

# Comparison between micro-dose GnRH agonist and GnRH antagonist protocol in poor responders undergoing intracytoplasmic sperm injection with embryo transfer

Moez Kdous, Ghaya Merdassi\*, Fethi Zhioua

Aziza Othmana Hospital, Department of Obstetrics and Gynecology, IVF Center, 1008, Tunis, Tunisia

## Email address:

kdousm@yahoo.fr (M. Kdous), merdassighaya@yahoo.fr (G. Merdassi), zhiouafethi@voila.fr (F. Zhioua)

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**Abstract:** Objective: To compare the effect of the multiple-dose GnRH antagonist protocol with the microdose GnRH agonist flare-up (MF) protocol in poor ovarian responders for ICSI. Design: Retrospective, Clinical study. Setting: IVF center. Patient(s): Two hundred eighty-six poor responder patients undergoing ICSI-embryo transfer cycle. Intervention(s): one hundred Forty four patients (144 cycles) included in group 1 (microdose GnRH-a flare-up protocol) and one hundred forty two patients (42 cycles) included in group 2 (multiple dose GnRH antagonist protocol). Main Outcome Measure(s): Serum E2 levels, number of growing follicles and mature oocytes, embryo quality, dose of gonadotropin used, cancellation, fertilization, implantation rate, pregnancy rate (PR) and live birth rate (LR). Results: Total gonadotropin dose used was significantly lower ( $2730 \pm 235.45$  vs  $3189 \pm 317.81$ ;  $P < 0.05$ ), duration of stimulation was significantly longer and E2 level was significantly higher in group 1. The mean number of oocytes retrieved, mature oocytes retrieved, embryos transferred and the rate of at least one top-quality embryo transferred was similar in the two groups. The implantation rate was significantly higher in the microdose flare-up group than in the multiple-dose antagonist group (18.79% vs 8.28%;  $P < .001$ ). Clinical pregnancy and live birth rates were similar in the two groups. Conclusion(s): We achieved comparable pregnancy and live birth rates in poor responders with the use of either GnRH antagonist or flare protocol. However, a significantly higher gonadotropin dose used and lower implantation rate in the antagonist group tips the balance in favor of the flare-up protocol.

**Keywords:** Cetorelix, GnRH Antagonist, IVF, Embryo, Micro-Dose GnRH Agonist Flare-Up, Poor Ovarian Response

## 1. Background

Despite considerable advances in assisted reproductive techniques (ART), management of poorresponder patients is still a challenge. Although there is lack of uniform definitions, poor response to controlled ovarian hyperstimulation (COH) can be generally defined as unsatisfactory ovarian response in terms of low number of follicles developed, low serum E<sub>2</sub> levels, and low number of oocytes retrieved despite adequate ovarian stimulation. During the ESHRE Campus Workshop, recently held in Bologna (Italy) on March 2010 [1] to discuss the definition and the diagnosis of poor responders, consensus criteria were established under the term of “Bologna Criteria”. According to this consensus, the definition of poor responders lies on two of the following three criteria:

- Advanced age  $\geq 40$  years or other risk factors of poor

ovary response (Genetic anomalies, pelvic infection and surgery, endometriosis and chemotherapy).

- Poor ovary reserve (AFC  $< 5$ -7 follicle or AMH  $< 0.5$ -1.1 ng/ml).
- A history of poor ovarian response to stimulation ( $\leq 3$  oocytes with a conventional stimulation protocol)

Many treatment modalities have been suggested to improve ART outcomes in poorresponders. At present, two protocols are popular: microdose GnRH-a flare-up and multiple dose GnRH antagonist protocols [2].

The aim of this study was to compare the efficacy of the microdose flare-up and multiple-dose antagonist protocols in patients with poor ovarian response undergoing intracytoplasmic sperm injection (ICSI) and embryo-transfer (ET) cycles.

## 2. Material and Methods

### 2.1. Patients and Study Design

A total of 286 patients, who were recognized as poorresponders, were enrolled from our computerized IVF database during the time period from July 2008 to December 2010.

- Inclusion criteria: two of the following three criteria inspired from "bologna criteria":
- Age  $\geq 40$  years
- Antral Follicle Count (AFC)  $\leq 5$  for the two ovaries.
- Failure of anterior ovarian stimulation: cycle cancellation (average gonadotrophins dose  $\geq 225$  UI/day); or less than 3 oocytes collected during an anterior cycle.
- Exclusion criteria:
- Polycystic ovary syndrome (PCOS) diagnosed according to the criteria set by the Rotterdam Conference 2003 [3],
- Stage III–IV endometriosis according to the revised American Fertility Society classification (1985),
- Inflammatory, autoimmune, or chromosomal disorders
- Endocrine or metabolic disease, including hyperprolactinemia
- Unique ovary.

During the same time interval, 144 patients (144 ICSI cycles) underwent controlled ovarian hyperstimulation (COH) using the microdose GnRH-a flare-up protocol, and 142 patients (142 ICSI cycles) underwent COH using the multiple dose GnRH antagonist protocol. The assignment of the patients to the microdose flare-up or multiple-dose antagonist protocols was made on the physician's preference.

### 2.2. Ovarian Stimulation and Patient Procedures

The first group (group 1) consisted of 144 patients in 144 cycles in whom triptorelin (Decapeptyl, 0.05 mg; Ipsen, Paris, France) was initiated on the 1<sup>st</sup> day of menstruation, followed by exogenous gonadotropins administered from the 2nd day of menstruation. The second group (group 2) consisted of 142 poor responders in 142 cycles in whom the exogenous gonadotropins were started on the first day of menstrual cycle, and later cetrotirelix 0.25 mg (Cetrotide, Serono, UK) was administered daily until the hCG injection once the leading follicle reached 14 mm in diameter. No oral contraceptive pretreatment was employed in all cases.

In both stimulation regimens, at least 300 IU of starting daily gonadotropins recombinant FSH (Gonal-F; Serono) was administered to all patients, with individual adjustments performed based on ovarian response. When the leading follicle reached 19 to 20 mm in diameter, 10,000 IU of hCG was administered; ultrasound-guided transvaginal oocyte retrieval was performed 36 hours later. ICSI was performed for all metaphase II oocytes according to our clinical policy, and ET for good-quality embryos was performed on day 3 for all patients. Luteal phase supplementation was performed

in both groups by administering 600 mg/day of intravaginal micronized progesterone. Supplementation with progesterone was continued for at least 2 weeks. Pregnancy test was done 2 weeks after embryo transfer and ultrasound scan to confirm the number of sacs and fetal viability was performed at 6 weeks gestation.

### 2.3. Outcome Measures

The main outcome measure in this study was the number of oocytes retrieved. Other outcome measures were Number of mature oocytes retrieved, number of fertilized oocytes, number of embryos transferred, stimulation duration (days), total dose of gonadotrophin IU, estradiol concentration and endometrial thickness on the day of hCG administration, cycle cancellation rate, fertilization rates (%) (Ratio of number of two pronuclear oocytes to number of cumulus-oocytes complexes), implantation rate (%) (Ratio of number of gestational sacs to number of embryos transferred), and clinical pregnancy rate (PR) per cycle and per ET (clinical pregnancy was diagnosed by the visualization of fetal cardiac activity on ultrasound scan).

### 2.4. Statistical Analysis

Data are expressed as the mean  $\pm$  SD or percentages. The  $\chi^2$ -test, Fisher's exact test, and Student's *t*-test were used for statistical analysis with SPSS software, version 15.0 for Windows (SPSS, Inc, Chicago, IL, USA).  $P < .05$  was considered statistically significant

## 3. Results

The baseline cycle characteristics for the two study groups are presented in Table 1. The groups were similar in respect to age, BMI, duration of infertility, basal FSH levels, basal E2 levels and AFC

No statistically significant differences between the protocol types were noted with respect to, cycle cancellation rates. In group 1, a total of 16 cycles were cancelled (4 cycles owing to premature LH surge, 8 to poor folliculogenesis, and 4 to fertilization failure), while in the multiple-dose antagonist group a total of 12 cycles were cancelled (4 cycles owing to arrested embryo development and 8 to poor folliculogenesis)

In group 1, total gonadotropin dose used was significantly lower ( $2730 \pm 235.45$  vs  $3189 \pm 317.81$ ;  $P < 0.05$ ), the duration of stimulation was significantly longer than in group 2 ( $11.39 \pm 0.79$  vs  $9.64 \pm 0.49$  days;  $P < .001$ ) and E2 level on the day of hCG administration was significantly higher ( $1534.27 \pm 1034.34$  vs  $876.08 \pm 519$ ;  $P < .001$ ). The mean number of oocytes retrieved, mature oocytes retrieved, embryos transferred and the rate of at least one top-quality embryo (Less than 15% fragmentation, blastomere number  $\geq 4$  with equivalent size) transferred was similar in the two groups (Table 2)

**Table 1.** The baseline characteristics of the two study groups.

	GnRH agonist flare	GnRH antagonist	P value
Number of cycles	144	142	
Age (years)	40,09±6,59	41,04±1,71	NS
BMI (Kg-m <sup>-2</sup> )	25,35±4,09	26.64±2,59	NS
Duration of infertility (years)	2.1 ± 2.5	3.1 ± 2.7	NS
Mean basal FSH (mIU/mL)	10,01±2,75	10,60±2,96 UI/L	NS
Mean basal E2 (pg/mL)	51.3 ± 19.7	52.2 ± 18.7	NS
AFC	3,8±1,16	3,7±0,96	NS

Note: Values are means (±SD) unless otherwise indicated. NS = not significant

**Table 2.** Comparison of cycle characteristics and embryological data in the GnRH antagonist and GnRH agonist flare groups.

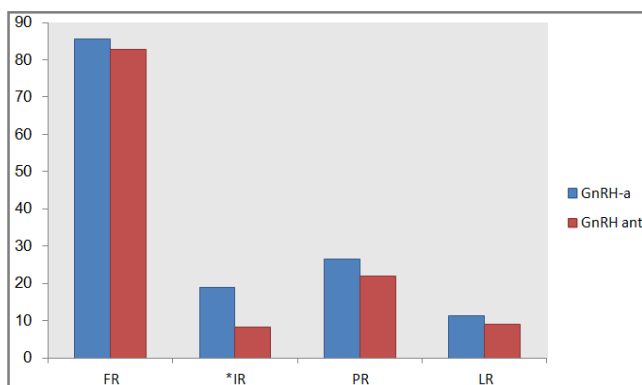
	GnRH agonist flare	GnRH antagonist	P value
Number of cycles	144	142	
Total gonadotropins used (UI)	2730±235,45	3189±317,81	<0.05
E2 level on the day of hCG administration, pg/mL	1534,27±1034,34	876.08±519	<0.001
Duration of stimulation (days)	11,39±0,79	9,64±0,49	<0.001
Endometrial thickness on the day of hCG administration, mm	11.1±2.8	11.5±2	NS
Number of oocytes retrieved	7,64±3,70	7,54±4,93	NS
No. of mature oocytes	5,88±4,38	5,41±4,10	NS
Number of embryos transferred	2.6± 2.3	2.4 ± 1.7	NS
Rate of at least one top-quality embryos transferred, %	49.6%	55.2%	NS
Cycle cancellation, %	9,09%	7,01%	NS

Note: Values are means (±SD) unless otherwise indicated. NS = not significant

**Table 3.** Fertilization, implantation, pregnancy and live birth rates in both groups of the study.

	GnRH agonist flare	GnRH antagonist	P value
Number of cycles	144	142	
Fertilization rate, %	85,71%	82.76%	NS
Implantation rate, %	18.79%	8.28%	<0.01
Clinical pregnancy/ET, %	26.60%	21.87%	NS
Clinical pregnancy/cycle, %	18.18%	15.90	NS
Live birth rate %	11,36%	9,09%	NS

NS = not significant

**Figure 1.** Fertilization (FR), implantation (IR), pregnancy (PR) and live birth rates in GnRH-a and GnRH-antagonist groups. Blue bars, GnRH-a; Red bars, GnRH antagonist. \* $P<0.01$ 

The fertilization rate was not significantly different between groups. Although the mean number and the quality of transferred embryos was similar in the two groups, the implantation rate was significantly higher in the microdose flare-up group than in the multiple-dose antagonist group (18.79% vs 8.28%;  $P<0.001$ ). However, the clinical pregnancy

and the live birth rates were similar in the two groups (Table 3, Fig 1)

## 4. Discussion

Microdose flare-up and GnRH antagonist protocols are currently the most common COH protocols used in poor ovarian responders. With the microdose flare-up (MF) protocol, the initial release of endogenous gonadotropins induced by low-dose GnRH-a administration in the early follicular phase enhances ovarian response to the subsequent administration of high-dose exogenous gonadotropins. Several studies have reported improved ovarian response and clinical outcomes using this protocol [4], [5], [6], [7] and [8]. The GnRH antagonist regimen allows for a more natural recruitment of follicles in the follicular phase in an ovary that has not been suppressed by the absence of FSH and LH caused by GnRH-a [9]. In addition, the introduction of the GnRH antagonist during stimulation prevents premature LH surges. So, the results for GnRH antagonists for poorresponders indicate the possibility of reducing the amount of gonadotropins, the length of stimulation and the

number of cancelled cycles. To our knowledge, there are only a few studies comparing flare-up versus the GnRH antagonist protocol in poorresponders [10], [11], [12], [13], [14] and [15]. There is still controversy on the efficacy of these two regimens for poorresponders.

Deplacido and al [13] suggested that the antagonist protocol compared with the short GnRH-a protocol led to a significant improvement in the oocyte quality and maturation process, which in turn resulted in a significant increase in the mean number of mature oocytes. Conversely, no statistically significant difference in the total number of cumulus oocyte complexes (COCs) retrieved ( $6.79 \pm 3.89$  vs.  $6.54 \pm 3.08$ , respectively) was observed between groups. Schmidt and al [12] in a study comparing the effect of microdose leuprolide versus ganirelix revealed that there were no significant differences in the two ovarian stimulation protocols in terms of number of oocytes retrieved, fertilization and clinical pregnancy rates.

Akman and al. [10] reported a significant increase in the total number of oocyte retrieved and a non significant increase in the total gonadotropin dose used and fertilization and clinical pregnancy rates in the microdose flare-up group compared with the antagonist group. In addition, Malsumi and al. [11] supported the former study findings by reporting a significant increase in the total number of oocytes retrieved, the number of high-quality embryos, and a significant decrease in the total gonadotropin dose used in the flare-up group. Our data are well correlated with those of De placido and Schmidt, and in contrast with those of, Akman and Malsumi that found significant differences in the number of oocytes retrieved.

We did not find a difference in terms of number of mature oocytes ( $5.88 \pm 4.38$  vs  $5.41 \pm 4.10$  oocytes ; NS). Our results are confirmed by the meta analysis of Franco [16] (WMD:0.07, 95% CI de -0.38 à 0.53). Nevertheless, De Placido [13], in his prospective randomized study reported a significant increase in the mean number of mature oocytes with the antagonist protocol ( $6.09 \pm 3.36$  vs  $5.02 \pm 1.86$ ,  $P < 0.05$ ). The authors concluded that their results could be related to the LH supplementation in the GnRH antagonist protocol. The addition of LH in GnRH-ant cycles (where > 80% of LH release is abolished in the first 24 hours), could be crucial for sustaining E<sub>2</sub> synthesis and paracrine production of peptides that are necessary for oocyte differentiation, while, during the short flare-up protocol an excessive increase of LH may induce follicle degeneration.

In the present study, although the amount of ampules units of FSH administered were significantly ( $P < .005$ ) higher in the GnRH antagonists than in the flare-up group, the serum E<sub>2</sub> levels on day of hCG administration were significantly higher ( $P < .005$ ) in the flare-up than the GnRH-antagonist groups. Similar results are reported in some published studies [10], [17] and [18]. This can be easily explained by the fact that a flare-up regimen with GnRH agonist induces a greater additional gonadotropin stimulus consequently, a reduction in the number of FSH ampules. Our data are in contrast with those of Akman and al. [10] that found no significant

differences in the number of FSH ampules administered. However, in the last study, in the GnRH agonist regimen, an oral contraceptive was started on cycle day 1 of the previous cycle for 21 days. It is probable that this OP pituitary suppression prior to stimulation in a flare-up regimen may explain an increase in the number of FSH ampoules used.

We found that the duration of ovarian stimulation was significantly longer in the agonist group flare-up. Although this did not have an overall impact on the total gonadotropin dose used, we believe that it might be the reason for a significantly higher peak E<sub>2</sub> in this group. This difference, however, is in agreement with previous findings [13] and [19] and is not unique to poorresponders [20].

A comparison of both groups in terms of fertilization rate showed no significant difference. This result is in agreement with previously reported data [10] and [12] except of Malmusi [21] who finds a significantly higher fertilization rate in the short protocol (84% vs 63%;  $p < 0.01$ ). However, a significant increase in the implantation rate was reported in our study with the microdose GnRH agonist group (18.79% vs 8.28%;  $P < 0.01$ ). To our knowledge this result has been shown in only 2 previous randomized study [22] and [23]. This high implantation rate that resulted from the microdose GnRH analogue used in the follicular phase may be explained by a reduction in endothelial nitric oxide synthase levels that may cause improvement in the implantation rates for poorresponders in IVF cycles. Furthermore, GnRH antagonists are a potent inhibitor of the cell cycle because they decrease the synthesis of locally produced growth factors in a dose-dependent manner ([24], [25], [26], [27], [28] and [29]). Binding sites for GnRH have been demonstrated in ovary, testis, uterus, and human endometrium of fertile patients ([26], [27] and [30]). Given that the GnRH receptors are present in all these cells and tissues, an interaction between the GnRH antagonist and the GnRH receptor is possible and manifested as lower implantation rates [31]. The GnRH antagonist decreases the production of E<sub>2</sub> by the granulosa compartment; hence, the circulating concentrations of this steroid may be insufficient to develop an ideal endometrium to maintain the life of the incipient human embryos [31]. These effects may explain the lower implantation and pregnancy rates in IVF cycles.

In the present study, similar cycle cancellation rates are showed in the two groups and a higher, but not significantly higher, clinical pregnancy and live birth rates were observed in the GnRH-a compared with GnRH-antagonist group. our findings appear to confirm the conclusion of some already-published reviews indicating lower pregnancy and live birth rates in GnRH-antagonist cycles as compared with GnRH-a cycles ([32] and [33] [34]). However, in a retrospective analysis of poorresponders, the clinical pregnancy and implantation rates appeared higher after GnRH-antagonist treatment than with GnRH-a treatment, but these were not statistically significantly different [35], indicating an existing controversy in this topic.

Our review included 286 patients, who received either the GnRH-a flare or the GnRH antagonist protocol, based on

physician preference. One of the limitations of our study was that the individual practitioners selected whether the GnRH-a flare or the antagonist protocol was used. Although the patients cannot be randomized in a retrospective analysis such as this, the two patient populations had similar baseline characteristics, which makes it possible to compare outcomes between the two groups. Our data in this study, we noted a significantly lower gonadotropin dose used, higher duration of stimulation, higher E2 level on the day of hCG administration and higher implantation rate with the microdose flare-up group.

## 5. Conclusion

In this study, we achieved comparable pregnancy and live birth rates in poor responders with the use of either GnRH antagonist or flare protocol. However, a significantly higher gonadotropin dose used and lower implantation rate in the antagonist group tips the balance in favor of the flare-up protocol. To our opinion, there is no one controlled ovarian hyper stimulation (COH) protocol which is best suited for all poor responders. Prediction of compromised response prior to cycle initiation by a thorough assessment of ovarian reserve as well as a careful review of past response should therefore allow for selection of the appropriate protocol for each individual patient. Furthermore, choosing the protocol should be also based on other practical considerations such as cost, ease of use, and ability to administer the GnRH antagonist in a timely fashion. Further prospective research is needed to assess the benefits of modifications in both GnRH antagonist and GnRH flare protocols in poor responders.

## Authors' Contributions

MK carried out the statistic tests and drafted the manuscript. MG participated in the design of the study and helped to draft the manuscript. ZF participated in the design and the draft. All authors read and approved the final manuscript.

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