A Novel Stimulation Protocol for Poor-Responder Patients: Combining the Stop GnRH-ag Protocol with Letrozole Priming and Multiple-Dose GnRH-ant: A Proof of Concept

Raoul Orvieto^{a,b} Ravit Nahum^a Adva Aizer^a Jigal Haas^a
Michal Kirshenbaum^a

^aDepartment of Obstetrics and Gynecology, Infertility and IVF Unit, Chaim Sheba Medical Center, Tel Hashomer, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; ^bThe Tarnesby-Tarnowski Chair for Family Planning and Fertility Regulation, at the Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel

Keywords

Poor responders · Controlled ovarian hyperstimulation · POSEIDON · Stop protocol · GnRH-antagonist

Abstract

Objective: The objective of this study was to examine whether the combined Stop GnRH-agonist (GnRH-ag), letrozole priming, and multiple-dose GnRH-antagonist (GnRH-ant) protocol may improve in vitro fertilization/intracytoplasmic sperm injection cycle in poor ovarian responders (PORs). **Design:** This was a historical cohort, proof of concept study under tertiary setting at University affiliated Medical Center. **Patients:** Five PORs fulfilling the POSEIDON Group 4 criteria were included. **Main Outcome Measures:** Number of oocytes retrieved, number of top-quality embryos (TQEs), and controlled ovarian hyperstimulation (COH) variables were the main outcome measures. **Results:** The combined Stop GnRH-ag, letrozole priming, and multiple-dose GnRH-ant COH protocol revealed significantly higher number of follicles >13 mm on the day of hCG administration and higher number of oocytes retrieved, with non-significantly more TQEs and a reasonable clinical pregnancy rate. **Conclusions:**

The combined Stop GnRH-ag, letrozole priming, and multiple-dose GnRH-ant COH protocol is a valuable tool in the armamentarium for treating POSEIDON Group 4 patients. Further large prospective studies are needed to elucidate its role in POR and to identify the specific characteristics of women (before initiating ovarian stimulation) that will aid both fertility specialists' counseling and their patients in adjusting the appropriate COH protocol.

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Introduction

Controlled ovarian hyperstimulation (COH) is a crucial step in the success of in vitro fertilization (IVF)-embryo transfer; however, in a subgroup of “poor-responder” patients, COH yields a very small number of follicles, if any [1]. Several strategies are offered for the treatment of patients with poor ovarian response to COH [1–9]. Nevertheless, in spite of the multiplicity of strategies, no clear conclusion has been established on which regimen would be the ideal COH protocol for patients defined as poor ovarian responders (PORs) [10].

In 1998, Faber et al. [11] were the first to introduce the stop GnRH-agonist (GnRH-ag) protocol aiming to improve treatment outcome in patients with poor ovarian response. The stop protocol combines downregulation with GnRH-ag starting at the luteal phase, cessation of GnRH-ag therapy with the onset of menstruation, and high-dose gonadotropin administration. This short-term ovarian suppression which begun in the luteal phase and discontinued with the onset of menses was demonstrated to yield favorable pregnancy results in PORs [11]. Recently, our group demonstrated that combining the Stop GnRH-ag protocol with GnRH-antagonist (GnRH-ant) protocols in PORs who previously failed several IVF treatment cycles revealed significantly higher number of oocytes retrieved and top-quality embryos (TQE), with a reasonable clinical pregnancy rate (16.6%) [9].

Androgens pretreatment in PORs were demonstrated to significantly improve the ongoing pregnancy/live birth rates (LBRs) [12]. The rationale of androgens pretreatment, in addition to being precursors for estrogen synthesis, is their trophic role in ovarian follicular development and granulosa cell proliferation by augmentation of FSH receptor expression on granulosa cells [13], with the consequent increase in the number of pre-antral and antral follicles [14–16]. Based on the aforementioned observations, letrozole, an aromatase inhibitor, was offered to PORs, demonstrating an improved ovarian response to FSH in patients undergoing ovulation induction and intrauterine insemination [17] and significantly increasing follicular fluid testosterone and androstenedione, resulting in a higher number of oocytes retrieved and a higher implantation rate in low responder patients with a previous canceled IVF cycle [18].

In 2003, Baerwald et al. [19, 20] in their ultrasonographic studies reported 2 or 3 follicular waves during the intraovulatory period of healthy women. They suggested that follicles developing during the luteal phase may have the potential to ovulate in the presence of a luteinizing hormone (LH) surge, offering new possibilities for ovary stimulation, such as starting gonadotropins for ovarian stimulation at any time during the menstrual cycle or using double stimulation in both the follicular and luteal phases – the “DuoStim” [21].

In 2016, the POSEIDON group suggested a new concept of low prognosis patients, aiming to improve their management and promote a more personally tailored approach [22]. One of the most frustrating subgroups of patients, as defined by the POSEIDON group, is Group 4: “patients >35 years with poor ovarian reserve prestimulation parameters (antral follicle count [AFC] <5, AMH <1.2 ng/mL).”

Based on the aforementioned observations, in the Chaim Sheba Medical Center, we started offering POSEIDON Group 4 patients a novel protocol, combining a modified Stop GnRH-ag protocol with letrozole priming, aiming to recruit 2 successive follicular waves while improving follicular sensitivity to FSH. Assessing this new potentially promising treatment protocol will aid both fertility specialists’ counseling and their patients in adjusting the appropriate treatment strategy for patients with poor ovarian response.

Patients and Methods

We reviewed the computerized files of all consecutive women admitted to our IVF unit, at the Chaim Sheba Medical Centre, between November 2019 and February 2020. Inclusion criteria included patients with poor ovarian response [23] to conventional multiple-dose GnRH-ant IVF/ intracytoplasmic sperm injection (ICSI) cycles (control cycle), who also fulfilled POSEIDON Group 4 criteria and underwent a subsequent COH, using the combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant protocol (study cycle), within 3 months of the previous failed conventional IVF/ICSI cycle. The study was approved by the institutional research ethics board of Sheba Medical Center.

In the initial conventional COH (control cycle), gonadotropins were started on day 2–3 of the menstrual cycle (corresponding to stimulation day 1) in variable doses, with a minimal daily dose of 300 IU, depending on patient’s age and/or ovarian response in previous cycles. Continuing dose was adjusted according to serum E2 levels and vaginal ultrasound measurements of follicular diameter obtained every 2 or 3 days. GnRH-ant treatment (0.25 mg/day, Cetorelix, Cetrotide, Serono International SR, Geneva, Switzerland or Orgalutran, NV Organon, Oss, The Netherlands) was started when a follicle reached 13 mm and/or E2 levels exceeded 400 pg/mL.

In the study protocol, patients received triptorelin (Lapidot, Netanya, Israel) 0.1 mg/day, starting in the midluteal phase and discontinued with the onset of menses and after confirmation of downregulation by serum E2 levels and vaginal ultrasound measurements. In the following 5 days, patients received letrozole 5 mg/day and thereafter were stimulated with high-dose gonadotropin (Fig. 1). Once the leading follicle had reached a size of 13 mm and/or E2 levels exceeded 400 pg/mL, co-treatment with the GnRH-ant 0.25 mg/day was initiated and continued up to and including the day of hCG administration. The criteria for final follicular maturation trigger were at least 2 follicles with the diameter of 17 mm with appropriate peripheral E2 levels. Oocytes were retrieved 36 h after dual trigger (hCG and GnRH-ag).

Following a positive pregnancy test, ongoing pregnancies were confirmed by the presence of gestational sac with fetal heart rate on ultrasound in 6–8 weeks’ gestation. Routine IVF or ICSI was performed, as appropriate. All patients received luteal support with progesterone. Embryos classification was based on the individual embryo scoring parameters according to pre-established definitions [24]. A TQE was defined as 3 or more blastomeres on day 2 and 7 or more blastomeres on day 3, equally sized blasto-

Fig. 1. Illustration of the combined Stop GnRH-ag, letrozole priming, and multiple-dose GnRH-ant COH protocol. GnRH-ant, GnRH-antagonist; GnRH-ag, GnRH-agonist; COH, controlled ovarian hyperstimulation; LH, luteinizing hormone.

Table 1. Clinical characteristics of the IVF cycles in the 2 study groups

	Study cycles	Control cycles	<i>p</i> values
Number of cycles	5	5	
Length of stimulation, days	12.2±6.1	9.8±2.6	ns
Total dose of gonadotropin used, IU	6,240±3,978	4,380±2,209	ns
Peak E2 levels on day of hCG administration, pmol/L	2,975±762	2,289±2,490	ns
Peak progesterone levels on day of hCG administration, nmol/L	2.38±0.45	3.12±1.3	ns
Number of follicles >13 mm administration	3±0	1.8±0.5	0.005 on day of hCG
(Range)	(3)	(1–2)	
Number of oocytes retrieved	3.8±2.4	2.0±1.2	0.04
(Range)	(2–7)	(1–3)	
Number of TQE	1.2±1.5	0.4±0.5	0.2
(Range)	(0–3)	(0–1)	
Number of embryos transferred	1.8±0	1.2±0.5	0.07
(Range)	(1–2)	(1–2)	

TQE, top-quality embryo; IVF, in vitro fertilization.

meres and <15% fragmentation. All other characteristics defined poor embryo quality.

Data on patient age and infertility treatment-related variables were collected from the computerized clinical files. Outcome was assessed in terms of COH characteristics, cancellation rates, amount of gonadotropin required to COH, duration of stimulation, number of retrieved oocytes, number of TQE, number of embryos transferred, and pregnancy rates and compared between the

previous conventional (Conventional group) and the combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant protocol (Study group) IVF/ICSI cycles.

Results are presented as means ± SD. Comparison of continuous variables between the 2 groups was conducted using Mann-Whitney U test or Student's *t* test as appropriate. χ^2 or Fisher's exact tests were used for comparison of categorical variables. Significance was accepted at a probability value of <0.05.

Results

Five PORs (age 39.9 ± 1.6 years) during a conventional IVF/ICSI cycle, with AFC of 2.8 ± 0.58 (range: 2–4), who underwent a subsequent combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant cycle, were evaluated. Patients' BMI and day-3 FSH were 22.8 ± 2.9 kg/m² and 9.3 ± 7.5 IU/L, respectively.

The clinical characteristics of the IVF cycles in the 2 study groups are shown in Table 1. There were no in-between group differences in COH variable during the combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant cycle (study cycle) and the preceding conventional IVF/ICSI cycles (control cycle), regarding the duration of stimulation (12.2 ± 6.1 vs. 9.8 ± 2.6 days, respectively), the total dose of gonadotropins used ($6,240 \pm 3,978$ vs. $4,380 \pm 2209$ IU, respectively), peak estradiol ($2,975 \pm 762$ vs. $2,289 \pm 2,490$ pmol/L, respectively), and progesterone (2.38 ± 0.45 vs. 3.12 ± 1.3 nmol/L, respectively) levels.

The Study group patients achieved significantly higher numbers of follicles >13 mm in diameter on the day of triggering final follicular maturation (3.0 ± 0 vs. 1.8 ± 0.5 , $p < 0.005$, respectively), significantly more oocytes retrieved (3.8 ± 2.4 vs. 2.0 ± 1.2 , $p < 0.04$, respectively), and nonsignificantly higher number of TQE (1.2 ± 1.5 vs. 0.4 ± 0.5 , $p = 0.2$, respectively) and number of embryos transferred (1.8 ± 0 vs. 1.2 ± 0.5 , $p = 0.07$, respectively) (Table 1). No patients conceived following the previous conventional IVF/ICSI cycles, while 2 ongoing pregnancies (40.0%) were recorded in the Study cycles.

Discussion

In the present proof of concept study of POSEIDON Group 4 PORs (patients >35 years with poor ovarian reserve prestimulation parameters, e.g., AFC <5) [22], the combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant cycle provided significantly higher number of follicles and oocytes retrieved and nonsignificantly more TQEs and embryos for transfer, as compared to their previous IVF attempt. Two clinical pregnancies (pregnancy rate, 40%) were recorded. However, it should be emphasized that the increased pregnancy rate in the combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant cycle is biased due to the study design, which offered this protocol to poor-responder patients who had failed a previous IVF attempt.

The observed increase in only 1 oocyte retrieved (3.8 instead of 2.0) was already shown to increase the cumulative LBR per cycle, in all age-groups, by approximately 25% [25]. Moreover, any improvement in patients' response to COH, such as from low (1–3 oocytes) to suboptimal response (4–9 oocytes), was also demonstrated to improve cumulative LBR [26]. Therefore, the additional 2 oocytes retrieved and 1 TQE in the present study of POSEIDON Group 4 patients undergoing the combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant cycle may explain the observed improvement in the IVF outcome with a reasonable LBR.

The rationale behind the sequential treatment of the combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant protocol stems from the advantages of its components: (a) The long GnRH-ag protocol pretreatment results in better synchronized response and a scheduled cycle [27, 28]. (b) Since continuing the GnRH-ag during COH is often associated with a significant increase in the number of gonadotropin ampoules required for achieving adequate follicular development, its cessation might improve ovarian response and avoids the need of increasing gonadotropin daily dose. GnRH-ag causes suppression of pituitary LH secretion for as long as 10 days after the last dose of the agonist [29]. (c) Maintaining pituitary suppression provides the “5-day pause,” allowing the development of additional follicular wave while enabling letrozole priming [20, 21]. The consequent increase in intrafollicular androgens levels might augment FSH receptor expression on granulosa cells with the consequent increase in the number of FSH-sensitive antral follicles [13–16]. (d) The Stop GnRH-ag together with the multiple-dose GnRH-ant provide immediate LH suppression, eliminating premature LH surge and might improve the quality of the embryos generated. (e) In poor ovarian response, GnRH-ant downregulation has an additional advantage in which final oocyte maturation may be triggered by GnRH-ag together with hCG (double trigger), with improved IVF outcome [30].

A limitation of our analysis is its retrospective design and the small sample size. However, based on our patients' selection process, only consecutive patients fulfilling the inclusion criteria were enrolled, which considerably decreases the likelihood of selection bias. In addition, the combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant cycle outcomes were compared to the previous COH-IVF of the same patients; this method may eliminate any matching hurdles.

In conclusion, we chose to focus on a specific population among all poor responders (POSEIDON Group 4)

with 3 or less oocytes following conventional COH for IVF with high daily dose gonadotropins (>300 IU), because these patients are the most challenging patients. In the present study, the combined Stop GnRH-ag and letrozole priming with multiple-dose GnRH-ant protocol was demonstrated to be a valuable tool in the armamentarium for treating this “genuine” poor ovarian responders. Further large prospective studies are needed to elucidate its role in poor ovarian response and to identify the specific characteristics of women (before initiating ovarian stimulation), which will aid both fertility specialists’ counseling and their patients in adjusting the appropriate COH protocol.

Statement of Ethics

This study was approved by the institutional research ethics board of Sheba Medical Center.

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Conflict of Interest Statement

The authors have nothing to declare.

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Author Contributions

R.O. was the principal investigator, designed the study, performed the statistical evaluations, wrote the first draft, took part in discussions regarding the results, and edited it in all its revisions. R.N., E.Z., and J.H. participated in designing the study, edited and proof read the paper, and took part in discussions regarding the results. M.K. participated in designing the study, retrieved the data, assisted in writing the paper, edited and proof read the paper, and took part in discussions regarding the results. All authors read and approved the final manuscript.

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