Gineco.eu [14] 58-61 [2018]
DOI: 10.18643/gieu.2018.61

Mild stimulation versus conventional ovarian stimulation in a cohort of poor responders

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Abstract

Much attention is being directed nowadays toward individualized medicine. Worldwide clinicians use different types of protocols for ovarian stimulation during human assisted reproduction techniques. In the last few years, researchers have tried to find the most appropriate stimulation protocol taking into consideration the ovarian response, clinical outcome, cost and complications. Mild stimulation has been proposed for patients with poor ovarian response, instead of the conventional protocol, because of its better safety profile and reduced costs. We studied 92 patients with poor ovarian response that underwent procedures in the department of Human Assisted Reproduction of Clinical Hospital of Obstetrics and Gyneacology "Prof. Dr. Panait Sârbu" from Bucharest, Roamnia between between January 2015 and June 2017. Our goal was to compare the outcomes of the procedures in patients that received conventional protocol or mild stimulation. The results favored the conventional protocol in terms of number of oocytes retrieved but showed no difference in terms of β-human chorionic gonadotropin positivity, clinical pregnancy rate and abortion rate.
Keywords: *poor ovarian response, mild stimulation, conventional ovarian stimulation, clinical pregnancies*

Introduction

In the last decade, there has been a lot of research regarding the appropriate doses of gonadotropines (Gns) used for ovarian stimulation. Much attention is being directed nowadays toward individualized medicine. The challenge is how to design a clinically useful algorithm, guided by the patients history and the initial screening tests. When establishing the desired outcome, the following should be taken into consideration: the most appropriate ovarian response, clinical outcome, cost and complications. The optimal number of retrieved oocytes is still heavily debated, but some recent studies have suggested between 8-14 for controlled ovarian stimulation and between 3-8 for mild stimulation. Many researchers have demonstrated lately that a bigger number of oocytes does no necessarily result in increased pregnancy rates[1].

In 2007, the International Society for Mild Approaches in Assisted Reproduction clarified the nomenclature regarding the available *in vitro* fertilization (IVF) protocols for ovarian stimulation. They proposed a simplified and revised terminology in order to replace the old terms and obtain a consistency in clinical practice, research articles and communication with patients: Natural cycle IVF (unstimulated, spontaneous cycle), modified natural cycle IVF (Seminatural, controlled natural cycle), mild IVF (soft, miniamal, 'friendly' IVF), and conventional IVF (standard controlled ovarian stimulation)[1].

The term of mild stimulation is used to describe a method when follicle stimulating hormone (FSH) or human menopausal gonadotropin (hMG) is administered at a lower doses, and/or for a shorter duration in a gonadotropin releasing hormone (GnRH) antagonist co-treated cycle, or when oral compounds, anti-estrogens or aromatase inhibitors are used either alone or in combination with Gns with the aim of collecting between 2 and 7 oocytes[1,2].

This protocol is associated with a better safety profile, in terms of the incidence of ovarian hyperstimulation syndrome and venous thrombembolism, is less expensive and patient-friendly. Despite the obvious benefits, the acceptance of mild stimulation is hindered by the insecurity of doctors regarding fewer oocytes and embryos and its corellation to pregnancy rate and increased risk of cycle cancellation[3].

There are different approaches to conventional IVF: long protocol – GnRH agonist for pituitary down-regulation followed by conventional doses of FSH or hMG, short protocol - GnRH agonist for the initial flare-up effect and down-regulation after followed by conventional doses of FSH or hMG or short protocol- GnRH antagonist used with conventional doses of FSH or hMG from early days of menstrual cycle. The aim of this protocol is to obtain a large number of oocytes (≥8), in order to maximize the number of embryos available for transfer and/or cryopreservation.

The European Society of Human Reproduction and Embryology established in 2011 the Bologna criteria for defining poor ovarian reserve (POR). At least two of the following three features must be present:maternal

Received: *March 26, 2018*
Revised: *April 19, 2018*
Accepted: *April 27, 2018*

age ≥40 years or any other risk factor that can diminish the ovarian reserve (i.e. genetic disorder or acquired disease);The retrieval of ≤3 oocytes after controlled ovarian stimulation; Positive marker for POR such as antimüllerian hormone (AMH)<1.1 ng/ml or antral follicle count (AFC)<7 follicles.

Two episodes of poor ovarian response after controlled ovarian stimulation with maximal doses are enough to define the patient as being a poor responder, even if the patient does not meet the other criteria[4].

The aim of this study is to evaluate the differences between mild stimulation and conventional ovarian stimulation in patients with poor ovarian stimulation that undergo IVF procedures in terms of number of oocytes retrieved, clinical pregnancy rate and abortion rate.

Methods

Patient population and study design. Before starting the IVF, patients were evaluated by hormonal exams on day 3 of menstrual cycle (FSH and AMH) and by transvaginal ultrasound in order to assess the AFC. They were classified as expected poor responders in accordance with the Bologna criteria that we have mentioned before. We decided to correlate the low AMH with the elevated value of day 3 FSH (≥10 IU/l), in the absence of a history of poor ovarian response.

In consequence, for this study, we have chosen the patients by the following criteria: age ≥40 years; patient with autoimmune thyroiditis, fragile X syndrome, Turner syndrome; history of POR after conventional ovarian stimulation (≤3 oocytes retrieved); AMH between 0.1-1.1; AFC<7.

Along with the hormonal profile (AFC, AMH) and the patients history, we recorded two demographic parameters, such as: age and body mass index (BMI).

According to the internal rules of the IVF Unit, patients with basal FSH >20 IU/l, AMH<0.1 and age >43 years were not considered for the present study.

Overall, we selected 92 women matching the inclusion criteria, that had one or two IVF procedures between January 2015 and June 2017 in the IVF Department of Clinical Hospital of Obstetrics and Gynaecology "Prof. Dr. Panait Sîrbu", Bucharest.

We divided the patients into 2 subgroups: 45 women in subgroup A that were treated with a mild regimen and 47 women in subgroup B that received conventional ovarian stimulation. The study was performed in order to compare the two subgroups. In order to avoid potential sources ofbias, we included all women that matched the inclusion criteria in the above mentioned period.

Mild stimulation. The patients in subgroup A were mild stimulated. They administered clomiphen citrate 100–150 mg/day or letrozole 2.5–5 mg/day orally from day 3 of menstrual cycle until day 7 and added 75–150 IU/day of gonadotropins (hMG) and GnRH antagonist (Cetrorelix) until the day of human chorionic gonadotrophin (hCG) administration (Figure 1).

Conventional ovarian stimulation. The patients in subgroup B were stimulated according with the short protocol GnRH antagonist. They started administering stimulation injections with gonadotropins225–325 IU/day (recombinant FSH and/or hMG) from day 2 of menstrual cycle, for 8-10 days. We performed a transvaginal ultrasound in the fifth day of stimulation and GnRH antagonist (0.25 mg/day) was added to the treatment when the leading follicle reached a diameter of ≥14 mm on average. In the eigth day of stimulation we repeated the ultrasound and blood exams were performed (estradiol, luteinizing hormone (LH) and progesterone) in order to establish the day of hCG administration.

We requested serum measurement of estradiol, LH and progesterone, when two or more follicles reached a mean diameter of 17 mm, in order to help us decide the triggering time. The cycle was cancelled when there was no follicle bigger than 10 mm diameter at the first ultrasound check. In case of monofollicular development,

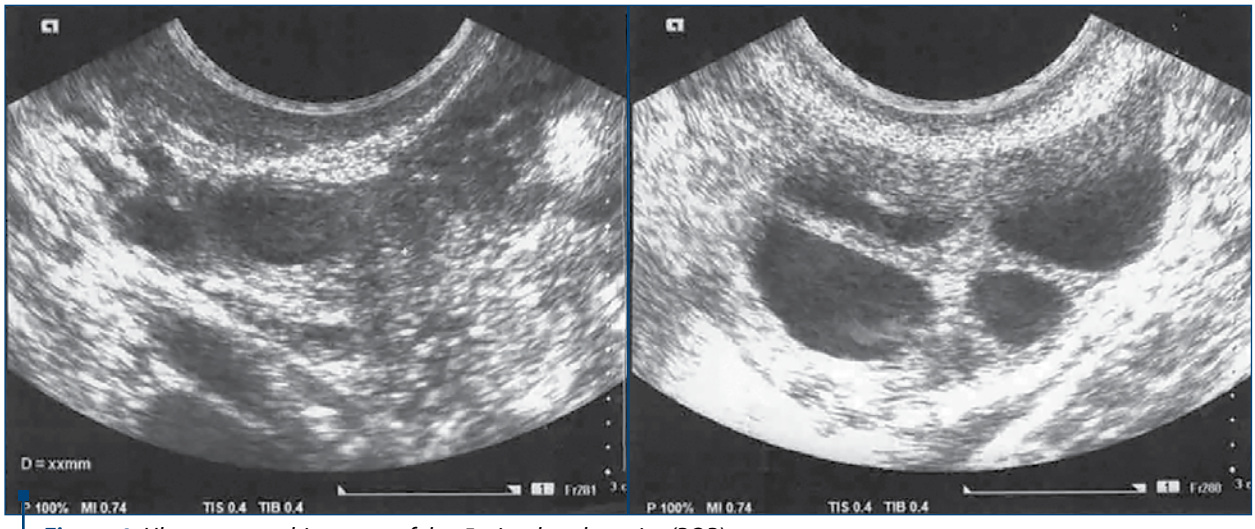

Figure 1. *Ultrasonographic aspect of day 5 stimulated ovaries (POR)*

patients gave their approval for the continuation of the procedure. Transvaginal ultrasound-guided oocyte aspiration was performed approximately 36 h after hCG injection. Depending on the embryo quality, the transfer was performed after 72 hours or 5 days.

Outcome measures. The primary outcome was the number of clinical pregnancies, which we define as the presence of an intrauterine sac with fetal heart activity through transvaginal ultrasound scan at four weeks after the embryo transfer, following a positive β-hCG test. Secondary outcomes were the number of oocytes retrieved, day 3 embryos, blastocysts, cycles without retrieved oocytes, implantation rate and abortion rate. Embryos were assesed according to morphological criteria based on the overall blastomere number and appearance and the degree of fragmentation.

Power calculation. Data analysis. All the information has been analysed with the SPSS 20.0 for Windows statistical package. For the analysis of association between variables, we have used Student's t-test and Chi square test. For the aim of the present study, the statistical significance was settled at a p-value <0.05.

Results

We excluded 6 patients because of the lack of ovarian response (4 from subgroup A – 8.88% and 2 from subgroup B – 4.25%). The decision was made if, at the ultrasound performed after five days of stimulation, no follicle was over 10 mm. The cycle cancellation rate because of no response was higher when the mild protocol was used.

The study cohort consisted of 86 patients, characterized as poor responders. The clinical characteristics were comparable between the two groups and are presented in Table 1. The patients in subgroup A had a slightly increased basal FSH in the third day of menstrual cycle (10.34 comparedto 9.36) and a lower AFC in the second day of menstrual cycle (4.15 compared to 5.6) but this data was not statistically significant. The age, BMI and AMH did not differ between subgroups.

The outcomes are summarized in Table 2. There was only one person in each group with failed retrieval of oocytes. A higher number of oocytes were withdrawn from patients in subgroup B, who received conventional ovarian stimulation (4.09 versus 2.76, p<0.05). Despite

Table 1 Baseline characteristics of the patients enrolled in the study

	Subgroup A (n=41)	Subgroup B (n=45)	*p*
Age (years)	38.34±2.6	37.89±3.4	0.059
BMI	22.22±2.86	22.33±3.03	0.59
Basal FSH (IU/L)	10.34±3.99	9.36±3.93	0.6
AFC	4.15±1.57	5.6±1.72	0.74
AMH (ng/ml)	0.66±0.48	0.67±0.52	0.54

Data are expressed as mean ± standard deviation
BMI=body mass index, FSH=follicle stimulating hormone, AFC=antral follicle count, AMH=anti-mullerian hormone.

Table 2 Outcome of IVF cycles

	Subgroup A (n=41)	Subgroup B (n=45)	p
Oocytes retrieved	2.76±1.41	4.09±2.57	0.033
No. of embryos	1.85±1.15	2.09±1.29	0.75
No. of day 3 embryos	1.05±1.09	1.11±1.11	0.53
No. of blastocysts	0.78±1.03	0.98±1.63	0.021
Fertilization rate(%)	67	51	0.93
β-hCG positive (%)	24	29	0.61
Clinical pregnancy rate (%)	13	16	0.49
Abortion rate (%)	12	13	0.49

Data are expressed as mean ± standard deviation
β-hCG=β human chorionic gonadotropin

the fact that the patients in subgroup B had more oocytes retrieved, the total number of embryos did not differ significantly (1.85 versus 2.09). The number of blastocysts obtained from the two subgroups was similar (0.78 versus 0.98, p<0.05). Despite the fact that the number of oocytes was higher in patients stimulated conventionally, with high doses of gonadotropins, the fertilization rate significatly lower (51% in subgroup B versus 67% in subgroup A). There was no difference between subgroups with regards to β-hCG positivity (24% versus 29%), clinical pregnancy rate (13% versus 16%) and abortion rate (12% versus 13%).

Discussion

In this case-control study, we evaluated the efficiency of the mild stimulation protocol for IVF over the conventional ovarian stimulation (short protocol GnRh antagonist) in a group of patients classified as poor responders according to Bologna criteria and basal FSH. The results included embryological and clinical outcomes following one of the procedures of IVF. The number of oocytes retrieved favored the conventional protocols. Regarding the β-hCG positivity, clinical pregnancy rate and abortion rate, the results were similar between the two subgroups.

In addition, the Gn intake was significantly lower for the patients mildly stimulated (75-150 IU versus 325-425 IU). The increased Gn dose had a direct impact on the number of retrieved oocytes, a finding consistent with one of the study previously published[5]. Other studies reported similar number of oocytes, despite high doses of Gns used[6,7]. The most used stimulation protocol for poor responders has been the long protocol in the last years, but, unfortunately, it failed to show better pregnancy rates and the economical costs of the procedures were elevated[8,9,10].

The mild protocol has been proposed ever since 1985[11]. Clomiphen citrate and letrozole are relatively inexpensive, with good compliance because of the oral administration. When they are used during the early follicular phase, they stimulate the secretion of endogenous Gn and, as a result, they promote multiple follicular growth[12]. The addition of a low dose of exogenous Gn (75-150UI/day) may effectively counterbalance any antiestrogenic effect of the clomiphene citrate on the endometrium. The premature ovulation and/or follicular luteinisation can be counteracted with the help of GnRH-antagonists.

The basic concept of the mild stimulation protocol is that, because of gentle stimulation, only the healthier follicles with higher quality eggs are encouraged to develop[13]. Two recent large meta-analysis on more than 3000 women, showed comparable clinical pregnancy rates and live birth rates, with lower doses of Gns used and a significant reduction of the economical costs[14,15].

A landmark randomized controlled trial showed that the number of euploid embryos after conventional IVF was no higher than that after mild IVF, despite twice the number of embryos retrieved with the conventional stimulation[16].

One limitation of our study was the lowered clinical value of the presented results attributed to the lack of randomization and the small cohort size.

Conclusions

It is better, in terms of safety and costs, to predict a poor ovarian response before the beginning of stimulation and individualize the stimulation protocol, than to be taken by surprise. Our results can be directly applicable in daily clinical practice and may lead to a lower budget for IVF as high dosages of gonadotrophins are not necessary in women with POR. According to the preexisting literature on this topic and the results of our study, we consider the mild stimulation a more cost-effective strategy than the conventional ovarian stimulation. ■

Conflict of interests: The authors declare no conflict of interests.

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