

Research Article

# Ultrashort Flare GnRH Agonist with GnRH Antagonist (MDA/Ant) Protocol Compared with Clomiphene Citrate/ Gonadotropins (CC/GND) for Poor Responder Patients

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## Abstract

**Background:** The ultrashort flare GnRH agonist/ GnRH antagonist protocol (MDA/Ant) has recently been advocated as a useful option for poor ovarian response (POR). POR patients with repeated IVF failures were offered stimulation with MDA/Ant (Group 1) or clomiphene citrate/gonadotropins (CC/Gnd; Group 2).

**Objective:** The aim of this study was to compare Group 1 versus Group 2 in a POR population, from January 1st, 2010 until October 1st, 2014.

**Design:** Retrospective Cohort Analysis.

**Methods:** A total of 116 IVF cycles were included in the study. Group 1 received 21 days of oral contraceptives (OCP's), and were then treated with leuprolide acetate 40 mcg twice a day for the first 3 days, followed by high dose gonadotropins with a flexible start Gonadotropin Releasing Hormone (GnRH) antagonist. Group 2 received CC 100mg x 5 days, and on CC day 4 rec-FSH 600 IU was added.

**Results:** No differences were found in age, body mass index (BMI), day 3 follicle stimulating hormone (FSH), or previous number of failed cycles. There were no differences noted in clinical pregnancy rate or live birth rate. Group 2 required a significantly lower amount of total gonadotropins, but Group 1 had a significantly lower rate of cycle cancellation.

**Conclusions:** Although a higher dose of gonadotropins was required, the significantly lower cancellation rate when compared with Group 2 suggests that the MDA/Ant regimen may be a useful alternative protocol for poor responder patients.

**Keywords:** Assisted Reproductive Technologies (ART); *In-vitro* Fertilization (IVF); Stimulation protocols; Poor responders

## Introduction

Assisted reproductive techniques for the infertile couple are responsible for more than 5 million live births worldwide [1]. The success of these techniques has increased significantly over the years, due to a number of factors, including a better understanding of controlled ovarian hyperstimulation (COH), accompanied by an expansion in the number of protocols in the physician's armamentarium, and ultimately, its successful application in a varied patient population.

Stimulation protocols are tailored to optimize a patient's chances of a successful pregnancy; however, poor responders continue to be a subgroup of patients that have proven very difficult to treat. While many strategies are currently being used to improve the COH response in POR patients, there does not seem to be a particular protocol that has established improved outcomes over any other [2]. Many of the studies evaluating COH protocols for poor responders tend to compare the patient's index cycle to their previously failed cycle, and this approach may challenge the proper evaluation of which treatment may be best for these patients.

The use of a microdose gonadotropin releasing hormone (GnRH) agonist followed by a GnRH antagonist protocol has been recently reported as a useful alternative for the treatment of this difficult group of patients [3]. This novel protocol has been advocated as being able to combine the benefits of oral contraceptive pills (OCP's) pretreatment, the early follicular phase endogenous FSH release resulting from the short microdose GnRH agonist flare effect, with the advantages attributed to the GnRH antagonist protocols. The MDA/Ant regimen has only been compared against the standard microdose flare protocol

in one recent study [4]; however, there are no other published studies comparing it against a different COH protocol.

For women with multiple failed IVF cycles, the clomiphene citrate/ gonadotropins (CC/Gnd) protocol has been shown to result in better embryo quality and improved pregnancy rates when compared with their own previous cycles [5]. At our center, it has been our practice to administer the combination of CC/Gnd to patients who have failed several previous attempts, particularly if they have manifested a poor response to stimulation.

The objective of this study was to compare *in-vitro* fertilization (IVF) outcomes between the recently described MDA/Ant protocol (Group 1) versus the more established CC/Gnd protocol (Group 2) in a poor responder population.

## Materials and Methods

A retrospective analysis was performed of all cycles that included

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the use of MDA/Ant and CC/Gnd protocols at a university-based infertility clinic during the period of January 1, 2010 and October 1, 2014. Due to the retrospective nature of this study, the use of either protocol was based on physician's preference. Approval was obtained by the Institutional Review Board of the University of Connecticut. This study included a total of 116 IVF cycles with anticipated poor response, defined as: previous poor response to ovarian stimulation ( $\leq 4$  oocytes retrieved), or previously canceled cycle(s) due to inadequate ovarian response after aggressive ovarian stimulation (defined by no follicular development or  $< 4$  developing follicles).

In Group 1 (n=69), patients took OCPs for approximately 21 days (range 15-25 days). Three days after the last pill, patients began taking microdose leuprolide acetate 40 mcg twice a day (BID) for 3 days. The next day, patients stopped the microdose leuprolide administration, and started recombinant follicle stimulating hormone (FSH) (rec-FSH; Follistim, Organon Pharmaceuticals, West Orange, NJ; or Gonal-F, Serono Pharmaceuticals, Rockland, MA) with or without the addition of human menopausal gonadotropins (hMG) (Menopur, Ferring Pharmaceuticals, Tarrytown, NY) for a total gonadotropin dose of 450-600 IU/day, at physician discretion. The gonadotropin regimen was maintained daily and adjusted individually according to serum estradiol (E2) concentrations and ovarian response as noted by ultrasound. When follicles reached  $\geq 14$  mm in mean diameter and/or E2  $\geq 350$  pg/ml, patients started ganirelix acetate (Ganirelix; Organon Pharmaceuticals, West Orange, NJ) 0.25 mg subcutaneous (SC) daily. When at least 2 leading follicles reached  $\geq 17$  mm in diameter, human chorionic gonadotropin (hCG) 5,000-10,000 IU was administered, and oocyte retrieval was performed 35 hr later.

Stimulation in Group 2 (n=47) started on the second day of menses with CC 100 mg PO daily x 5 days. On the fourth day of CC, patients started rec-FSH with or without the addition of hMG for a total gonadotropin dose of 450-600 IU/day. Individual adjustments in dose were made in the same fashion as mentioned above. When follicles reached  $\geq 14$  mm in mean diameter and/or E2  $\geq 350$  pg/ml, patients started ganirelix acetate (Ganirelix; Organon Pharmaceuticals, West Orange, NJ) 0.25 mg SC daily. When 2 leading follicles reached  $\geq 20$  mm in diameter, hCG 5,000-10,000 IU were administered, and oocyte retrieval was performed 35 hr later.

Embryos were transferred on the third or fifth day after retrieval, with the number of embryos depending on embryo quality and patient's age according to the American Society of Reproductive Medicine (ASRM) guidelines [6]. All patients received 50 mg of progesterone (P) in oil intramuscular (IM) daily for luteal support, which was initiated the day after oocyte retrieval. This was continued until a negative serum pregnancy test ( $\beta$ hCG) was obtained. If the serum pregnancy test was positive ( $\beta$ hCG  $> 5$  IU/L), P was continued at least until a transvaginal ultrasound confirmed fetal heart activity. Clinical pregnancy was defined as a normal gestational sac measured with a transvaginal ultrasound after 5 weeks.

The primary outcome measure was live birth rate. Secondary outcome measures included: total amount of gonadotropins used, number of oocytes retrieved, number of mature oocytes retrieved, cycle cancellation rate, implantation rate, and clinical pregnancy rate.

The  $\chi^2$ -test, Fisher's exact test, and independent Student's t-tests were used for statistical analysis as appropriate, and a p value  $\leq 0.05$  was considered to be statistically significant. Data are presented as the mean  $\pm$  SD. All statistical analyses were performed in SPSS software (SPSS Inc, Chicago, IL).

## Results

A total of 116 IVF cycles were included in this study, 69 cycles in Group 1 and 47 cycles in Group 2. Amongst the two treatment groups, there were no differences in patient characteristics including age, BMI, day 3 FSH, or the number of previously failed IVF cycles (Table 1).

There were no differences noted in the total number of days of stimulation, peak estradiol (E2) levels, mean number of total oocytes and mature oocytes obtained (Table 2). Group 1 required a significantly higher amount of gonadotropins when compared with Group 2 ( $6,644 \pm 1,451$  vs.  $4,423 \pm 1,360$  IU,  $p < 0.01$ ). The cancellation rate was significantly lower in Group 1 when compared with Group 2 (17% (12/69) vs 36% (17/47),  $p = 0.03$ ). Criteria for cycle cancellation were either lack of response to gonadotropins, or less than four developing follicles observed during stimulation. Of those cycles where a transvaginal oocyte retrieval was performed, 21% in Group 1 and 30% in Group 2 (12/57 vs. 9/30,  $p = 0.4$ ) did not undergo embryo transfer due to either lack of oocytes retrieved (Group 2, n=3), failed embryo development (Group 1, n=6; Group 2, n=3), or failed fertilization (Group 1, n=6; Group 2, n= 3).

There were no differences noted in the number of embryos transferred in Group 1 vs Group 2 ( $2.5 \pm 1.2$  vs  $2.4 \pm 1.4$ ,  $p = 0.7$ ), as well as no differences noted in the fertilization, implantation, clinical pregnancy, or the live birth rates (see Table 2).

## Discussion

To our knowledge, this is the first study to compare the novel MDA/Ant protocol with an established treatment (CC/Gnd) in poor responder patients. The definition of poor responder patients remains varied in the literature, and the heterogeneity of inclusion criteria has made the comparison between various treatment approaches difficult. More recently, the Bologna criteria has attempted to give a standardized definition, so that results from the literature may be compared

	Group 1 (MDA/Ant) (n=69)	Group 2 (CC/Gnd) (n=47)	P-Value
Age (years)	39.3 $\pm$ 3.5	39.9 $\pm$ 3.6	NS
BMI	25.5 $\pm$ 4.9	27.9 $\pm$ 6.4	NS
Day 3 FSH (mIU/mL)	9.2 $\pm$ 3.9	9.8 $\pm$ 3.8	NS
Previous No. of Failed Cycles	2.8 $\pm$ 2	2.4 $\pm$ 1.2	NS

Table 1: Characteristics of Patients Undergoing Group 1 vs. Group 2.

	Group 1 (n=69)	Group 2 (n=47)	P-Value
Number of Days of Stimulation	11 $\pm$ 2.4	10 $\pm$ 2.3	NS
Amount of Total Gonadotropins (IU)	6,644 $\pm$ 1,451	4,423 $\pm$ 1,360	$< 0.01$
Peak E2	1150 $\pm$ 602	1035 $\pm$ 590	NS
Mean Oocytes Retrieved	6.4 $\pm$ 3.4	5.7 $\pm$ 3.2	NS
Mean Mature Oocytes Retrieved	4.8 $\pm$ 2.7	3.9 $\pm$ 2.8	NS
Mean Embryos Transferred	2.5 $\pm$ 1.2	2.4 $\pm$ 1.4	NS
Cycle Cancellation Rate (%)	17 (12/69)	36 (17/47)	0.03
Fertilization Rate (%)	67 (184/274)	66 (75/113)	NS
Implantation Rate (%)	48 (12/29)	42 (5/12)	NS
CPR/ per ET (%)	29 (12/41)	29 (5/17)	NS
CPR/ per initiated cycle (%)	17 (12/69)	11 (5/47)	NS
Live Birth Rate (LB/ ET %)	24 (10/41)	18 (3/17)	NS
LBPR/ per initiated cycle (%)	14 (10/69)	6 (3/47)	NS
Spontaneous Abortion Rate (%)	17 (2/12)	40 (2/5)	NS

Table 2: IVF Outcomes for Group 1 vs. Group 2.

uniformly [7]. Poor responder patients represent a challenging treatment group for the reproductive endocrinologist. They typically require large amounts of gonadotropins when compared to normal or high responders, and often their cycles may lead to a low number of oocytes retrieved, with consequently few embryos available for transfer, or even cycle cancellation, resulting in a disappointing outcome for the patient and physician, as well as the potential need for multiple IVF cycles.

Many different strategies have been developed to help treat this difficult group of patients. Some of these include increasing the dose of gonadotropins, decreasing the dose of the GnRH analog used for pituitary suppression, the use of OCP's combined with microdose GnRH agonist, GnRH antagonists, as well as the use of oral medications (letrozole and clomiphene citrate) in combination with high dose gonadotropins [2].

In 1995, Benadiva et al. published a study of 93 poor responder patients who were stimulated with a combination of CC and gonadotropins [5]. Their results showed significantly increased pregnancy rates for these patients when compared with previous cycles. This protocol takes advantage of the cumulative effect of circulating clomiphene citrate along with the administration of high dose gonadotropins. The idea behind using the combination treatment is that CC stimulates the endogenous release of FSH and LH through negative hypothalamic-pituitary feedback. These endogenous gonadotropins are then potentiated by the administration of exogenous FSH/LH administered during the second part of the COH, maximizing the ovarian stimulatory effect.

The concept of using a short-term microdose GnRH agonist followed by a GnRH antagonist during stimulation has recently been presented as an additional alternative protocol for poor responders [3,4,8,9]. This protocol combines the benefit of the initial stimulatory effect of the agonist flare on endogenous FSH, with the LH suppression provided by the antagonist. In 2008, Orvieto et al. [9] published a study assessing poor responder patients with repeated IVF failures and poor embryo quality. In this study, the authors compared the use of the ultrashort GnRH-agonist combined with the flexible GnRH-antagonist in 21 poor responder patients with the patient's previous cycle. They found a significantly greater number of follicles larger than 14 mm, a higher number of oocytes retrieved and embryos transferred, and a reasonable clinical pregnancy rate (14.3%) [9]. In a follow up manuscript, the same authors reported that the use of the ultrashort flare GnRH-agonist combined with flexible multidose GnRH-antagonist for patients with repeated IVF failures again showed improvement when compared to the patient's previous cycle [8]. Both studies included a small number of patients, and the comparison to the patient's previous failed cycle could not allow for a meaningful interpretation of the potential benefit of this treatment.

These were then followed by a preliminary study published in 2010 by Berker et al. [4] which demonstrated that the agonist-antagonist protocol was not inferior to the microdose flare-up protocol in 82 women using ICSI. The primary outcome was the mean number of mature oocytes, and the authors found that despite the need for an increased amount of total gonadotropins with the MDA/Ant protocol, there were no differences with respect to the number of eggs fertilized, number of embryos transferred, as well as implantation and clinical pregnancy rates. While this has been the only study to date which has compared the MDA/Ant protocol to another established protocol for poor responders, neither group received pre-treatment with oral contraceptives, as initially described by Scott et al. [10] and others [11,12], and therefore the results may not be generalizable.

The benefits of using either a GnRH antagonist protocol or a microdose leuprolide acetate protocol for poor responders have been explored by other authors. Mahutte et al. [13] described several advantages with the use of GnRH antagonists for poor responders including a shorter duration of stimulation, a decrease in the total amount of gonadotropins, lower cost, and a shorter interval between successive treatment cycles. In addition, it has been shown that the pretreatment with oral contraceptives, which is used in a microdose leuprolide acetate protocol, suppresses the luteal-follicular FSH rise [14]. The benefits associated with combining these two protocols include synchronization of the follicular cohort by pretreatment with OCP's, blunting of the LH flare resulting in the rise of early follicular progesterone and androgen levels [15], and the incorporation of GnRH antagonists to the protocol provides immediate LH suppression with the possible improvement in embryo quality [16].

The aim of this study was to evaluate IVF outcomes after the microdose GnRH agonist/ antagonist protocol versus the combination of CC/gonadotropins in a poor responder population. Our results demonstrated that while a higher dose of gonadotropins was required, the significantly lower cancellation rate when compared with CC/Gnd suggests that the MDA/Ant regimen may be a useful alternative for this difficult group of patients. The higher dose of gonadotropins may be explained by the fact that during the first three days of stimulation, clomiphene citrate is used alone in the absence of injectable gonadotropins. The decrease in the cancellation rate seen with MDA/Ant may have to do, at least in part, with the use of pre-treatment with OCP's allowing for a more uniform follicular recruitment.

This study is retrospective and is limited by its small size, and therefore may result in a Type II error. A post-hoc sample size calculation was performed, using live birth rates per initiated cycle, and 220 patients would have been required to achieve a power of 0.80 with a p-value of 0.05. Further studies are required to confirm which regimen should be considered the most effective treatment in this patient population. In addition, it may be useful to add another control group treated with MDA/Ant protocol, but less total gonadotropins. Nevertheless, it is the first to compare the use of this novel protocol versus CC/gonadotropins for poor responders. As poor responders may fail several cycle attempts, having alternative choices to offer may allow improvement in outcomes for this difficult group of patients. This study reaffirms the benefit of the microdose agonist / antagonist protocol as an effective treatment option among COH protocols for poor responder patients undergoing ART. Further studies are required to confirm which regimen should be considered the most effective treatment in poor responder patients.

## References

1. <https://www.eshre.eu/Guidelines-and-Legal/ART-fact-sheet.aspx>
2. Surrey ES, Schoolcraft WB (2000) Evaluating strategies for improving ovarian response of the poor responder undergoing assisted reproductive techniques. *Fertil Steril* 73: 667-676.
3. Orvieto R, Laufer N (2014) Ultrashort flare gonadotropin-releasing hormone (GnRH) agonist/GnRH antagonist protocol: a valuable tool in the armamentarium of ovulation induction for in vitro fertilization. *Fertil Steril* 102: 1254-1255.
4. Berker B, Duvan CI, Kaya C, Aytaç R, Satiroglu H (2010) Comparison of the ultrashort gonadotropin-releasing hormone agonist-antagonist protocol with microdose flare-up protocol in poor responders: a preliminary study. *J Turk Ger Gynecol Assoc* 11: 187-193.
5. Benadiva CA, Davis O, Kligman I, Liu HC, Rosenwaks Z (1995) Clomiphene citrate and hMG: an alternative stimulation protocol for selected failed in vitro fertilization patients. *J Assist Reprod Genet* 12: 8-12.
6. Practice Committee of American Society for Reproductive Medicine; Practice Committee of Society for Assisted Reproductive Technology (2013) Criteria for number of embryos to transfer: a committee opinion. *Fertil Steril* 99: 44-46.

7. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, et al. (2011) ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod* 26: 1616-1624.
8. Orvieto R, Nahum R, Rabinson J, Gemer O, Anteby EY, et al. (2009) Ultrashort flare GnRH agonist combined with flexible multidose GnRH antagonist for patients with repeated IVF failures and poor embryo quality. *Fertil Steril* 91: 1398-1400.
9. Orvieto R, Kruchkovich J, Rabinson J, Zohav E, Anteby EY, et al. (2008) Ultrashort gonadotropin-releasing hormone agonist combined with flexible multidose gonadotropin-releasing hormone antagonist for poor responders in in vitro fertilization/embryo transfer programs. *Fertil Steril* 90: 228-230.
10. Scott RT, Navot D (1994) Enhancement of ovarian responsiveness with microdoses of gonadotropin-releasing hormone agonist during ovulation induction for in vitro fertilization. *Fertil Steril* 61: 880-885.
11. Surrey E, Bower J, Hill D, Ramsey J, Surrey M (1998) Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. *Fertil Steril* 69: 419-424.
12. Schoolcraft W, Schlenker T, Gee M, Stevens J, Wagley L (1997) Improved controlled ovarian hyperstimulation in poor responder in vitro fertilization patients with a microdose follicle-stimulating hormone flare, growth hormone protocol. *Fertil Steril* 67: 93-97.
13. Mahutte NG, Arici A (2007) Role of gonadotropin-releasing hormone antagonists in poor responders. *Fertil Steril* 87: 241-249.
14. Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, et al. (1993) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod* 8: 1061-1066.
15. Takahashi K, Mukaida T, Tomiyama T, Goto T, Oka C (2004) GnRH antagonist improved blastocyst quality and pregnancy outcome after multiple failures of IVF/ICSI-ET with a GnRH agonist protocol. *J Assist Reprod Genet* 21: 317-322.
16. Keltz MD, Gera PS, Skorupski J, Stein DE (2007) Comparison of FSH flare with and without pretreatment with oral contraceptive pills in poor responders undergoing in vitro fertilization. *Fertil Steril* 88: 350-353.