Short gonadotropin-releasing hormone agonist versus flexible antagonist versus clomiphene citrate regimens in poor responders undergoing *in vitro* fertilization: a randomized controlled trial

M. SCHIMBERNI[1], F. CIARDO[1], M. SCHIMBERNI[1], A. GIALLONARDO[1], V. DE PRATTI[1], M. SBRACIA[1]

[1]Bioroma, Centre for Reproductive Medicine, Clinica Valle Giulia, Rome, Italy

Abstract. – OBJECTIVE: Poor responders represent a frustrating condition for couples undergoing IVF and clinicians, and their treatment remains disputed. To assess the efficacy and the most suitable protocol, we conducted a randomized controlled trial comparing three different protocols of ovarian stimulation in poor responder women: clomiphene citrate (CC) plus a high dose of gonadotropins and GnRH antagonist, flexible GnRH antagonist protocol and a short GnRH agonist protocol.

PATIENTS AND METHODS: Between July 2014 and December 2015 we enrolled 250 poor responders in a previous IVF cycle at least 3 months before. We divided into three groups: group A, 68 women treated with clomiphene citrate and FSH plus antagonist; Group B, 71 patients treated with FSH plus antagonist; Group C, 75 patients treated with FSH plus GnRH agonist.

RESULTS: The GnRH agonist protocol showed a significantly higher pregnancy rate (29.3% vs. 5.9% vs. 14.1% respectively) than the clomiphene and the GnRH antagonist protocol, number of mature oocytes collected, estradiol levels and endometrial thickness. The cost of medications for each baby born was lower for the GnRH agonist protocol than for the others; the implantation rate was significantly lower in the clomiphene group (4.8%) than in the GnRH antagonist group (9.3%) and the GnRH agonist groups (19.2%). No significant differences emerged for total FSH administered, days of stimulation, numbers of oocytes retrieved and embryos transferred.

CONCLUSIONS: This study demonstrates that short GnRH agonist protocol should be the first choice in poor responders; instead, clomiphene citrate should be avoided due to its very low success rate and high costs.

Key Words:
    Clomiphene citrate, Flexible antagonist, IVF, *In vitro* fertilization, Poor responders.

Introduction

Poor ovarian response affects a large number of women undergoing IVF with a reported occurrence in the literature ranging from 5% to 18%[1], even though the definition and classification of this condition are disputed. In order to overcome the differences in definition, recently, the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine in a consensus conference established that poor ovarian response to controlled ovarian hyperstimulation (COH) may been diagnosed when at least two of the following three features are present: advanced maternal age ($\geq$ 40 years) or any other risk factor for poor ovarian response; a previous poor ovarian response ($\leq$ 3 oocytes with a conventional stimulation protocol); an abnormal ovarian reserve test[2]. This condition may be due to several factors, including chronological age, diminished ovarian reserve, severe endometriosis, smoking and prior ovarian surgery and genetic factors[1]. Poor ovarian response to COH remains a challenge for clinicians and a source of distress for patients due to the high cycle cancellation rate and a low chance of pregnancy. Multiple strategies have been suggested for enhancing the outcomes of these patients, but there is no ideal stimulation regimen. A simple approach is to increase the dose of the gonadotropin administration, but the results regarding pregnancy rate are very low[3]. Another commonly used stimulation regimen is the microdose GnRH agonist protocol which takes advantage of a flare-up, the initial rise in endogenous gonadotropins that follows the agonist administration in the early fol-

licular phase, and prevents a premature LH surge resulting in fewer cycle cancellations[4,5]. However, their application in poor responders, even if in 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 outcomes[6]. Recently, GnRH antagonists were introduced in ART treatment. They are effective in preventing a premature LH surge and allow a natural recruitment of follicles in the follicular phase, offering a potential alternative in the treatment of that patients[7]. However, randomized studies evaluating the efficacy of this regimen in poor responders did not show any improvements in pregnancy rates. An Italian review conducted by Marci et al[9] suggested the necessity to adopt a hormonal and ultrasound monitoring together with GnRH-antagonist administration due to its impact on ovarian steroidogenesis.

The addition of oral agents such us clomiphene citrate (CC) to gonadotropins in these patients has been suggested. Some authors[10] have tried CC in addition to a low dose of gonadotropins in mild stimulation regimens, demonstrating that, despite a small number of oocytes retrieved, good quality embryos were produced with a subsequent improvement in the fertilization rate, clinical pregnancy rate and live birth rate. The only study[11] that evaluated the efficacy of CC in addition to high doses of gonadotropins in poor responders showed an improvement in the number of oocytes retrieved, embryos transferred and biochemical pregnancy; however, the clinical pregnancy rate and live birth rate remained low and showed no measurable increase.

The aim of this study was to compare the efficacy of three different protocols of ovarian stimulation in poor responder women: CC plus a high dose of gonadotropins and GnRH antagonist, flexible GnRH antagonist protocol and a short GnRH agonist protocol, in order to assess the most suitable protocol in poor responder patients.

Patients and Methods

This randomized controlled trial was conducted at the Bioroma IVF program, Rome Italy, between July 2014 and December 2015. Two hundred and fifty patients, poor responders in a previous IVF cycle at least 3 months before at our center and undergoing a new IVF attempt were enrolled in the study. Patients with at least

two of the following criteria were defined as poor responders: I) age > 40 years old; II) basal follicular stimulation hormone (FSH) > 12 mIU/ml; III) three or fewer oocytes retrieved in the previous IVF cycle; IV) low estradiol levels on the day of human chorionic gonadotropin (hCG) administration (< 1500 pmol/ml). Patients with a body mass index higher than 30, biochemical and ultrasound evidence of polycystic ovary syndrome, stage III-IV endometriosis, inflammatory, autoimmune or metabolic disorders, those who had taken infertility medications (gonadotropins, clomiphene citrate) within the past two months were excluded from the study.

The study was conducted according to the Helsinki Human Rights criteria and was approved by Institutional Review Board. All patients received adequate counseling and signed an informed consent form. The study was registered with ClinicalTrials.gov with the number NCT02201914.

Computer-assisted randomization was used. A block randomization scheme was used to ensure equally sized groups and all members of the study team were blinded to the randomized group, at least until randomization was carried out. The women were allocated to the following three groups of treatment during the previous menstrual cycle after evaluation for inclusion criteria:

1) Clomiphene citrate plus gonadotropins in a flexible GnRH antagonist protocol (Group A): the patients received clomiphene citrate (Clomid, Bruno Farmaceutici, Rome, Italy) 100 mg daily starting on day 2 for 5 days and 450 IU recombinant FSH (Gonal-F, Merk-Serono, Europe) daily starting on day 5; Cetrorelix 0.25 mg (Cetrotide, Merk-Serono, Europe) was administered daily when one or more follicles reached 13-14 mm in diameter until the human chorionic gonadotropin (hCG) injection;

2) Standard flexible GnRH antagonist protocol (Group B): the women received an initial daily dose of 450 IU recombinant FSH (Gonal-F, Merk-Serono, Europe) starting on day 3; Cetrorelix 0.25 mg (Cetrotide, Merk-Serono, Europe) was administered daily when one or more follicles reached 13-14 mm in diameter until the hCG injection;

3) Short GnRH agonist protocol (Group C): the women received short-acting Triptorelin (Decapeptyl, Ipsen, Rome, Italy) 0.05 mg daily starting on day 1 until the hCG injection and 450 IU recombinant FSH (Gonal-F, Merk-Serono, Europe) daily starting on day 2.

The study was modified from the original protocol registered for economic reasons, since instead of recFSH plus recLH (Pergoveris, Merck-Serono, Europe) only recFSH (Gonal-F, Merck-Serono) was administered.

All patients started from the 7th day of the cycle a daily monitoring of follicular diameter by transvaginal ultrasound scan and a blood test to evaluate plasmatic estradiol levels until the hCG injection. After that, gonadotropin doses were adjusted according to the ovarian response.

Final oocyte maturation was triggered with 10.000 IU of hCG (Gonasi HP 10000, IBSA, Rome, Italy) when the dominant follicles reached a maximum diameter of 18-20 mm. Oocyte retrieval was performed under transvaginal ultrasound control 34-36 hours after the hCG injection. Intracytoplasmic sperm injection (ICSI) was performed in all cases for all metaphase II oocytes in order to obtain a good fecundation rate and to maximize the chances of embryo transfer. Embryo assessment was carried out on the day of embryo transfer (3 days after oocyte retrieval). Scoring was based on developmental stage and morphology using the established criteria[12,13]. Only embryos of grade A and B were transferred. After the transfer, all patients received luteal support with vaginal Progesterone administration (Prometrium 200 mg, Rottapharm, Monza, Italy).

Primary outcomes evaluated were clinical pregnancy rate and implantation rate; secondary outcomes were total doses of administered gonadotropins, days of stimulation, estradiol levels and endometrial thickness at oocyte retrieval, number of dominant follicles, all retrieved and metaphase II oocytes, total embryos obtained, number of grade A and B embryos obtained and total number of transferred embryos.

Clinical pregnancy was defined as the presence in the uterine cavity of a gestational sac with fetal heartbeat by ultrasound 5 weeks after embryo transfer (ET). Implantation rate was calculated as the ratio of the observed gestational sacs to the number of embryos transferred.

Statistical Analysis

Sample-size calculation was based on previous experience on poor responder patients, expecting an observed difference of 20% among the protocols in pregnancy rate for a power of 80% an alpha of 5%, 62 women needed to be recruited into each arm. Dropout rate from the study is generally reported as ranging between 4.5% and 4.8%, assuming and adjusting for a worst case scenario of 10% attrition the number of 78 patients were needed to be recruited in each arm.

For continuous variables, if they were normally distributed, they were summarized as means and SDs; if they were non-normally distributed, then medians and interquartile ranges were to be reported. Dichotomous data were reported as percentages.

Analysis of variance was used for each continuous variable across all three interventions (A, B, C), and, if this was found to be significant (at a $p < 0.05$), pairwise comparisons were performed with Student's t-test when appropriate. For dichotomous variables, differences among groups were assessed by using the χ^2-test and Fisher's exact test when appropriate. All statistical analyses were performed using SPSS (Statistical Package for Social Science) software, version 12.0 for Windows (SPSS Inc., Chicago, IL, USA); $p < 0.05$ was considered statistically significant.

Results

A total of 250 patients poor responders in a previous in vitro fertilization (IVF) cycle and candidates for a new IVF attempt during the study time were initially selected. Two hundred and thirty-nine women met the inclusion criteria and were enrolled in the study. After counselling, 5 (3.7%) declined further treatment. The 234 patients remaining were randomized into 3 groups of 78 women each. Six women in group A, 4 in group B and 2 n group C did not conclude the study, withdrawing from the study before initiating ovarian stimulation. In 4 patients in group A, 3 in group B and 1 in group C oocyte retrieval was not performed because of no follicular growth, an arrest of follicular growth or a premature luteinization, leaving 68, 71 and 75 patients in group A, B and C respectively for data analysis (Figure 1).

Demographic characteristics of IVF patients are reported in Table I. No statistically significant differences were found in the median age, body mass index, duration of infertility, basal FSH and infertility causes between the three groups.

The pregnancy rate was 5.9% in the clomiphene citrate group, 14.1% in the flexible GnRH antagonist protocol and 29.3% in patients treated with the short GnRH agonist protocol ($p = 0.028$); the pregnancy rate of the short GnRH agonist protocol was statistically significant compared to both the clomiphene citrate and flexible GnRH antag-
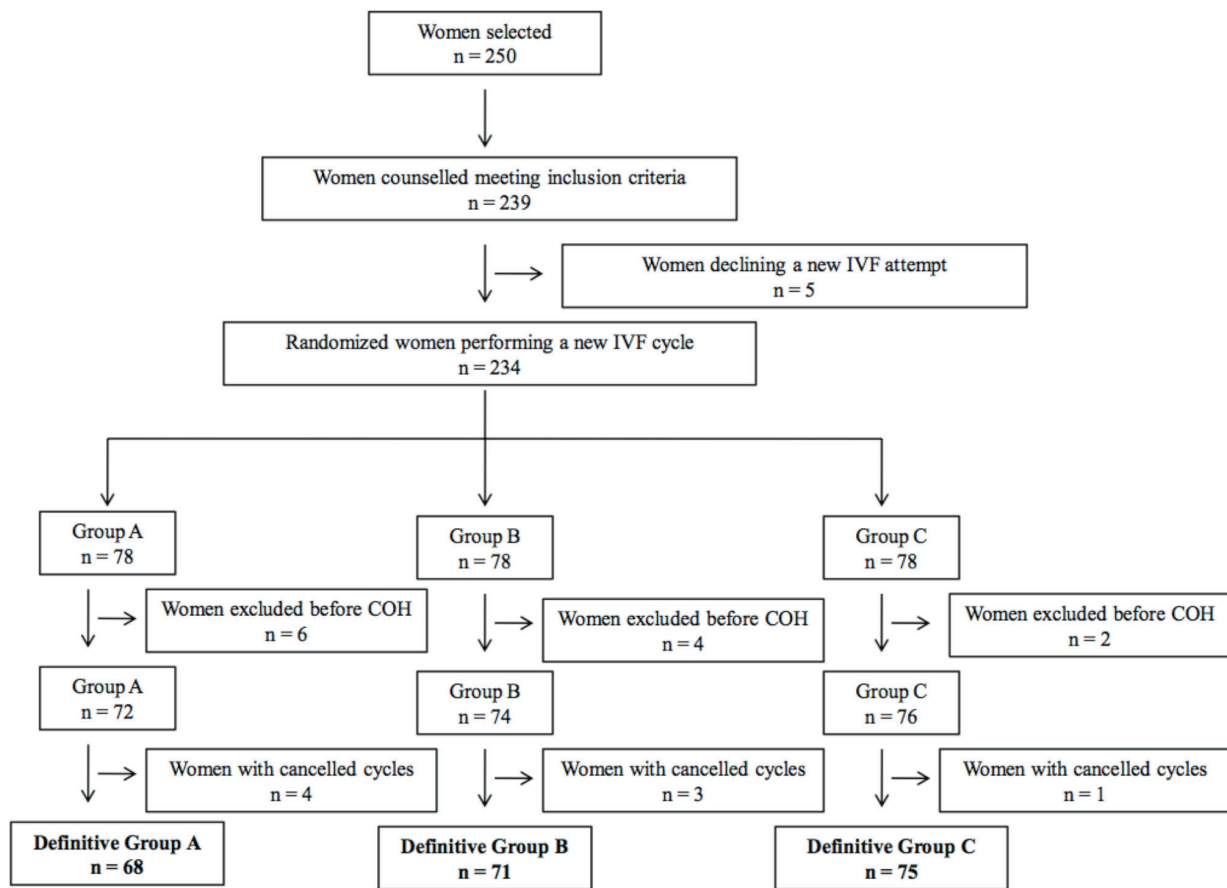
Figure 1. Flow chart of the study.

Table I. Demographic characteristics of IVF patients.

	Group A	Group B	Group C	*p*-value
Total number of patients	68	71	75	
Age (yrs)	40.4 ± 2.7	40.8 ± 1.8	41.0 ± 1.74	0.199
Body mass index (kg/m2)	24.4 ± 3.8	25.1 ± 3.6	24.8 ± 3.4	0.422
Duration of infertility (months)	45.5 ± 3.8	44.4 ± 4.1	45.1 ± 3.5	0.191
Basal FSH (mUI/mL)	16.65 ± 2.7	16.24 ± 3.9	15.74 ± 2.6	0.192
Infertility causes				
- Tubal factor (%)	24.2	21.5	26.4	ns
- Male factor (%)	20.8	23.6	22.6	ns
- Ovulatory factor (%)	30.5	31.8	29.5	ns
- Unexplained (%)	24.5	23.1	21.5	ns

Data are expressed as the mean ± SD

onist groups ($p < 0.001$ and $p = 0.029$). Similarly, the implantation rate was significantly lower in the clomiphene group (4.8%) than in the GnRH antagonist group (9.3%) and in the GnRH agonist group (19.2%) (C *vs.* B $p = 0.040$, and C *vs.* A $p = 0.0032$). A sample size calculation showed that a minimum of 127 patients per group was needed to detect an absolute difference 15.2% (29.3% *vs.* 14.1%) between the two groups, at a level of significance of 0.05 and at least a power of 80%.

No statistically significant differences were observed for total FSH administered, total LH

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administered, days of stimulation, number of oocytes retrieved and number of embryos transferred. The difference in E2 levels at hCG day was statistically significant between group C *vs.* group B and group C *vs.* group A ($p < 0.001$); the differences in endometrial thickness was statistically significant between group C *vs.* group B and group C *vs.* group A ($p < 0.001$); the difference in the number of dominant follicles obtained was statistically significant between group C *vs.* group B and group C *vs.* group A ($p < 0.01$); the difference in the number of metaphase II oocytes retrieved was statistically significant between group C *vs.* group B and group C *vs.* group A ($p < 0.015$); the difference in the total number of embryos obtained was statistically significant between group C *vs.* group A and group B *vs.* group A ($p < 0.01$); the difference in the number of grade A and B embryos obtained was statistically significant between group C *vs.* group B ($p < 0.001$). All data sets are reported extensively in Table II.

The cost per cycle in medication was € 2,719 ± 1,060 in the CC group, in the flexible GnRH antagonist group, it was € 2,633 ± 1,526 and, in the short GnRH agonist group, it was € 2,579 ± 1,716. The differences were not statistically significant.

The cost for each baby born in the CC group was € 42,647, in the flexible GnRH antagonist group it was € 18,432 and in the short GnRH agonist group, it was € 10,030.

Discussion

Poor ovarian response to stimulation in IVF cycles is a challenging and frustrating condition[2] for both clinician and patient, due to its poor prognosis in terms of pregnancies and live births. Although in the literature a large number of papers have been published in which many stimulation protocols suggested should be considered as the best in these women but no conclusive results on this issue have been reached.

Our study evaluated in a group of poor responders the efficacy of three different protocols, flexible GnRH antagonist, short protocol with GnRH agonist and a clomiphene citrate protocol, showing that the micro flare up with GnRH agonist should be preferred in these patients. Our data showed that in poor responder patients a short GnRH agonist protocol gave better results in terms of mature oocytes collected, clinical pregnancy rate and implantation rate, with a comparable amount of gonadotropins used among the three groups. Furthermore, a short GnRH-agonist protocol showed significantly higher estradiol levels and endometrial thickness than the other two protocols; moreover, the cost for each baby born was lower for the short GnRH agonist protocols than for the others.

In the past years, several works[14-17] have been published on the effectiveness of a long GnRH agonist, a short GnRH agonist and a flexible GnRH antagonist protocol in poor responder pa-

Table II. Primary and Secondary outcomes in IVF patients.

	Group A	Group B	Group C	p-value
Total number of patients	68	71	75	
Dosage of total FSH (IU)	3041 ± 1325	2939 ± 1908	3111.7 ± 2145	0.851
Dosage of administered LH (IU)	1580 ± 1491	2150 ± 1741	2133.3 ± 1711	0.072
Duration of stimulation (days)	10.6 ± 2.1	10.3 ± 1.9	11.0 ± 2.0	0.107
E2 on hCG day (pg/mL)	1045.18 ± 583.7	903.64 ± 651.9	1480 ± 823.8	0.001 (C vs. B, C vs. A)
Endometrial thickness (mm)	8.4 ± 1.6	8.7 ± 2.3	10.2 ± 1.74	0.001 (C vs. B, C vs. A)
No. of dominant follicles	4.12 ± 2.03	4 ± 2.2	5.2 ± 2.21	0.01 (C vs. B, C vs. A)
No. of retrieved oocytes	3.8 ± 2.9	3.41 ± 1.9	3.8 ± 2.39	0.542
No. of metaphase II oocytes	2.31 ± 2.05	2.3 ± 1.7	3.13 ± 2.13	0.015 (C vs. A, C vs. B)
No. of total embryos	1 ± 1.2	2 ± 1.8	1.8 ± 1.7	0.01 (C vs. A, B vs. A)
No. of grade A and B embryos	1.25 ± 1.78	0.8 ± 0.72	1.7 ± 1.78	0.001 (C vs. B)
No. of transferred embryos	1.22 ± 0.8	1.5 ± 1.12	1.66 ± 1.63	0.107
Clinical pregnancy rate	4/68	10/71	22/75	
	5.9%	14.1%	29.3%	0.0291 (C vs. B), 0.001 (C vs. A, B vs. A)
Implantation rate (%)	5.7%	9.3%	19.2%	
	4/83	10/107	24/125	0.040 (C vs. B) 0.0032 (C vs. A)

Data are expressed as the mean ± SD

tients, always with contradictory results. Most of them concluded that there is no evidence for the superiority of one over the others.

Our data on poor responder women suggested that short GnRH agonist protocol performs better than the others. These data confirmed what is reported by other authors; Demirol and Gurgan[18] concluded that the short protocol seems to have a better outcome in poor responders than the multiple dose antagonist protocol with a significantly higher number of mature oocytes retrieved and implantation rate. Malmusi et al[19] reported that the short protocol appears to be more effective than the GnRH antagonist protocol in terms of mature oocytes retrieved, fertilization rate and top-quality embryos transferred. Ozcan-Cenksoy et al[20] suggested that for the stimulation of poor responder patients the short protocol is better than GnRH antagonist/aromatase inhibitor letrozole and GnRH antagonist/clomiphene citrate protocols regarding maximum estradiol levels, numbers of mature oocytes retrieved and cancellation rate. Our previous study[21] also showed that short GnRH agonist protocols worked better in women aged 40 years or more with respect to the long protocol.

In a recent paper, Sunkara et al[22] reported that in poor responder patients long GnRH-agonist and flexible GnRH-antagonist protocols worked better than short GnRH agonist protocols in terms of oocytes harvested and amount of gonadotropins administered. These results conflict with our data, even though in our research we included a larger number of patients (70 per group in our study versus 31 per group in the other one), with an older age, since in the paper by Sunkara et al[22], women aged more than 40 years were excluded.

The possible explanation for our findings may be that during the first two or three days of the menstrual cycle when there are the selection and growth of cohort of follicles moving from the primordial to preantral stage, the flare-up effect of the short protocol may boost follicle growth increasing the number of follicles growing in the recruited cohort. Especially in older women, where there is an anticipation of recruitment and selection of follicle cohort in each menstrual cycle, due to the reduction of inhibin B levels for the follicle reserve reduction associated with age and the consequent precocious FSH peak during the late luteal phase of the previous cycle, the short GnRH-agonist protocol may allow a relatively larger number of follicles to be recruited and grow to the antral stage[23,24].

Our findings showed in the clomiphene citrate patients that the clinical pregnancy rate and the implantation rate were statistically significantly lower than ones observed in patients treated with the other two protocols.

Recently, for a more patient-friendly IVF with fewer injections and a lower dose of gonadotropins, a new interest in the use of CC, especially in poor responder patients, has been raised. All studies concluded that the mild protocol can be considered a valid alternative for these patients, regarding reduced doses of gonadotropins used and a shorter duration of the stimulation regimen and, therefore, more cost effective and patient friendly than conventional IVF, even though the overall pregnancy and live birth rates remained low in these cases[2,8].

A Cochrane review by Gibreel et al[25], evaluating the efficacy of CC with gonadotropins (with or without a mid-cycle antagonist) versus gonadotropins alone in GnRH agonist protocols in normal responder patients, concluded that there was no evidence to indicate that CC differed significantly from the standard protocol in terms of live birth or pregnancy rates.

To our knowledge, only one work[11] has evaluated the efficacy of the association of CC to a high dose of gonadotropins in 48 patients. In this study, the supplementation with CC showed significant improvements in estradiol levels, number of dominant follicles, oocytes retrieved, number of embryos transferred and biochemical pregnancy rates; however, the overall clinical and live birth rate remained low. In this study, patients had undergone at least 2 consecutive different IVF cycles using in the first one a flexible GnRH antagonist protocol with a fixed dose of gonadotropin, and those who failed in the next one, the same gonadotropin dose was used with the adjunct of clomiphene citrate. In this way, each patient served as her own control for the two different regimens. However, also in Josanovic's[11] study, the women treated with CC showed a very low overall clinical pregnancy and live birth rate. Our research, the only controlled randomized trial comparing CC with other stimulation protocols in poor responder patients, showed that clomiphene citrate regimens should be avoided in these women.

Conclusions

We showed that the short GnRH agonist protocol with its flare-up effect should be the first choice in poor responder women especially in

cases of women 40 years old or more, whereas the flexible GnRH antagonist protocol seems to be less effective in these patients. Instead, the association of CC to high doses of gonadotropins in the treatment of poor responder patients should be avoided due to its very low success rate and the high cost per baby born.

Conflicts of interest

The authors declare no conflicts of interest.

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