Microdose flare-up vs. flexible-multidose GnRH antagonist protocols for poor responder patients who underwent ICSI

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Summary

Purpose: To compare the performance of microdose flare-up (MF) and flexible-multidose gonadotropin-releasing hormone (GnRH) antagonist protocols in poor responder patients who underwent intracytoplasmic sperm injection (ICSI). *Materials and Methods:* One hundred and 12 consecutive patients (217 cycles) suspected to have poor ovarian response were enrolled. Group 1 (MF GnRH agonist group) constituted 64 patients (135 cycles) who underwent MF GnRH agonist protocol. Group 2 (flexible-multidose GnRH antagonist group) constituted 48 patients (82 cycles) who underwent flexible-multidose GnRH antagonist protocol. *Results:* The duration of stimulation (d) (11.5 ± 2.1 vs. 10.4 ± 2.7, $p < 0.01$) and the total dose of gonadotropin used (IU) (5,892.9 ± 1,725.7 vs. 4,367.5 ± 1,582.1, $p < 0.05$) were significantly lower in Group 2 when compared to Group 1. The numbers of retrieved oocyte-cumulus complexes (4.5 ± 3.6 vs. 5.9 ± 4.9, $p < 0.05$), metaphase II oocytes (3.6 ± 3.1 vs. 4.9 ± 4.2, $p < 0.05$), two pronucleated oocytes (2.6 ± 2.3 vs. 4.0 ± 3.4, $p < 0.05$), the number of available embryos at day 3 (2.6 ± 2.2 vs. 4.2 ± 3.2, $p < 0.05$) and the rate of embryos with ≥ seven blastomeres and < 10% fragmentation at day 3 (35.9% vs. 65.1%, $p < 0.05$) were significantly lower in Group 1 when compared to Group 2. The number of embryos transferred (2.2 ± 1.3 vs. 2.4 ± 0.9), the clinical pregnancy per embryo transfer (16.3% vs. 25.8%), and the implantation rate (8.6% vs. 12.2%) were comparable between groups. *Conclusions:* Although the flexible-multidose GnRH antagonist protocol produced better oocyte and embryo parameters, the clinical pregnancy rate and the implantation rates were comparable between the flexible-multidose GnRH antagonist and MF protocols in poor responder patients.

Key words: Microdose flare-up; Poor responder; GnRH antagonist; IVF.

Introduction

There is strong evidence that ovarian response is one of the most important prognostic factors for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) success. Poor ovarian response is associated with a high cancellation rate and low pregnancy rates [1]. However, the debate surrounding which stimulation protocol should be preferred in poor responders is still ongoing. A variety of stimulation regimens have been used, including the use of high doses of gonadotropins, supplementation with exogenous luteinizing hormone (LH), decreased gonadotropin releasing hormone (GnRH) agonist doses, flare regimes, the use of growth hormone or growth hormone-releasing factor, transdermal testosterone, aromatase inhibitors, GnRH antagonists, and microdose flare regimes [2-6].

The microdose flare-up (MF) protocol is one of the stimulation protocols used in poor ovarian responders. The flare effect induced by low-dose GnRH agonist administration in the early follicular phase enhances ovarian response to the subsequent administration of high-dose exogenous gonadotropins. GnRH antagonist protocols are another alternative stimulation protocol for poor responders [7,8]. GnRH antagonists do not suppress endogenous gonadotropin secretion at the stage of follicular recruitment and may increase the ovarian response in patients with diminished ovarian reserve to exogenous gonadotrophins [9].

In this study, the aim was to compare the performance of MF GnRH agonist and flexible-multidose GnRH antagonist protocols in poor responder patients who underwent ICSI.

Materials and Methods

One hundred and twelve consecutive patients (217 cycles) suspected to have poor ovarian response were enrolled from a computerized IVF database during the time period from 2002 to December 2012. Inclusion criteria were: (1) bilateral antral follicle count less than 6; (2) patients who underwent a flexible-multidose GnRH antagonist cycle or MF GnRH agonist cycle; (3) fresh ICSI cycles; (4) cycles in which ejaculate sperm used for ICSI. Assessment of antral follicle count was performed in the early follicular phase without any preceding medical treatment one to three months before the scheduled COH cycle. The total number of follicles in both ovaries, each measuring two to nine mm in diameter at transvaginal ultrasound, was defined as the antral follicle count. The author divided these cycles into two groups according to the pituitary suppression protocol employed. Group 1 (MF GnRH agonist group) constituted 64 patients (135 cycles) who underwent MF GnRH agonist protocol. Group 2 (flexible-multidose GnRH antagonist group) constituted 48 patients (82 cycles) who underwent flexible-multidose GnRH antagonist protocol.

Group 1 (MF GnRH agonist group) patients underwent controlled ovarian hyperstimulation using the microdose leuprolide

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Table 1. — *The baseline characteristics of the microdose flare-up protocol (MF) and the flexible-multidose GnRH antagonist protocol.*

Variable	Group I (MF)	Group II (GnRH antagonist)	*p* value
No. of patients	64	48	
No. of cycles	135	82	
Rate of first ICSI attempt (%)	47.4	58.5	NS
No. of canceled cycles (n, %)	31 (23.0)	16 (19.95)	NS
Female age (y)	35.9 ± 4.3	35.4 ± 4.9	NS
Body mass index (kg/m^2)	25.8 ± 4.1	26.2 ± 3.9	NS
Duration of infertility (m)	117.8 ± 81.1	114.6 ± 88.0	NS
No. of antral follicle count	3.3 ± 1.5	3.5 ± 1.4	NS

NS: Non-significant

acetate flare-up regimen. After a 21-day course of an oral contraceptive, leuprolide acetate 40 mg subcutaneous (s.c.) twice daily was commenced three days after the last pill and continued until the day of human chorionic gonadotropin (hCG) administration. Two days after initiation of leuprolide acetate, a daily s.c. injection of recombinant follicle stimulating hormone (FSH) or urinary FSH was commenced. The starting dose of gonadotropin was determined based on the age of the woman, antral follicle count at baseline transvaginal ultrasonography, body mass index (BMI), and previous ovarian response, if available. Ovarian response was monitored with frequent serum estradiol (E2) measurements and transvaginal ultrasonography, as described previously [10].

Group 2 (flexible-multidose GnRH antagonist group) patients underwent controlled ovarian hyperstimulation consisting of GnRH antagonist with or without oral contraceptive pretreatment and gonadotropin using the step-down protocol. When desensitization was achieved, as evidenced by plasma estradiol (E2) levels of ≤ 50 pg/ml, the absence of ovarian follicles and endometrial thickness ≤ six mm on transvaginal ultrasound examination [11], a daily s.c. injection of recombinant FSH or urinary FSH was commenced. The starting dose of gonadotropin was determined based on the age of the woman, antral follicle count at baseline transvaginal ultrasonography, BMI, and previous ovarian response, if available. Ovarian response was monitored with frequent serum E2 measurements and transvaginal ultrasonography, as described previously [10], were undertaken. If the serum E2 level was > 600 pg/ml and/or if a leading follicle exceeding 14 mm in diameter were present, GnRH antagonist 0.25 mg was initiated as daily injections up to the day of oocyte pick-up. The assignment of the patients to the groups was made on the physician's preference.

The criterion for hCG administration was the presence of three or more follicles exceeding 17 mm in diameter. Oocyte retrieval was carried out under local anesthesia using vaginal ultrasound-guided puncture of follicles 36 hours after hCG administration. Standard procedures were carried out for gamete-embryo handling and cleavage-stage embryo or blastocyst (day 5) embryo transfer was performed under abdominal ultrasonography guidance in all cases using a soft catheter . The luteal phase was supported by daily vaginal progesterone suppositories starting one day after oocyte pick-up.

Clinical pregnancy was defined as the presence of an intrauterine gestational sac by transvaginal ultrasonography. Symptomatic patients with moderate or severe ovarian hyperstimulation syndrome (OHSS) were hospitalized [12].

The statistical analyses were performed using Statistics Package for Social Sciences version 17.0. The chi-squared and Fisher exact tests were used to analyze nominal variables in the form of frequency tables. Normally distributed (Kolmogorov-Smirnov test) parametric variables were tested by student t test. Non-normally distributed metric variables were analyzed by Mann-Whitney U test, and p values of < 0.05 were considered statistically significant. Values were expressed as mean ± SD, unless stated otherwise. The Institutional Review Board of the university approved the study protocol.

Results

Both groups were comparable regarding the women's age, BMI, and the duration of infertility (Table 1). The duration of stimulation (d) (11.5 ± 2.1 vs. 10.4 ± 2.7, $p < 0.01$) and the total dose of gonadotropin used (IU) (5,892.9 ± 1,725.7 vs. 4,367.5 ± 1,582.1, $p < 0.05$) were significantly lower in Group 2 when compared to Group 1 (Table 2).

The numbers of retrieved oocyte-cumulus complexes (4.5 ± 3.6 vs. 5.9 ± 4.9, $p < 0.05$), metaphase II oocytes (3.6 ± 3.1 vs. 4.9 ± 4.2, $p < 0.05$), two pronucleated oocytes (2.6 ± 2.3 vs. 4.0 ± 3.4, $p < 0.05$), the number of available embryos at day 3 (2.6 ± 2.2 vs. 4.2 ± 3.2, $p < 0.05$), and the rate of embryos with ≥ seven blastomeres and < 10% fragmentation at day 3 (35.9% vs. 65.1%, $p < 0.05$) were significantly lower in Group 1 when compared to Group 2 (Table 2).

The number of embryos transferred (2.2 ± 1.3 vs. 2.4 ± 0.9), the clinical pregnancy per embryo transfer (16.3% vs. 25.8%), and the implantation rate (8.6% vs. 12.2%) were comparable between groups (Table 3).

Discussion

In the current study, although the duration of stimulation (d) (11.5±2.1 vs. 10.4±2.7, $p < 0.01$) and the total dose of gonadotrophin used (IU) (5,892.9 ± 1,725.7 vs. 4,367.5 ± 1,582.1, $p < 0.05$) were significantly lower in the GnRH antagonist group when compared to MF group (Table 2), the numbers of retrieved oocyte-cumulus complexes (4.5 ± 3.6 vs. 5.9 ± 4.9, $p < 0.05$), metaphase II oocytes (3.6 ± 3.1 vs. 4.9 ± 4.2, $p < 0.05$), two pronucleated oocytes (2.6 ± 2.3 vs. 4.0 ± 3.4, $p < 0.05$), the number of available embryos at day 3 (2.6 ± 2.2 vs. 4.2 ± 3.2, $p < 0.05$), and the rate of embryos with ≥ seven blastomeres and < 10% fragmentation at day 3 (35.9% vs.

Table 2. — *The controlled ovarian hyperstimulation response of the microdose flare-up protocol (MF) and the flexible-multidose GnRH antagonist protocol.*

Variable	Group I (MF)	Group II (GnRH antagonist)	*p* value
Duration of stimulation (d)	11.5 ± 2.1	10.4±2.7	< 0.01
Total dose of FSH used (IU)	5,892.9 ± 1,725.7	4,367.5 ± 1,582.1	< 0.01
E2 level on the day of hCG administration (pg/mL)	1,334.8 ± 952.2	1,310.3 ± 899.6	NS
No. of follicles >17 mm in diameter at hCG administration	2.1 ± 1.3	2.2 ± 1.7	NS
No. of follicles 15–17 mm in diameter at hCG administration	1.5 ± 1.4	1.5 ± 1.7	NS
No. of follicles 10–14 mm in diameter at hCG administration	2.7 ± 2.6	3.5 ± 3.9	NS
Endometrial thickness at hCG administration (mm)	9.8 ± 2.6	10.2 ± 2.0	NS

NS: Non significant

Table 3. — *The embryological data and pregnancy outcome of the microdose flare-up protocol (MF) and the flexible-multidose GnRH antagonist protocol.*

Variable	Group I (MF)	Group II (GnRH antagonist)	*p* value
No. of oocyte-cumulus complexes	4.5 ± 3.6	5.9 ± 4.9	< 0.05
No. of metaphase II oocytes	3.6 ± 3.1	4.9 ± 4.2	< 0.05
Metaphase II oocytes/total oocytes (%)	79.9	80.2	NS
2-pronucleated/metaphase II oocytes (%)	69.7	77.9	< 0.01
No. of 2 pronucleated oocytes	2.6 ± 2.3	4.0 ± 3.4	< 0.01
No. of available embryos at day 3	2.6 ± 2.2	4.2 ± 3.2	< 0.01
Rate of embryos with ≥7 blastomeres and <10% fragmentation at day 3 (%)	35.9	65.1	< 0.01
No. of embryos transferred	2.2 ± 1.3	2.4 ± 0.9	NS
Clinical pregnancy/embryo transfer (%)	16.3	25.8	NS
Implantation rate (%)	8.6	12.2	NS
Multiple pregnancy rate (%)	11.8	11.8	NS
Miscarriage rate (%)	5.9	11.8	NS

NS: Non significant

65.1%, $p < 0.05$) were significantly higher in the GnRH antagonist group when compared to the MF group (Table 2). However, the clinical pregnancy and implantation rates were comparable between groups. According to the current study, the use of GnRH antagonist may have a possible advantage with regards to the number of oocytes, the number of embryos available at day 3, and the embryo quality when compared to the MF protocol in poor responders. However, this positive effect did not translate into increased clinical pregnancy and implantation rates in the current study.

Scott and Navot [13] first reported the use of the MF agonist protocol in poor ovarian responders (34 patients). The hypothesis was that the flare effect induced by low-dose GnRH agonist administration in the early follicular phase enhances ovarian response to the subsequent administration of high-dose exogenous gonadotropins. They noted a higher number of retrieved oocytes, a lower cancellation rate, and higher pregnancy rates in patients receiving the MF protocol. After this report, Schoolcraft *et al.* [14], Surrey *et al.* [15] and Detti *et al.* [16] reported similar results.

The hypothesis for the usage of GnRH antagonists in the poor ovarian response is that the GnRH antagonists do not suppress endogenous gonadotrophin secretion at the stage of follicular recruitment and may increase the ovarian response in patients with diminished ovarian reserve to exogenous gonadotrophins. Cheung *et al.* [17] compared the GnRH luteal long protocol (31 patients) with GnRH antagonist fixed multi-dose protocol (32 patients) in poor ovarian responders. There were no significant differences in the cycle cancellation rates, duration of stimulation, consumption of gonadotrophins, and mean numbers of mature follicles, oocytes, and embryos obtained. The implantation rates were similar, but the number of embryos transferred was significantly higher for the antagonist group (2.3 ± 0.6 vs. 1.5 ± 0.8, $p = 0.01$). The pregnancy rates were also higher in the antagonist group, but the difference was not statistically significant.

In a recently published meta-analysis (14 studies included), Pu *et al.* [18] compared the performance of GnRH antagonist protocols with GnRH agonist protocols in poor ovarian responders. They reported that GnRH antagonist protocols resulted in a statistically significant lower duration of stimulation compared with GnRH agonist protocols, but there was no significant difference in the number of oocytes retrieved or the number of mature oocytes retrieved. Moreover, no significant difference was found in the cycle cancellation rate (odd ratio (OR): 1.01, 95% CI:

0.71–1.42) or clinical pregnancy rate (OR: 1.23, 95% CI: 0.92, 1.66). In this meta-analysis, MF protocol's results were present in only three studies. Six studies were with short multiple GnRH agonist protocol, three studies were long multiple GnRH agonist and two studies were flare-up multiple GnRH agonist protocols. The authors did not make a subgroup analysis comparing MF protocol with GnRH antagonist protocol.

There is limited data in the literature comparing the effect of GnRH MF protocol and GnRH antagonist protocol in poor ovarian responders. Demirol and Gurgan [19] compared the efficacy of the MF and multiple-dose antagonist protocols for poor responder patients in ICSI cycles. They noted that the total gonadotropin used (IU) (3,675 ± 748 vs. 4,200 ± 775, $p < 0.05$) was significantly higher in the GnRH antagonist group when compared to MF group. The number of metaphase II oocytes retrieved was significantly lower in GnRH antagonist protocol (4.3 ± 2.1 vs. 3.1 ± 1.1, $p < 0.05$). They also noted that although the clinical pregnancy rates (28.6% vs. 15%) were comparable between groups, the implantation rates were significantly higher in the MF group (22.1% vs. 11.0%, $p < 0.05$).

Kahraman *et al.* recently compared the efficacy of microdose GnRH agonist flare-up (21 patients) and multiple dose GnRH antagonist protocols (21 patients) in patients who have a poor response to a long luteal GnRH agonist protocol. They noted that the mean serum E2 concentration on the day of hCG administration was significantly higher in the microdose GnRH agonist group than in the GnRH antagonist group (1,904.8 ± 768.2 vs. 1,362.5 ± 587.4 pg/ml). The clinical pregnancy rate per started cycle of microdose GnRH agonist and GnRH antagonist groups were 14.2% and 9.5%, respectively ($p > 0.05$). There were no statistically significant differences in the other ovulation induction characteristics, fertilization, and implantation rates.

Wang *et al.* [20] in 2008 compared the outcomes achieved by GnRH antagonist (63 patients) and MF (58 patients) in IVF-ET for patients with poor ovarian responses. They noted the total amount of gonadotropin used, the numbers of retrieved oocytes, and the number of embryos transferred were the same between groups. However, the clinical pregnancy rates were comparable between groups. The cycle cancellation rate was lower in the GnRH antagonist when compared to MF groups.

There is no consensus on the definition of poor responders and the test to diagnose it. The basal FSH, E2, inhibin B measurements on day 3 of menstruation, a history of poor ovarian response to controlled ovarian hyperstimulation, anti-Müllerian hormone, ovarian volume measures, and antral follicle count are the most commonly used tests to predict poor ovarian response [21]. Antral follicle count is performed by transvaginal ultrasonography in the early follicular phase and it is considered to have the best discriminating potential for a poor ovarian response compared to the total ovarian volume and basal serum levels of FSH, E2, and inhibin B on day 3 of the cycle [22]. Therefore, the authors only used antral follicle count to predict poor ovarian response in the current study. There is also no clear consensus in the literature on the cut of value for antral follicle count to predict poor ovarian response. However, the majority of the physicians agreed that an antral follicles count of less that six at both ovaries should be considered as a predictor of a poor response to IVF/ICSI.

In conclusion, the current study noted that although flexible-multidose GnRH antagonist protocol produced better oocyte and embryo parameters, the clinical pregnancy rate and the implantation rates were comparable between flexible-multidose GnRH antagonist protocol and MF protocols in poor responder patients.

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