“Mild” vs. “long” protocol for controlled ovarian hyperstimulation in patients with expected poor ovarian responsiveness undergoing in vitro fertilization (IVF): a large prospective randomized trial

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Abstract

Background This large prospective, randomized study was designed to compare the “mild” protocol with clomiphene citrate, low-dose gonadotropins and a GnRH-antagonist (CC/Gn/GnRH-ant protocol) with the “long” protocol with a GnRH-agonist and high-dose Gn for the controlled ovarian hyperstimulation (COH) of patients with expected poor ovarian responsiveness undergoing IVF.

Materials and Methods A total of 695 women with clinical, endocrine and ultrasound characteristics suggesting a low ovarian reserve and a poor responsiveness to COH were recruited and randomly assigned to receive the CC/Gn/GnRH-ant “mild” protocol (mild group, $n=355$) or the “long” protocol with high-dose Gn (long group, $n=340$).

Results The “mild” stimulation led to significantly shorter follicular phase, lower consumption of exogenous Gn and lower peak estradiol level than the “long” regimen. With the “long” protocol, significantly less cycles were cancelled due to the lack of ovarian response; further, it obtained significantly more oocytes, more mature oocytes, more embryos, and a

thicker endometrium. As for the final IVF outcome, however, the two stimulation regimens obtained comparable implantation rate, clinical pregnancy rate, and ongoing pregnancy rate at 12 weeks.

Conclusions In conclusion, the “mild” CC/Gn/GnRH-ant stimulation protocol is a valid alternative to the long protocol with high Gn dose as it obtains a comparable success rate and requires significantly less medications, with an obvious economical advantage.

Keywords Controlled ovarian hyperstimulation · In vitro fertilization outcome · Mild stimulation protocols · GnRH-antagonist · Clomiphene citrate

Introduction

Among patients undergoing IVF, some variables like age, anti-Müllerian hormone (AMH) and day 3 follicle-stimulating hormone (FSH) circulating levels, and antral follicle count (AFC) allow identifying patients with poor ovarian reserve who will likely obtain a few oocytes in response to controlled ovarian stimulation (COH) [1]. These patients may be defined as “expected poor responders”, and represent a subgroup of IVF patients in which several different COH regimens have been used trying to optimize oocyte yield. Unfortunately, to date no convincing evidence to support the use of a specific COH protocol has been provided, and the overall COH results in these challenging patients remain disappointing [2, 3].

Most COH regimens currently used for expected poor responders are based on using a high daily dose (300–450 IU/die) of exogenous gonadotropins (Gn): the most popular worldwide is still the “long” GnRH-agonist protocol, in

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Capsule “Mild” stimulation protocol for expected poor responders.

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which the pituitary is blocked in the luteal phase of the run-in cycle and Gn are given after the pituitary block has been achieved [4, 5]. Giving a high Gn dose obviously increases the cost of IVF, a consequence that would be acceptable if paralleled by an improvement in IVF outcome. Unfortunately, however, the available data suggest that increasing the daily Gn dose may increase the number of retrieved oocytes, but not the final success rate of IVF [6–8].

In the last years a new stimulation strategy, named “mild”, has been proposed as an alternative to the classical protocols. According to ISMAAR association, a COH is defined as “mild” either when (a) Gn are administered at a lower-than-usual dose and/or for a shorter duration together with a GnRH-antagonist, or when (b) oral compounds (e.g. anti-estrogens) are used either alone or in combination with Gn and GnRH-antagonists [9]. The “mild” protocols have a softer impact on the ovary, are better tolerated, imply a much lower risks, are quicker and cheaper as less medications are used for a shorter time [10, 11]. On the other side, the “mild” stimulation strategy leads to the retrieval of less oocytes, a reason for which it has been proposed mainly for young, good responding patients [10, 11].

Some studies have been planned to check whether the “mild” strategy may be applied also to expected poor responders: a combination of Clomiphene citrate (CC), Gn and GnRH antagonists (CC/Gn/GnRH-antag) was tested on expected poor responders in a few studies [12–14]. Unfortunately, the number of recruited patients was so low that these studies did not allow drawing well definite conclusions. The present study was designed to recruit a large cohort of expected poor responders and to compare in a prospective, randomized way the “mild” CC/Gn/GnRH-antag protocol with a classical, “long” protocol with GnRH-agonist plus Gn at high doses.

Materials and methods

Patients

Patients undergoing IVF were evaluated by hormonal exams performed on day 3 of their menstrual cycle (FSH, AMH and estradiol) and transvaginal ultrasound aimed at assessing the antral follicle count (AFC). They were classified as “expected poor responders”, and selected as eligible to enter the study, when the following criteria were satisfied: (a) circulating day 3 FSH between 10 and 20 IU/l in the presence of estradiol (E2) serum level <80 pg/ml; (b) circulating AMH between 0.14 and 1.0 ng/ml (measured using the commercially available Immunotech Beckman Coulter kit, with lowest detection limit 0.14 ng/ml); (c) AFC between four and ten. According to the internal rules of our IVF Unit, patients with basal FSH >20 IU/l, undetectable AMH levels, AFC <3 and aged more than 43 were excluded from the IVF program and consequently not considered for the present study.

Overall, in the last 4 years 763 women at their first IVF attempt matching the inclusion criteria were selected and proposed to enter the study; the trial was approved by the local ethical committee and all eligible patients received a detailed informed consent that they signed to allow recruitment. Sixty-seven eligible patients did not gave their consent refusing to be randomized; another patient accepted, but was later excluded because during pituitary suppression had a positive pregnancy test. Finally, 695 women were enrolled in the study and randomly assigned to the “Mild group” ($n=355$) or to the “Long group” ($n=340$), receiving a “mild” or classical COH protocol, respectively. Each patient was included in the study only for one IVF cycle; in case of repeated attempts, only the first of them was considered.

Controlled ovarian hyperstimulation

The “mild” CC/Gn/GnRH-antag protocol was performed administering orally CC (Serophene, Merck-Serono, Switzerland) 100 mg/day for 5 days (from the 2nd to 6th day of the menstrual cycle), and adding 150 IU/day of subcutaneously injected Gn (Meropur, Ferring, Germany or Pergoveris, Merck-Serono, Switzerland) from the 5th day of the cycle. A GnRH-antagonist (Cetrotide, Merck-Serono, Switzerland, or Orgalutran, MSD, Germany) was then given subcutaneously (0.25 mg/d) from the 8th day of the cycle until the day of hCG administration.

In the classical “long” protocol, 0.8 mg/d of a GnRH-agonist (Suprefact, Hoechst, Germany) were given intranasally from the 21st day of the run-in cycle for 14 days; then, at the beginning of Gn administration, the dose was reduced at 0.4 mg/d and continued during ovarian stimulation. After clinical (menstruation) and hormonal ($E2 < 50$ pg/ml) confirmation of the obtained pituitary block, exogenous Gn (Meropur, Ferring, Germany or Pergoveris, Merck-Serono, Switzerland) were administered at a starting daily dose of 300 IU, that was eventually increased up to a maximum of 450 IU/d after 1 week.

IVF cycle management

The ovarian response to COH was monitored by transvaginal ultrasound plus serum E2 measurement every second-third day from stimulation day 7 or 8. The cycle was cancelled when no more than one follicle >10 mm diameter was seen and serum E2 was <50 pg/ml the day of the first checkpoint. Ovulation was triggered by injecting subcutaneously 10,000 IU of hCG (Gonasi HP, IBSA, Switzerland) when the leading follicle reached 18 mm, with appropriate serum E2 levels.

Transvaginal ultrasound-guided oocyte aspiration was performed approximately 36 h after hCG injection under local anaesthesia (paracervical block). Either IVF or ICSI was

performed according to the clinical indication. After 2 days of in vitro culture, embryos were scored using the evidence-based, 1–10 points scale score by to Holte et al. [15] and transferred in utero using a soft catheter (Sydney, Cook, Australia). According to the rules of our IVF Unit, no more than two embryos were transferred in utero; in rare cases, spare embryos were kept in culture to be frozen on day 5, but it never happened to get a blastocyst from these second-choice embryos. The luteal phase was supported with 180 mg/d natural progesterone (Crinone 8, Merck-Serono, Switzerland) for 15 days from the day of ET.

Pregnancy was assessed by serum hCG assay after 15 days from ET and then confirmed by transvaginal ultrasound after two further weeks. Only cases with ultrasound confirmation of pregnancy were considered as clinical pregnancies.

Power calculation, randomization and data analysis

The power of the study was calculated according to the primary outcome, that was the number of oocytes retrieved. Considering that in expected poor responders a cycle cancellation rate around 10 % may be predicted, we calculated that 350 patients per study arm was the number needed to detect a difference of 20 % in the primary outcome with 85 % statistical power (beta error 15 %) and a significance level at $p < 0.05$.

Secondary outcomes were the following: totally administered Gn dose, length of the ovarian stimulation, fertilization rate (FR), clinical (ultrasound-confirmed) pregnancy rate (CPR) per started cycle, per oocyte pick-up and per ET, implantation rate (IR), abortion rate, ongoing pregnancy rate (OPR) at 12 weeks gestational age.

Randomization was performed using a computerized algorithm without any restriction. No blocks were used since the size of the study group was estimated to be large enough to ensure a balanced distribution of patients between groups. Allocation concealment was obtained using sequentially-numbered dark envelopes: until they were opened at the time of allocation, both physicians and patients were blinded to the study. Patients whose cycle was cancelled and did not undergo oocyte pick-up were considered as lost to follow-up and not included in the analysis of the primary outcome (number of retrieved oocytes) (Fig. 1).

Finally, the data analysis was performed on 640 patients, 309 in the “Mild group” vs. 331 in the “Long group” (Fig. 1). Quantitative variables were subjected to normality test of Shapiro-Wilk. Statistical comparison between groups was performed using the Fisher exact test, Yeats’ corrected Chi square test, Mann–Whitney test or Student’s *t* test, as appropriate. All analyses were performed with statistical software STATA® SE 9.2. Significance was defined as a p value < 0.05 . Categorical variables are presented as absolute numbers and percentages, and quantitative variables are presented as mean $\pm$ SD.

Results

The clinical characteristics of patients recruited and randomized appear in Table 1, whereas Table 2 includes only patients whose cycle was not cancelled after the first checkpoint, performed on day 7–8. In both Tables clearly appears that patients in the two subgroups were comparable as far as age, body mass index (BMI), endocrine and ultrasound-detectable indexes of ovarian reserve are concerned (Table 1 and 2). The mean age of the patients in the two subgroups is above the average of IVF patients in our IVF Unit (36.4 years); accordingly, and due to the inclusion criteria, day 3 FSH and AMH circulating levels, as well as the antral follicle count, may definitely be considered within the range that is considered predictive of poor ovarian responsiveness to COH.

Overall, the cycle cancellation rate for insufficient response was significantly higher when the “mild” strategy was used (13 % vs. 2.7 %) (Table 3). More information on the patients whose cycle was cancelled are given in Table 4. Patients stimulated with the “mild” protocol had significantly shorter follicular phase, received a significantly lower amount of exogenous Gn, and reached a lower peak estradiol level than patients stimulated with the “long” protocol (Table 3). Moreover, the endometrium at oocyte pick-up was significantly thinner than that observed in patients in the “long group”, both because circulating E2 was lower and because CC is known to exert a peripheral anti-estrogenic action able to affect endometrial proliferation. The “mild” strategy was more frequently associated with the failed retrieval of oocytes (that occurred in patients having a slightly higher age than the mean of the corresponding group, but similar basal FSH and AFC), and the “long” protocol allowed retrieving significantly more oocytes and more mature (MII) oocytes, finally leading to have more embryos available for transfer (ET) (Table 3).

Overall, IVF outcome was corresponding to what we could expect to observe in these kind of patients, who are known to have a poor prognosis. The clinical pregnancy rate per completed treatment (CPR/ET) was 23.2 % and 19.9 % for the “mild” and “long” groups, respectively (Table 5), with no significant differences between the two stimulation regimens. Also the implantation rate was similar in the two groups (15.2 % in the “mild” group vs. 12.3 % in the “long” group). Similarly, the abortion rate did not significantly differ among groups, and finally the ongoing pregnancy rate/ET at 12 weeks was comparable with either stimulation regimen (17.8 vs 16.8 % in the “mild” and “long” groups, respectively) (Table 5).

Discussion

Stimulating women with a poor ovarian reserve is one of the most frustrating aspects of IVF, as most COH protocols proposed to improve IVF outcome in these patients gave

Fig. 1 Flow diagram of the study according to CONSORT guidelines

disappointing results [2]. In the last years, the most frequently used COH regimen for expected poor responders has been the “long” protocol with a high daily Gn dose (300–450 IU/d) [4]. Unfortunately, however, despite the higher number of retrieved oocytes this regimen failed to improve the final IVF success rate, implying, on the other side, a relevant increase of economical costs of IVF procedure [6–8].

The “mild” protocol combining CC, Gn and a GnRH-antagonist (CC/Gn/GnRH-ant protocol) has been proposed several years ago for patients undergoing IVF [16, 17]. CC is a relatively inexpensive medication with a very good compliance due to oral administration; when administered in the early follicular phase it stimulates the secretion of endogenous Gn [18], overcoming follicular dominance and promoting multiple follicular growth. The later addition of a low dose of exogenous Gn (150 IU/d) may effectively counterbalance any undesired anti-estrogenic effect on the endometrium. The occurrence of

premature ovulation and/or follicular luteinisation may be counteracted by giving daily GnRH-antagonists.

Indeed some studies compared the outcome of the “mild” CC/Gn/GnRH-ant treatment with a standard “long” protocol in patients undergoing IVF: two recent meta-analysis, respectively including seven trials with 702 participants [19] and 12 studies with 2,536 patients [20], showed that the two protocols obtained comparable clinical pregnancy rates and live birth rates, with a significant reduction of the total Gn dose and of the economical costs in favour of the CC/Gn/GnRH-ant regimen. Most patients included in these studies, however, had a normal or wide ovarian reserve and belonged to the “high responders” category.

To date it remains to be proven that the CC/Gn/GnRH-ant protocol could be applied even to patients with poor ovarian reserve as a “mild” alternative to the classical protocol with

Table 1 Baseline characteristics of the patients recruited and randomized

	Mild group (n=355)	Long group (n=340)	<i>p</i>
Age (years)	38.5±3.4	37.5±3.6	ns
BMI	22.8±3.8	23.1±4.3	ns
Basal FSH (IU/L)	12.4±4.4	13.7±2.9	ns
AMH (ng/ml)	0.71±0.44	0.68±0.35	ns
Antral Follicle Count (AFC)	5.3±2.7	6.2±2.8	ns

Data are expressed as mean ± SD

BMI body mass index, AMH anti-Mullerian hormone

Table 2 Baseline characteristics of the patients analyzed

	Mild group (n=309)	Long group (n=331)	<i>p</i>
Age (years)	38.4±3.3	37.6±3.6	ns
BMI	22.7±3.6	23.0±4.2	ns
Basal FSH (IU/L)	12.4±4.6	13.5±2.8	ns
AMH (ng/ml)	0.70±0.39	0.69±0.37	ns
Antral Follicle Count (AFC)	5.3±2.6	6.1±2.7	ns

Patients whose cycle was cancelled for insufficient ovarian response at the first checkpoint are not included in calculations. Data are expressed as mean ± SD

BMI body mass index, AMH anti-Mullerian hormone

Table 3 Outcome of controlled ovarian hyperstimulation

	Mild protocol (n=309)	Long protocol (n=331)	<i>p</i>
Started cycles	355	340	
Cycle cancellation rate (%)	13	2.7	<0.01
Completed cycles	309	331	
Stimulation length (days)	10.9±1.6	12.1±2.0	<0.05
Total amount of Gn (IU)	2237±1215	5265±2083	<0.001
Peak E2 level (ng/ml)	1088±661	1562±879	<0.01
Endometrial thickness at OPU (mm)	7.6±2.1	10.4±2.1	<0.01
OPUs	309	331	
Cycles without retrieved oocytes (%)	9.4	1.6	ns
Retrieved oocytes/OPU	2.7±2.3	4.8±3.3	<0.01
MII oocytes/OPU	2.2±1.9	4.0±2.8	<0.01
Fertilization Rate (%)	66.5	63.5	ns
Transferred embryos	1.8±1.7	2.7±2.3	<0.01
Embryos scored ≥8 points (%)	57.6	54.8	ns
ETs	202	261	

Data are expressed as mean ± SD. Data refer to analyzed cycles, that were only those who were completed; cancelled cycles are not included in the analysis. The embryo score by Holte et al. [15] is expressed in a point scale from 0 to 10

Gn gonadotropins, E2 estradiol, OPU oocyte pick-up, MII metaphase II, ET embryo transfer

high-dose Gn. A previous report [14] studied 145 IVF patients with a prior poor response and showed that the CC/Gn/GnRH-ant regimen was able to obtain better results than the “long” protocol; however, in this study a high Gn dose was associated to CC, rendering the results just suggestive of the possibility of using the CC/Gn/GnRH-ant protocol with a low Gn amount in poor responders. A small pilot study on 18 poor responders to whom the CC/Gn/GnRH-ant regimen was administered showed light improvements in the cycle cancellation rate and oocyte yield in comparison to a previous standard GnRH-agonist cycle, with a relevantly lower Gn consumption [12].

Table 4 Baseline characteristics of the patients whose cycle was cancelled for insufficient ovarian response at the first checkpoint (day 7–8 of the cycle)

	Mild group (n=46)	Long group (n=9)	<i>p</i>
Age (years)	38.1±3.2	38.6±3.9	ns
Basal FSH (IU/L)	12.6±5.2	11.9±2.5	ns
AMH (ng/ml)	0.59±0.30	0.62±0.27	ns
Antral Follicle Count (AFC)	5.4±2.9	6.7±4.2	ns
Total amount of Gn (IU)	338±129	351±148	ns

Data are expressed as mean ± SD

AMH anti-Mullerian hormone

Table 5 IVF cycle outcome

	Mild protocol (n=309)	Long protocol (n=331)	<i>p</i>
hCG positive tests	55	70	
Clinical pregnancies	47	52	
n. of gestational sacs	52	60	
CPR/started cycle (%)	13.2	15.3	ns
CPR/OPU (%)	15.2	15.7	ns
CPR/ET (%)	23.2	19.9	ns
Implantation Rate (%)	15.2	12.3	ns
abortions	11	8	
Abortion Rate (%)	23.4	15.3	ns
ongoing pregnancies >12 w	36	44	
OPR/ET (%)	17.8	16.8	ns

Data are expressed as mean ± SD. Data appearing in this Table refer to patients whose cycle was completed; only the CPT/started cycle refers to all started cycles (355 in the Mild protocol group and 340 in the Long protocol group), including those who were cancelled

hCG human chorionic gonadotropin, CPR clinical (ultrasound confirmed) pregnancy rate, OPU oocyte pick-up, ET embryo transfer, OPR ongoing pregnancy rate

Another study on 40 expected poor responders with a story of multiple IVF failures with the “long” protocol showed that the CC/Gn/GnRH-ant regimen obtained an ovarian response comparable to the previous ones, but a significantly higher ongoing pregnancy rate [13]. Unfortunately, the small size of these two studies did not allow drawing any definite conclusion.

We designed the present trial in order to compare the classical “long” protocol with high Gn dose to the “mild” CC/FSH/GnRH-ant protocol in a large cohort of expected poor responders. To the best of our knowledge, this is the largest prospective randomized trial comparing these two protocols in expected poor responders. Our study was planned before the publication of the Bologna criteria for the diagnosis of “poor responder” [21]; we selected eligible patients who were defined “expected poor responders” on the basis of clinical, endocrine and ultrasound parameters. The averagely low number of oocytes that we retrieved in both study arms (even when a high Gn dose was employed) suggests that the women included herein can be properly defined as “poor responders” even if not all meet the Bologna criteria.

We used hMG or a combination of recombinant FSH plus LH to stimulate the ovary because some LH activity was proven to be beneficial in poor responders [22]. Moreover, it was previously shown that when in CC/Gn/GnRH-ant cycles the circulating LH is less than one-third at the time of hCG than at the beginning of stimulation, both the pregnancy and implantation rates are significantly reduced [23].

In agreement with previous studies [19] we observed that the length of ovarian stimulation was significantly shorter, and that the total amount of administered Gn and the peak E2 level were

significantly lower in the “mild” group than in the long group. In the group that received CC, the endometrium was significantly thinner than in the “long” group, likely due to the peripheral anti-estrogenic effect of CC; the lower endometrial thickness, however, did not appear to negatively affect the implantation rate.

Patients treated with the “long” protocol showed a significantly lower rate of cancelled cycles and of cycles in which no oocytes were retrieved; they also obtained significantly more oocytes and more MII oocytes, and finally had significantly more embryos available for transfer, with a morphological quality comparable to those obtained in the “mild” group. Despite the advantages of having less cancelled cycles, a thicker endometrium, more oocytes and more embryos, they ultimately obtained a clinical pregnancy rate and an ongoing pregnancy rate at 12 weeks comparable to those of the “mild” group.

The success rate was similar in the two subgroups even when calculated per started cycle, despite a significantly higher cancellation rate in the “mild” group. These observations agree with previous reports, that showed that a high Gn dose is effective in getting more oocytes [8, 24] and avoiding cycle cancellation [8], but does not improve IVF results [6–8, 24]. This could be due to a negative impact of high Gn dose on endometrial quality [25] or, alternatively, to the fact that a high dose of exogenous Gn could force the recruitment of “resistant” follicles, hosting oocytes of poor quality from which poor competence embryos derive [26].

In conclusion, our study shows in a large cohort of expected poor responders that the “mild” CC/Gn/GnRH-ant protocol allows obtaining IVF results similar to those of a classical “long” protocol with a high dose of exogenous Gn. Indeed the latter lowers the risk of cycle cancellation and allows retrieving more oocytes and producing more embryos, but ultimately does not provide increased clinical pregnancy and ongoing pregnancy rates. While showing a similar success rate, the “mild” CC/Gn/GnRH-ant protocol is probably cheaper, as it requires much less exogenous Gn, whose cost is one of the major expenditures in human IVF. The results of the present study suggest that according to the search of an optimal balance between effectiveness and cost, using a high-dose Gn protocol in expected poor responders may be questionable if not paralleled by a relevant improvement in clinical outcome.

Competing interests The authors declare that they have no competing interests.

Authors' contribution AC, VS, FE and GB collected the data. AC provided the first draft of the manuscript. PD performed the statistical analysis. AR conceived the study, participated in its design and coordination, and helped to draft the manuscript. CB provided critical revision of the manuscript. All authors read and approved the final manuscript.

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