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Gonadotropin-Releasing Hormone (GnRH)-Antagonist Versus GnRH-Agonist in Ovarian Stimulation of Poor Responders Undergoing IVF

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***Purpose*:** The objective of this study was to compare the efficacy of GnRH-antagonists to GnRH-agonists in ovarian stimulation of poor responders undergoing IVF.
***Methods*:** Retrospective analysis of our data revealed that 56 patients underwent treatment with a GnRH-agonist according to the flare-up protocol. Patients failing to achieve an ongoing pregnancy ($n = 53$) were subsequently treated in the next cycle with a GnRH-antagonist according to the multiple-dose protocol. Main outcome measures included the clinical pregnancy and implantation rates.
***Results*:** While ovulation induction characteristics and results did not differ between the two protocols, the number of embryos transferred was significantly higher ($P = 0.046$) in the GnRH-antagonist than in the GnRH-agonist stimulation protocol (2.5 ± 1.6 vs. 2.0 ± 1.4, respectively). The clinical pregnancy and implantation rates per transfer in the GnRH-antagonist group appeared higher than in the GnRH-agonist, but did not differ statistically (26.1 and 10.7 compared with 12.2 and 5.9%, respectively). However, the ongoing pregnancy rate per transfer was statistically significantly higher ($P = 0.03$) in the GnRH-antagonist than in the GnRH-agonist group (23.9 vs. 7.3%, respectively).
***Conclusion*:** Applying GnRH-antagonists to ovarian stimulation protocols may offer new hope for IVF poor responder patients. However, further controlled randomized prospective studies with larger sample sizes are required to establish these results.

KEY WORDS: Cetrorelix; GnRH-agonist; GnRH-antagonist; IVF; Poor responders.

INTRODUCTION

Since the evolution of assisted reproductive technologies (ART), the management of poor responders has been one of the most difficult challenges with disappointing results. These patients, who encompass about 10% of the ART population, are characterized by a poor response to ovulation induction and high cancellation and failure rates, thus influencing significantly the overall IVF success rates, as well as its cost-effectiveness (1). Efforts to improve ovarian response in these patients vary and include the application of almost all the currently known stimulation protocols. However, definite conclusions regarding the ideal approach for their treatment cannot yet be made, mainly due to the great variability among the studies performed (2).

Recently, improved outcome has been reported with the application of the minidose GnRH-agonist flare-up protocol, which takes advantage of the initial pituitary stimulation (3,4). However, the application of GnRH-agonists in low responders, even in

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small doses and for a limited period, has been questioned as they may cause oversuppression of ovarian function, leading to a prolonged cycle and increased treatment costs, without improving the outcome (5). Furthermore, a possible direct deleterious effect of GnRH-agonists on the ovary by their direct binding to their ovarian receptors has also been suggested (6).

GnRH-antagonists, which were recently introduced in ART treatments, seem to overcome these disadvantages by avoiding ovarian suppression in the early follicular phase, which is a critical period for these patients with decreased ovarian reserves (7). On the other hand, they are effective in preventing premature LH surges when added to the ovarian stimulation regimen during the late follicular phase, and therefore may offer a potentially better alternative in the treatment of this group of patients (8).

The aim of this study was to compare the efficacy of GnRH-antagonists to GnRH-agonists in ovarian stimulation of poor responders undergoing treatment with assisted reproductive techniques.

MATERIALS AND METHODS

Patients

Fifty-six poor responder patients who underwent IVF at the Assisted Reproductive Units of the Hadassah University Hospitals at Ein Kerem and Mount Scopus from January 2001 to December 2002 were included in this retrospective study. The definition of a poor responder was based on data retrieved from previous IVF cycles of the patients, and had to include at least one of the following criteria: i) four or less oocytes retrieved; ii) low estradiol levels on the day of human chorionic gonadotropin (hCG) administration (<1500 pmol/L); iii) basal follicular stimulating hormone (FSH) levels > 12 mIU/mL. Clinical and endocrine characteristics of the patients are presented in Table I. All patients received adequate counseling regarding the stimulation regimens and signed informed consent forms.

Table I. Clinical and Endocrine Characteristics of the Patients

No. of patients	56
Age (years)	40.3 ± 4.6
No. of patients > 35 years of age (%)	49 (87.5)
No. of patients with baseline FSH > 12 mIU/mL (%)	11 (19.6)
Duration of infertility (years)	7.5 ± 5.0
Reason of infertility	
Mechanical	11
Endometriosis	1
Male	17
Unexplained	27
FSH on cycle day 3 (mIU/mL)	8.7 ± 3.5
LH on cycle day 3 (mIU/mL)	5.6 ± 2.8
Estradiol on cycle day 3 (pmol/L)	120.6 ± 61.3

Note. Values are means ± SD.

Stimulation Regimens

Retrospective analysis of our data during the study period revealed 56 poor responder patients who underwent treatment with a GnRH-agonist according to the short (flare-up) protocol. In each patient treatment with D-Trp6-LHRH 0.1 mg s.c. per day (Decapeptyl®, Ferring, Kiel, Germany) was initiated on the first day of menstruation followed by the administration of exogenous gonadotropins on cycle day 3. Patients failing to achieve an ongoing pregnancy under this protocol ($n = 53$) were subsequently treated in the next attempt with a GnRH-antagonist according to the multiple-dose protocol, in which exogenous gonadotropins were started on day 3 and later 0.25 mg s.c. of cetrorelix (Cetrotide®, Serono International) was added daily when the leading follicle reached 14 mm in diameter until the hCG injection. The time intervals between the application of the two stimulation protocols varied among the patients from a minimum of 2 months to a maximum of 15 months.

In both stimulation regimens, all patients received an initial gonadotropin dose of 450–600 IU of human menopausal gonadotropins (hMG) for the first 5 days, followed by individual adjustments in gonadotropin dose according to ovarian response and estradiol concentration. When the leading follicle reached 17–18 mm in diameter, 10,000 IU hCG was administered i.m., followed 34–36 h later by an ultrasound-guided transvaginal oocyte aspiration. All embryo transfers were performed on day 3. The luteal phase was supported with 900 mg of micronized progesterone (Utrogestan®, Besins Iscovesco, France) administered vaginally daily in three divided doses, initially for 2 weeks starting on the day following oocyte retrieval, and being continued for another 8 weeks in cases where a pregnancy was achieved.

Micromanipulation including intracytoplasmic sperm injection and assisted hatching was performed in all cycles.

Statistical Analysis

Data are expressed as means ±SD. For assessing the difference between the two protocols, the paired *t* test was applied for quantitative measures. For comparing the two protocols when the parameter was categorical

Table II. Cycle Characteristics with Pregnancy Outcome

	GnRH-antagonist	GnRH-agonist	*P* value
No. of cycles	53	56	
Cancellation rate (%)	5 (9.4)	5 (8.9)	ns
Premature ovulation (%)	3 (5.7)	1 (1.8)	ns
Low response (%)	2 (3.7)	4 (7.1)	ns
No. of oocyte retrievals (%)	48 (90)	51 (91)	ns
No. of FSH/hMG ampoules	60.4 ± 23.4	59.3 ± 24.2	ns
Estradiol on hCG day (pmol/L)	3250 ± 1704	3542 ± 2274	ns
No. of total oocytes retrieved	4.9 ± 3.6	4.4 ± 3.3	ns
No. of mature oocytes retrieved	3.3 ± 2.4	2.8 ± 2.1	ns
Fertilization rates (%)	86	76	ns
No. of embryo transfers (%)	46 (96)	41 (80)	0.02
No. of embryos transferred	2.5 ± 1.6	2.0 ± 1.4	<0.05
Implantation rate (%)	10.7	5.9	ns
Clinical pregnancy/transfer (%)	12/46 (26.1)	5/41 (12.2)	ns
Ongoing pregnancy/transfer (%)	11/46 (23.9)	3/41 (7.3)	0.03

Note. Values are means ± SD unless otherwise indicated. ns = not significant.

(pregnancy) the Mc-Nemar test was used. $P < 0.05$ was considered to be statistically significant.

RESULTS

Cycle characteristics with pregnancy outcome are summarized in Table II. The two studied protocols were similar in the cancellation rate, number of ampoules of gonadotropins used, estradiol concentrations on the day of hCG injection, and fertilization rates. While the total number of follicles ≥14 mm as seen on ultrasonography on the day of hCG administration was significantly higher ($P < 0.05$) in the GnRH-antagonist group (4.9 ± 2.4) than in the GnRH-agonist group (4.2 ± 2.3), the number of total and mature oocytes retrieved did not differ significantly (Table II). However, as the number of embryos reaching day 3 was significantly higher ($P < 0.05$) in the GnRH-antagonist group (2.7 ± 1.9) than in the GnRH-agonist group (2.0 ± 1.7), the number of embryo transfers, as well as the number of embryos transferred, was significantly higher in the former than in the latter group (Table II).

In the GnRH-agonist group five clinical pregnancies were achieved, two of which miscarried, resulting in three ongoing pregnancies. In the GnRH-antagonist group 12 clinical pregnancies were achieved, with only one miscarriage, resulting in 11 ongoing pregnancies. All pregnancies were singletons. While the clinical pregnancy/transfer and implantation rates were higher in the GnRH-antagonist than in the GnRH-agonist group, they did not reach statistical significance (Table II). However, the clinical pregnancy rates per retrieval and per initiated cycle were significantly higher ($P = 0.04$) in the GnRH-antagonist than in the GnRH-agonist group (25.0 and 22.6% compared with 9.8 and 8.9%, respectively). In accordance, the ongoing pregnancy rates per transfer, retrieval, and initiated cycle were significantly higher ($P < 0.05$) in the GnRH-antagonist than in the GnRH-agonist group (23.9, 23.0, 20.8% vs. 7.3, 5.9, 5.3%, respectively).

DISCUSSION

The introduction of GnRH-agonists revolutionized ART treatments improving significantly the success rates. However, it was soon realized that in low responders prior suppression with a GnRH-agonist resulted in excessive dampening of the ovarian response to hormonal stimulation, so that cancellation rates due to lack of ovarian response were unacceptably high or hormonal stimulation was excessively prolonged with increased cost and duration of treatment without a significant improvement in the yield of mature oocytes (9). Thus, several stimulation protocols, with a decreased dosage and duration of administration of the GnRH-agonist were proposed in an effort to improve the outcome of poor responders (2).

Experience to date shows that the short protocol has an important role in the treatment of low responders. Muasher reported a low cancellation rate (5%) in 150 cycles of poor responders using a flare-up GnRH-agonist protocol starting on cycle day 2 with

4–6 ampules of FSH starting on day 3 (5). Similarly, Yang *et al.* showed that in patients with an FSH/LH ratios, either ≥3.0, ≥2.5, or ≥2.0, the pregnancy rates were higher when using the short rather than the long protocol (10). Scott and Navot in 1994 offered a novel approach to the poor responder with the introduction of a microdose GnRH-agonist flare protocol, after pretreatment with oral contraceptives. They found that approximately 2% of the normal dose of GnRH-agonist (20-μg leuprolide acetate, BID) was able to stimulate significant endogenous gonadotropin release, and also to inhibit premature LH surges in 100% of cases. Compared with a luteal GnRH-agonist protocol, this microdose GnRH-agonist flare protocol improved stimulation outcomes in 90% of cases (11). Schoolcraft *et al.*, in a study of 32 poor responder patients, who were pretreated for 21 days with oral contraceptives, followed by the administration of a 40-μg dose of leuprolide acetate twice daily simultaneously with growth hormone (4 IU/day i.m.), reported a 12.5% cancellation rate, and an impressive 50% ongoing pregnancy rate per oocyte retrieval in a group of patients with a previously poor response (3). Other studies using this protocol also supported these findings reporting significantly decreased cycle cancellations, as well as increased clinical and ongoing pregnancy rates (4,12).

Recognizing the possible negative effects that GnRH-agonists may have in ovarian stimulation of poor responders, Faber *et al.*, recently, proposed the "stop-dose" protocol, in which the agonist was initiated in the midluteal phase, terminated by the onset of menses, and then followed by high-dose gonadotropin therapy. A 12.5% cancellation rate was reported as a result of inadequate response. However, the majority of the patients produced an adequate number of mature oocytes (approximately 10 oocytes per stimulation attempt), achieved three or more embryos per transfer resulting in an acceptable clinical pregnancy rate of 32% per transfer (13).

The introduction of GnRH-antagonists in ART protocols may offer an improved alternative in the treatment of low responders, overcoming any possible detrimental effects that GnRH-agonists may have on ovarian stimulation. Furthermore, evidence exists to support their effectiveness in preventing premature LH surges, which is one of the most common causes of cancellation in these patients (6). Craft *et al.* reported improved stimulation outcome in 18 poor responders treated with cetrorelix in combination with clomiphene citrate according to the multiple-dose protocol compared to previous treatment with GnRH-agonists. While the results were not statistically significant, a reduced rate of cancelled cycles, improved oocyte production with a lower FSH dosage, and higher clinical pregnancy rates per cycle were reported following the use of the antagonist compared to the agonist (14).

Further evidence supporting these observations was shown in a study comparing the efficacy of GnRH-antagonists in ovarian stimulation of 21 low responders compared to that of long protocol GnRH-agonists. It was demonstrated that the use of GnRH-antagonists resulted in significantly less ampoules of gonadotropins and a shorter duration of stimulation (15). In addition, since a deleterious effect of agonists directly on the ovary might be the reason for the failure in those with a limited ovarian reserve, Akman *et al.* compared ovarian stimulation in 20 low responders following the use of GnRH-antagonists to that without the addition of an agonist, and reported higher, though not statistically significant, clinical pregnancy and implantation rates in the antagonist group than in the group without an agonist (20 and 13.33% compared to 6.25 and 3.44%, respectively) (16). Contrary to these preliminary encouraging results, a recent prospective randomized trial comparing the efficacy of the flare-up protocol to the antagonistic multiple dose protocol in ovarian stimulation of 24 poor responders reported that of the parameters evaluated, only the estradiol concentrations on the day of hCG were higher in the GnRH-agonist group, whereas the clinical pregnancy and implantation rates among the groups did not show any significant difference, concluding that the impact of these two regimens in ovarian stimulation of poor responders seems to be the same (17).

Our study demonstrates that treatment with GnRH-antagonists yielded a higher clinical pregnancy rate compared to GnRH-agonists. While the cycle cancellation rates did not differ among the two treatment strategies, under the use of GnRH-antagonist, a higher, though not significant, number of mature oocytes were retrieved. Similarly, higher, but not significant, fertilization rates were obtained resulting in a significantly higher number of day 3 embryos available for transfer in cycles applying GnRH-antagonist (Table II). The combination of these eventually led to a higher number of patients reaching embryo transfer, which may offer an explanation for the higher pregnancy rates per cycle achieved in the GnRH-antagonistic cycles. While we understand the limitations of our study mainly due to its retrospective character, the large number of patients evaluated

in accordance with the fact that these patients previously failed under the use of GnRH-agonists, enhances our findings, which show that GnRH-antagonists may bring a new hope for poor responder IVF patients.

Intracytoplasmic sperm injection was used as the fertilization technique in all our patients. However, whether conventional IVF or ICSI should be applied to poor responders in order to increase the fertilization rate remains still an open question. Moreno *et al.* in a prospective study of 96 low responder patients reported no differences in the fertilization rate, number of embryos transferred, pregnancy, and implantation rates, after ICSI or conventional IVF (18), and concluded that in cases of non-male infertility the technique of fertilization is not related to the reproductive outcome of poor responders, and thus the routine use of ICSI is not indicated. Conversely, other recent reports have shown that ICSI can be used successfully on non-male factor couples to provide comparable or even higher fertilization rates and a superior quality of embryos than those obtained after standard IVF (19). Ludwig *et al.* in a retrospective analysis of ICSI cases where only one, two, or three oocytes were retrieved by follicular puncture reported that ICSI provided a constant fertilization rate regardless of the number of oocytes retrieved, and concluded that ICSI guarantees a successful treatment even if only as many oocytes are available as the number of embryos planned to be transferred (20). Since similar assumptions were made through our experience at our IVF Unit, we have adopted ICSI as the fertilization technique in poor responders.

In conclusion, 25 years after the introduction of ART the evaluation and treatment of poor responders remains a challenge, and requires constant scrutiny and modification of currently used stimulation protocols which should be achieved at a minimum increase in cost, duration of treatment, and patient's risks. Whereas, further controlled randomized prospective studies with a larger sample size are required to establish our results, it seems that the addition of GnRH-antagonists in cycles of poor responders undergoing IVF may offer an alternative approach to the ovarian stimulation of this group of patients.

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