist protocol, and the long GnRH agonist protocol to the fixed GnRH antagonist protocol in poor responders.[8,9] These studies found no significant difference in clinical pregnancy rates between agonists and antagonists. Previous retrospective studies in poor responder patients have reported a lower cancellation rate, lower gonadotropin consumption, shorter duration of stimulation, and better oocyte retrieval in antagonist cycles, compared to previous cycles using a GnRH agonist.[10,11] In a prospective study comparing the microdose

flare up agonist protocol to the antagonist protocol in IVF poor responders, reported no statistically significant differences between the two groups concerning stimulation, laboratory and pregnancy outcomes.[12] A recent systematic review addressing interventions for poor responders included an analysis of a variety of GnRH analogue schedules, versus GnRH antagonist; low-dose GnRH flare-up versus natural cycle; multiple dose antagonist versus mini-dose long agonist; flare-up agonist versus modified long agonist and long agonist versus modified long agonist. There was no overall difference in oocyte yield, pregnancy rates and cancellation rates.[13] Our results were similar as above; the mean number of oocyte-cumulus complexes, metaphase II oocytes, and two-pronucleated oocytes, number of embryos transferred, implantation and clinical pregnancy rates were similar among three groups. Prapas et al. reported a randomized controlled trial, treated with the long GnRH agonist protocol, and GnRH antagonist protocol. Although clinical pregnancy rates per transfer cycle were not different between the two groups the clinical pregnancy rate per cycle initiated was significantly higher in the agonist compared to the antagonist group.[14]

There is insufficient evidence to recommend GnRH agonist or GnRH antagonist protocols for patients considered "normal responder" and "poor responder". In conclusion, we can assume that ovarian stimulation with GnRH antagonists is a safe, efficient and acceptable treatment.

## Normal ve Düşük Yanıtlı Hastalarda Kontrollü Ovarian Hiperstimülasyon Protokollerinin IVF Sonuçlarının Karşılaştırılması

**AMAÇ:** Normal over yanıtlı hastalarda luteal long agonist ve antagonist protokollerinin, düşük over yanıtlı hastalarda luteal long agonist, antagonist ve mikrodoz flare-up protokollerinin IVF/ICSI sonuçlarına etkisini belirlemek

**GEREÇ VE YÖNTEM:** Bu çalışma Ocak 2005-Aralık 2007 tarihleri arasında Hacettepe Üniversitesi Tıp Fakültesi Tüp Bebek Ünitesinde normal ve düşük over yanıtlı hastalarda yapılmış retrospektif bir değerlendirmedir. Normal over yanıtlı (birinci kol) luteal long agonist protokol ile stimüle edilmiş (193 hasta 300 siklus) ve antagonist protokol ile stimüle edilmiş (215 hasta 300 siklus) hastalar değerlendirilmiştir. Düşük over yanıtlı (ikinci kol) mikrodoz flare-up protokolü ile stimüle edilmiş (27 hasta 74 siklus), luteal long agonist protokol ile stimüle edilmiş (20 hasta 32 siklus) ve antagonist protokol ile stimüle edilmiş (21 hasta 45 siklus) hastalar değerlendirilmiştir.

**BULGULAR:** İlk kolda klinik gebelik, implantasyon ve çoğul gebelik oranları arasında iki stimulasyon protokolü arasında fark saptanmamıştır. İkinci kolda; klinik gebelik, implantasyon ve çoğul gebelik oranları arasında 3 grup arasında fark saptanmamıştır.

**SONUÇ:** Normal ve kötü yanıtlı olgularda agonist protokolün antagonist protokole üstünlüğü gösterilmemiştir.

**Anahtar Kelimeler:** GnRH antagonist, GnRH agonist, Microdoz flare-up, IVF

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