Mini-Dose Long Gonadotropin-Releasing Hormone (GnRH) Agonist Versus Agonist Flare Stimulation Protocol for in Vitro Fertilization Poor Responders

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Abstract

**Aim of the Work:** To compare 2 stimulation protocols, mini-dose long gonadotropin releasing hormone (GnRH) agonist versus agonist flare for in vitro fertilization poor responders.

**Design:** Prospective comparative nonrandomized clinical trial.

**Setting:** Kasr Al Ainy IVF center, Cairo University Teaching Hospital from February 2013 to July 2013 on 50 women undergoing IVF/ICSI fulfilling the criteria of poor responders.

**Material and Methods:** Patients were allocated into 2 groups, group 1 (n=25) received mini-dose long agonist and group 2 (n=25) received agonist flare protocol.

**Main Outcome:** Number of oocytes retrieved (primary outcome), duration of stimulation (days), peak E2 level on the day of hCG injection, number of fertilized oocytes, number of transferred embryos and pregnancy rate/cycle.

**Results:** Both groups were comparable regarding age, body mass index and duration of infertility (years). The difference in basal FSH, duration of stimulation (days) doesn’t reach statistical significance (p-value 0.833 and 0.373 respectively). There was high statistical difference between both groups regarding peak E2 on day of hCG injection, number of oocytes retrieved, number of fertilized oocytes, number of transferred embryos; which is higher in mini-dose agonist group (p-value 0.000).

Pregnancy rate/cycle was higher in mini-dose agonist group (9/25 vs. 6/25) however this difference doesn’t reach statistical significance (p-value 0.355) which may be attributed to small sample size or advanced maternal age.

**Conclusion:** Mini-dose long GnRH stimulation protocol appears to be more beneficial for poor responders than GnRH agonist flare.

**Key Words:** Mini-dose GnRH long agonist — GnRH agonist flare — Poor responders — IVF/ICSI.

Introduction

POOR response is one of the frustrating problems which are faced in COH (controlled ovarian hyper stimulation); it occurs in 10-25% of all IVF cycles [3]; and unfortunately associated with high cancellation rate, low pregnancy rate and in young women it is the first sign of ovarian aging and early menopause.

There is still no universal definition of poor responders and several criteria were used which is badly reflected on its management [2].

Poor response is due to poor ovarian reserve which may be due to woman’s age 40ys, previous ovarian surgery or irradiation, chromosomal anomalies such as Turner Syndrome, endometriosis, benign or malignant ovarian tumors, chemotherapy, decreased number of FSH receptors available in granulosa cells [3], the presence of a special FSH receptor-binding inhibitor in the follicular fluid, and the presence of autoimmune antibodies against granulosa cells [4]; and can be predicted from number of developing follicles and/or the number of oocytes retrieved after conventional stimulation protocol, total dose of gonadotrophins used for ovarian stimulation; Eric et al. [3], define poor response as number of mature follicles <2-5; number of mature follicles

**Abbreviations:**
GnRH : Gonadotropin releasing hormone.
IVF : In vitro fertilization.
ICSI : Intracytoplasmic sperm injection.
HCG : Human chorionic gonadotropin.
FSH : Follicular stimulating hormone.
E2 : Estradiol.
COH : Controlled ovarian hyper stimulation.
AFC : Antral follicle count.
HMG : Human menopausal gonadotropin.
oocytes retrieved 3; Single dominant follicle; Mean daily gonadotropin dose 300 IU; Total gonadotropin dose >40 ampules. And also poor response can be predicted if patient age 40ys [6], previous cancelled IVF cycle due to poor response [7], peak E2 level at time of trigger <300 to 500pg/ml, day 3 FSH >15miu/ml [8], poor or no response to clomiphine challenge test, Anti-mullerian hormone (AMH) level which correlates with antral follicles [9].

Sonographic assessment of ovarian reserve includes decreased antral follicle count, decreased ovarian volume and decreased stromal blood flow [10,11].

Treatment of poor responders to COH who are undergoing IVF remains a challenge and many protocols were proposed to improve the outcome including increase the dose of gonadotropins; but it was found ineffective, use of recombinant purified FSH instead of HMG, luteal initiation of gonadotropins depending on the recruitment of the growing follicles starts in the luteal phase but the results was disappointing [8,12,13].

GnRH agonist flare including short, ultra short and luteal phase agonist including long protocol, agonist stop, mini-dose agonist, antagonist were also tried [14-16].

Addition of adjuvant therapy to the stimulation protocol as growth hormone, corticosteroids, testosterone, aromatase inhibitors and DHEA (dehydroepiandrosterone) were also tried but unfortunately the improvement of the pregnancy rate is quite low and up till now no single protocol is universally perfect for poor responders [17-19].

The aim of our study is to compare 2 stimulation protocols (mini-dose long GnRH agonist vs. agonist flare) in poor responders regarding peak E2 (estradiol) level at time of HCG injection, duration of stimulation (days), number of oocytes retrieved, fertilization rate, number of embryos transferred and pregnancy rate.

Material and Methods

Our study was prospective non randomized comparative study which was conducted in Kasser Al Ainy IVF center, cairo university teaching hospital from February 2013 to July 2013 on 50 women fulfilling the criteria of poor response to COH and candidate for IVF. Group 1 (n=25 women) received mini-dose long GnRH agonist and group 2 (n=25 women) received GnRH agonist flare.

Inclusion criteria: Ovarian volume <3 [20], antral follicle count (AFC) <6 [21,22], day 3 FSH >9IU/l [23] and history of previous cancelled IVF trial due to poor response.

Exclusion criteria: Single ovary, severe endometriosis, endocrine or metabolic disorders.

Our study was approved by the ethical comity of obstetrics and gynecology department of cairo university and fulfilling the ethical considerations in accordance with the Declaration of Helsinki for medical research involving human subjects. An informed consent was taken from all women whom agreed to participate in our study.

Stimulation protocol: In both groups pretreatment with an oral contraceptive pills (OCP) (Gynera, Schering-Plough) in the cycle preceding stimulation. In group (1) triptorelin (decapeptyl 0.1mg, Ferring, Malmo, sweden) was taken s.c daily from day 21 of OCP cycle until menstruation, followed by 0.05mg daily, in group (2) treptorelin (decapeptyl 0.1mg, Ferring, Malmo, sweden) was administrated s.c daily from 1St of withdrawal bleeding till the day of hCG administration.

In both groups gonadotropin (hMG) (Menogon, ferring pharmaceuticals, Germany) was initiated day 3 of the cycle in a dose of 450IU daily intramuscular injection. Once 2 or more leading follicles reach 17-18mm hCG 10,000IU (Pregnyl; NV Organon) was administrated IM.

Cycle monitoring: Serial transvaginal ultrasound to assess follicular growth and serum E2 were done starting day 6 of the cycle and onward, with adjustments of gonadotropin dose and monitoring frequency based on patient response.

Oocyte retrieval: Was performed 35 hours from HCG injection using transvaginal ultrasound guided double lumen needle aspiration under general anesthesia followed by ICSI.

Embryo transfer: 2-3 days after ovum pick up depending on the number and quality of available embryos using labotec catheter (Labotec, Gottingen Germany) under ultrasound guidance.

Luteal phase support: Using progesterone vaginal pessaries (Cyclogest, Alpharma, UK) 400mg twice daily from the day of egg collection till the day of the pregnancy test and continued till 12 weeks gestation if pregnancy is documented.

Pregnancy test was done 2 weeks after oocyte pick-up and ultrasound was done at 6-8 weeks gestation to document gestational sac and fetal pulsation.
In this study a pregnancy was defined as a positive serum HCG test and a sac on ultrasound scan, or an ectopic pregnancy.

The primary outcome is number of oocytes retrieved, secondary outcomes are serum E2 level at time of hCG injection, duration of stimulation, fertilization rate per oocytes, number of embryos transferred and pregnancy rate/cycle.

**Statistical analysis:**

Data were statistically described in terms of mean ± standard deviation (±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t-test for independent samples in comparing 2 groups when normally distributed and Mann Whitney U test for independent samples when not normally distributed. For comparing categorical data, Chi square ($\chi^2$) test was performed. Exact test was used instead when the expected frequency is less than 5. p-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

**Results**

50 infertile women fulfilling the criteria of poor responders to COH were enrolled in the study and assigned into 2 groups, group 1 (n=25 women) received mini-dose long GnRH agonist, group 2 (n=25 women) received agonist flare protocol.

Both groups were comparable regarding age, duration of infertility and body mass index (BMI). Mean age in group (1) was 39.04±1.76 vs. 39.04±1.76 in group (2), p-value 0.750.

Duration of infertility was 3.24±1.2 in group (1) vs. 3.48±1.4 in group (2) with p-value 0.521.

BMI was 25.75±1.69 in group (1) vs. 25.96±1.699 in group (2) with p-value 0.660 (Table 1).

There is no statistical difference between both groups regarding basal FSH level (10.68±1.14 vs. 10.75±1.25; p-value 0.833) and days of stimulation (10.5±1.35 vs. 10.8±1.22; p-value 0.373).

There was difference reaching statistical significance between both groups regarding serum E2 levels on the day of hCG injection (784±1.66pg/ml, 534±1.3pg/ml respectively with p-value 0.00), number of oocytes retrieved (4.52±1.9 vs. 2.36±1.3; p-value 0.00), number of fertilized oocytes (2.8±1.5, 1.4±1.0; p-value 0.00) and number of transferred embryos (2.5±1.12 vs 1.4±1.0; p-value 0.00) (Table 2).

The difference in clinical pregnancy/cycle in both groups doesn’t reach statistical significance (9/25 vs. 6/25; p-value 0.355) (Table 3).

Among group (1) one ET (embryo transfer) was cancelled due to failure of fertilization. Among group (2) 4 ET were cancelled due to failure of fertilization.

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<th>Table (1): Demographic characteristics in both groups.</th>
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<td><strong>Mini-dose long agonist protocol</strong> (n=25)</td>
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<td>Age (years)</td>
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<td>Duration of infertility (years)</td>
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' Statistically non significant.

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<th>Table (2): Clinical and laboratory findings in both groups.</th>
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<td><strong>Mini-dose long agonist protocol</strong> (n=25)</td>
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<td>FSH level</td>
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<td>Duration of stimulation (days)</td>
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<td>E2 level at time of hCG injection (pg/ml)</td>
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<td>Number of oocytes retrieved</td>
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* Statistically significant.

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FSH: Follicular stimulating hormone.

hCG: Human chorionic gonadotropin.

E2 : Estradiol.

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<th>Table (3): Clinical pregnancy in both groups.</th>
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Discussion

Although the lack of uniform definition of poor responders the most common criteria are elevated day 3 FSH, low antral follicle count, low peak E2, large dose of gonadotropins is required for stimulation, low number of oocytes retrieved and history of previous cancelled cycle due to poor response [24].

Unfortunately poor responders are associated with low pregnancy rate and high cancellation rate, many protocols were tried to improve the pregnancy rate and implantation rate in poor responders including increasing the dose of gonadotropins, GnRH agonist (longstop, micro-dose flare protocols), antagonist protocol, addition of adjuvant therapy to stimulation protocols (growth hormone, testosterone, DHEA, aromatase inhibitors) but non them is ideal for all patients [25].

The comparison between short and long protocols in COH for IVF has been studied for many years; In long protocol down regulation of the pituitary was achieved by mid-luteal administration of GnRHa injection and once down regulation have been achieved exogenous gonadotropins is commenced while GnRH a is continued till the day of hCG injection.

The short protocol takes the privilege of initial stimulation of GnRHa on pituitary gonadotropins to promote follicular growth; in general population long protocol is found more superior and more efficient than short protocol however its use in poor responders is controversial [26,27].

Feldberg et al., 1994 stated that decreasing the dose of luteal GnRHa prior to ovarian stimulation (mini-dose GnRHa protocol in which Three treatment protocols of midluteal Decapeptyl (D-Trp6) were compared: [1] a single-dose of 3.75mg; [2] 0.5mg daily until menstruation, followed by 0.1mg daily; and [3] 0.1mg daily until menstruation, followed by 0.05mg daily) decreases the extent of endogenous gonadotropins suppression and at the same time enough to prevents premature ovulation and associated with increased serum E2 level, number of oocytes retrieved, number of embryos available for transfer, implantation, pregnancy rate and decreases the cancelation rate in poor responders; This finding was confirmed by Olivennes et al., 1996 [28,29].

Our study is prospective non randomized study to compare 2 stimulation protocols (mini-dose long GnRHa vs. GnRH agonist flare) in poor responders.50 women fulfilling the criteria of poor responders were recruited and allocated into 2 groups: group (1) (n=25 women) received mini-dose long GnRH a and group (2) (n=25 women) received agonist flare protocol.

Both groups were comparable regarding age, BMI and duration of infertility.

There was no difference of statistical significance between both groups regarding basal FSH and days of stimulation (p-value 0.833 and 0.373 respectively) but there was a highly statistically significant difference between both group regarding serum E2 level on the day of HCG injection, number of oocytes retrieved, number of fertilized oocytes and number of transferred embryos (p-value 0.00) which goes in agreement with Hamed et al., 2008; Marco et al., 2005; Madani et al., 2012; Belaisch et al., 1989 and Toth et al., 1996 [30-34].

There was difference in pregnancy rate/cycle between both groups higher in mini-dose long GnRH a than agonist flare (9/25 vs. 6/25) however this difference doesn’t reach statistical significance (p-value 0.355) this can be explained by small sample size and advanced maternal age (mean age was 39.04±1.76 in mini-dose long agonist and 39.04±1.76 in agonist flare) which is associated with poor implantation in addition.

In our study we found mini-dose long GnRHa is more beneficial for poor responders especially with advanced maternal age and this could be explained as older women has short follicular phase mostly due to early recruitment of follicles in the preceding luteal phase "advanced growth" so the available cohort of antral follicles is lower than younger women [35].

In agonist flare stimulation of follicular recruitment arrives too late because the follicular recruitment in older women had already started in the preceding luteal phase lead to lower number of recruited follicles in contrary to long protocol, mid luteal initiation of GnRHa lead to increased size of follicle cohort recruited and in addition longer period of stimulation allow additional growing follicles to enter the recruited cohort [31].

In addition in our study we used mini dose GnRHa long protocol which is stated by many studies that has better outcome than conventional long protocol [28,29,36].

Our results was in disagreement with Padilla et al., Spandorfer et al., and Akman et al., whom stated that short protocol is more effective in poor
responders. However, Ye H et al., 2001; stated that both protocols are equal [37-40] which may be attributed to the difference in sample size or the difference in the criteria if IVF oor responders.

**Conclusion:** Mini-dose long GnRHa is more beneficial for poor responders especially with advanced maternal age than GnRH agonist flare.

Authors declare no conflicts of interest.

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**References**


