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GROWTH HORMONE ADJUVANT TREATMENT IN POOR RESPONDERS

Which is the best IVF/ICSI protocol to be used in poor responders receiving growth hormone as an adjuvant treatment? A prospective randomized trial

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Abstract

This open label randomized study aims to define the best protocol to be used with growth hormone in poor responders, with comparison performed to delineate which protocol offers the best cycle outcomes. Two-hundred eighty-seven poor responders were included. The patients were randomly allocated into four groups receiving growth hormone (GH) as an adjuvant therapy added to either long or short agonist protocol, miniflare or antagonist protocols. The short/GH gave significantly lower mean number of oocytes when compared with the long/GH, antagonist/GH and miniflare/GH (4 ± 1.69 versus 5.06 ± 1.83, $4.95 +/ = 1.90$ and4.98 ± 2.51, respectively $p = 0.005$). Considering the number of fertilized oocytes, the long/GH showed significantly higher levels than short/GH and antagonist/GH (3.73 ± 1.47 versus 3.02 ± 1.52 and 2.89 ± 1.14, respectively). The main drawback is that it required significantly higher HMG dose and longer duration of stimulation. The long/GH was superior when compared with the three protocols regarding the number of oocytes retrieved and fertilized. But, when considering the clinical pregnancy rates, there was a difference in favor of the long/GH but not reaching a statistically significant value (ClinicalTrials.gov Identifier: NCT01897324).

Keywords

Antagonist protocol, growth hormone, IVF/ICSI, long agonist protocol, miniflare protocol, poor responders, short agonist protocol

History

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Introduction

Over the past decades, the need to improve the results of the assisted reproductive techniques has been the main aim of the researchers worldwide. With the increase in the percentage of delayed marriage and advanced maternal age, the problem of poor responders has markedly increased [1]. The incidence of poor ovarian response varies from 9% to 24% among different studies [2]. The definition of poor ovarian response (POR) was debatable, with no uniform agreement for many years. This problem was solved by the ESHRE according to a consensus done in 2011 [3]. A definition was reached as POR, including at least two of the following three features: (1) advanced maternal age ($\geq$40 years) or any other risk factor for POR, (2) a previous POR ($\leq$3 oocytes with a conventional stimulation protocol), (3) an abnormal ovarian reserve test (i.e. antral follicular count (AFC) <5–7 follicles, or anti-Mullarian hormone (AMH) <0.5–1.1 ng/ml).

Clinical pregnancy rates remains unfortunately low in patients with poor ovarian response despite the use of the different stimulation protocols. Thus, many options were suggested to improve the results, one of these options was the addition of growth hormone (GH) to the stimulation protocols [4].

It is well known now that the GH has a positive impact on the process of steroidogenesis and estrogen production, as well as follicular growth and oocyte maturation [5]. The addition of GH enhances the response of the granulosa cells to the gonadotrophins in both animal and human studies [6]. GH acts by increasing the local production of insulin-like growth factor-I that has an important role in the ovarian function [4]. In our study, we aimed to detect which protocol gave better results when added to GH in cases of poor responders.

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Material and methods

This was an open-labeled randomized prospective study involving patients undergoing ICSI cycles at two IVF centers (Kasr Al-Aini IVF Center, Faculty of Medicine, Cairo University, Cairo, Egypt; and Nile IVF Center, Giza, Egypt). Institutional Review Board approval was obtained prior to the initial start of the study.

The study population included 287 poor responder women who fulfilled the criteria defined by the ESHRE consensus in 2011 [3]. Females above the age of 45, with elevated FSH above 20 IU/l, having previous ovarian surgery, or having other causes of infertility (other than POR), with male factor of infertility, and finally couples refusing to get enrolled in the study were excluded.

From July 2013 to January 2015, all candidate couples were asked to sign an informed consent, with all the details of the study included and verbally explained. Patients were divided into four groups by disclosing the sealed envelopes and randomized by specific computer system:

(1) GnRH -a long protocol: triptorelin (Decapeptyl; Ferring, Switzerland) was started on day 21 of the preceding cycle at a dose of 0.1 mg/day, subcutaneously. On the second day of menstruation, human menopausal gonadotrophin (HMG) (75 IU, Merional, IBSA) was started, and this was associated with the reduction of triptorelin dose to 0.05 mg/day. This treatment was continued till the day of HCG administration.

Table 1. the demographic criteria of the four groups.

Characteristics	Long/GH ($n=74$)	Short/GH ($n=74$)	Antagonist/GH ($n=68$)	Miniflare/GH ($n=71$)
Age (years)	36.43 ± 5.8	38.14 ± 5.04	36.76 ± 6.34	36.38 ± 5.8
Duration of infertility (years)	7.51 ± 4.62	8.58 ± 4.25	6.97 ± 3.95	8.31 ± 4.76
Number of previous failed cycles	4.31 ± 2.31	4.11 ± 1.55	2.68 ± 1.44	4.75 ± 2.05
FSH (IU/l)	11.73 ± 1.93	11.75 ± 2.2	11.07 ± 2.61	11.74 ± 1.74
AMH (ng/ml)	0.47 ± 0.31	0.39 ± 0.25	0.36 ± 0.24	0.49 ± 0.32
AFC	4.85 ± 1.43	4.55 ± 1.62	4.53 ± 1.85	4.87 ± 1.72

AFC: antral follicular count, AMH: antimullerian hormone, FSH: follicle stimulating hormone, *n:* number. Data are expressed as mean ± SD.

Table 2. Comparison between the cycle characteristics of the studied groups.

Characteristics	Long/GH ($n=74$)	Short/GH ($n=74$)	Antagonist/GH ($n=68$)	Miniflare/GH ($n=71$)
HMG duration (days)	13 ± 1.47	13.5 ± 1.74	11.74 ± 1.85	12.79 ± 1.38
HMG dose	7443.74 ± 2741.7	7114.86 ± 2325.72	5175.15 ± 1714.34	6127.4 ± 3184.71
Endometrial thickness (mm)	11.46 ± 1.623	11.42 ± 1.59	12.06 ± 1.35	11.11 ± 1.83
E2	1129.8 ± 442.05	925.43 ± 488.73	665.74 ± 298.84	1110.9 ± 537.95
LH	2.91 ± 1.54	3.27 ± 1.57	5.88 ± 1.49	3.08 ± 1.69
Progesterone	0.79 ± 1.36	0.59 ± 0.35	0.7 ± 0.31	0.59 ± 0.35

E2: estradiol, LH: luteinizing hormone, *n*: number. Data are expressed as mean ± SD.

(2) GnRH a-short protocol: triptorelin 0.05 mg/day S.C. was started on day 1 of cycle, then HMG was administered starting from days 2 or 3 of the cycle.
(3) GnRH antagonist protocol: HMG IM daily was administrated from day 2 of the cycle. The GnRH antagonist (Cetrotide, Serono, Geneva, Switzerland) was given when the leading follicle was from 12 to 14 mm, at a daily dose of 0.25 mg subcutaneously.
(4) GnRH-a miniflare protocol: The patients were given oral contraceptive pills (OCPs) for 21 days, followed by 2 medication-free days. Triptorelin 0.05 mg/day S.C. was then started daily followed by HMG IM daily 3 days later.

GH (Norditropin, Novo nordisk) co-treatment was introduced on day 6 of HMG stimulation daily in a dose of 2.5 mg S.C. till the day of HCG administration. Growth of the follicles was monitored by Voluson 730 Pro (GE, Fairfield, CT) apparatus, starting from the eighth day of the cycle and then on alternate days, to adjust the dose of HMG given accordingly. The initial scanning involved assessment of the AFC that was done by a single expert sonographer to eliminate any inter-observer differences. When the leading follicle reached 18 mm or more, ovulation was induced with 10.000 IU HCG (Choriomon, IBSA) IM. Serum progesterone, LH and E2 were analyzed on the day of HCG administration. Oocyte retrieval was done 35–36 hours following HCG administration, while embryo transfer was performed 3 days later. Our protocol was the transfer of a maximum number of three embryos. Luteal phase support was given as follows: Cyclogest 400 mg (Alpharma, Barnstaple, UK) BD. The patients returned 12 days later to perform quantitative beta HCG estimation.

The main outcomes of the study were the mean number of mature oocytes retrieved and fertilized, while the secondary outcomes included HMG dose and duration of stimulation (days), endometrial thickness, estradiol, LH and progesterone levels on the day of HCG administration, number of MII retrieved, fertilization rates, numbers of embryos transferred, number of blastocytes, implantation rates, clinical pregnancy rates and the cycle cancellation rates.

Clinical pregnancy was defined as observation of fetal heart activity by vaginal ultrasound performed 5 weeks after positive beta HCG. Cycle cancellation included cases with no embryos transferred either due to with failed oocyte retrieval or failed fertilization.

Data were statistically described in terms of mean standard deviation (SD), and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using one way analysis of variance (ANOVA) test with *post-hoc* multiple 2-group comparisons For comparing categorical data, Chi-squared (2) test was performed. Exact test was used instead when the expected frequency is less than 5 p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL) version 15 for Microsoft Windows.

Results

The study started with 314 eligible poor responders that met our inclusion criteria. Using randomization through opening sealed envelopes, and specific computer system 78 patients were allocated to receive the long/GH protocol, 80 were allocated to receive short/GH protocol, 71 for the antagonist/GH and 71 for the miniflare/GH protocol. The final available patients for analysis were 287 patients, as 27 patients refused to get enrolled in the study. No patients were lost for follow up in both groups.

Table 1 represents demographic criteria of the patients involved in our study. There was no statistical significant difference regarding the age, duration of infertility, FSH, AMH, and AFC. But the number of previous failed cycles showed a statistical difference when comparing the antagonist versus the long or short or miniflare protocol ($p<0.001$).

The cycle characteristics are summarized in Table 2. Long/GH protocol required significantly higher HMG dose than the antagonist/GH and miniflare/GH protocol ($p<0.001$ and $p=0.013$ respectively). The HMG duration of stimulation was shortest with the antagonist protocol/GH than the rest of the used protocol (in long/GH and short/GH $p<0.001$, in miniflare/GH $p=0.001$). When comparing the endometrial thickness, it was found that the difference was statistically significant between the antagonist and the miniflare agonist protocol ($p=0.003$). Regarding hormonal profile, E2 level showed statistical

Table 3. The cycle outcomes of the four groups.

Characteristics	Long/GH ($n=74$)	Short/GH ($n=74$)	Antagonist/GH ($n=68$)	Miniflare/GH ($n=71$)
Oocytes retrieved[a]	5.06 ± 1.83	4 ± 1.69	4.95 ± 1.9	4.98 ± 2.15
M II[a]	2.42 ± 1.31	2.05 ± 1.18	2.26 ± 1.26	2.43 ± 1.36
Fertilized oocytes[a]	3.73 ± 1.47	3.02 ± 1.51	2.89 ± 1.14	3.57 ± 1.41
Fertilization rate (%)[b]	72.27 ± 17.07	73.7 ± 20.68	57.53 ± 19.02	67.3 ± 17.62
Blastocyst[a]	1.2 ± 1.11	0.86 ± 0.98	1.41 ± 1.17	1.32 ± 1.14
Implantation rate (%)[a]	42.42 ± 17.61	41.03 ± 12.93	42.86 ± 19.29	39.22 ± 13.09
Clinical pregnancy rate (%)[b]	36.7	23.2	25.9	30.4
Cancellation rate (%)[b]	20.3	23	20.6	21.1

n: number.
[a]Data are expressed in mean ± SD.
[b]Data are expressed in percentage (%).

significance when comparing the antagonist protocol versus the long/GH, short/GH ($p<0.001$) or miniflare/GH protocols ($p=0.004$). In respect to LH levels, there was significant difference when comparing the antagonist versus the long, short or miniflare protocol as well ($p<0.001$).

In our main outcome (Table 3), the mean number of oocytes collected was found to be significantly higher in long/GH followed by miniflare/GH, then antagonist/GH and lowest with the short/GH. The short/GH gave significantly lower number of oocytes when compared with the long/GH, antagonist/GH or miniflare/GH protocols ($p=0.010$, $p=0.035$ and $p=0.024$, respectively). Considering the number of fertilized oocytes, the outcome of the long/GH showed significantly higher levels than short/GH and antagonist/GH protocol ($p=0.037$ and $p=0.009$, respectively), but not significantly different when compared with the outcome of the miniflare/GH protocol. When considering the fertilization rates, there was statistical significance when comparing the antagonist/GH versus the long/GH and short/GH ($p<0.001$) and with the miniflare/GH ($p=0.037$), while there was no evidence of statistical significant difference when comparing the clinical pregnancy rates between the four groups.

Discussion

After performing a randomized trial with GH addition to the known main four stimulation protocols, we found out that the mean number of oocytes retrieved and fertilized were highest with the long/GH with significant difference when compared with the rest of the protocols. While the short/GH gave the least number of oocytes collected in comparison with the other three stimulation protocols.

Reviewing literature, a number of studies were found reaching the same results. In 2012, a study was conducted to compare the different stimulation protocols (without the addition of growth hormone). It was found out that four different protocols in poor responders had similar efficacy in improving clinical outcomes such as implantation, pregnancy rates and cancellation rate although GnRH-a long protocol retrieved more mature oocytes when compared to the miniflare protocol [7]. Another study in 2011, comparing the antagonist and the short agonist protocol, showed an increase in the number of oocytes retrieved in the antagonist than the short protocol (6.34 ± 5.540 versus 4.10 ± 3.30, $p=0.005$) [8]. In 2003, a comparison was set between the short agonist protocol and the antagonist protocol, and it ended up that the antagonist gave higher number of oocytes retrieved and fertilized, but the difference did not reach statistical significance [9].

On the contrary to, Cheung LP et al. conducted a study to compare the long agonist versus the antagonist protocol and it showed an increase in the number of oocytes retrieved in antagonist versus the long protocol but the results didn't reach statistical significance [10]. Also, Griesinger et al. found out that the antagonist protocol gave significantly more cumulus oocyte complex (COCs) when compared with the long agonist protocol, but no difference when compared with the short agonist protocol [11]. In the mentioned studies, growth hormone was not included as an adjuvant treatment.

Choosing the best protocol for ovarian stimulation will remain a dilemma. The use of GnRH agonists has been proposed to deal with poor responder patients, but it soon appeared to have many limitations, they cause excessive suppression of the ovarian function and response which led to increased the HMG dose and duration, they also cause premature LH surge which increases the cancellation rates [1]. Thus, the need for a different stimulation protocol was suggested to overcome the drawbacks of the agonist protocol in poor responders, which led to the use of the antagonist protocol [12,13]. The antagonist protocol led to prevention of premature LH surge without affecting the follicular recruitment process [14]. This goes well with our study that demonstrated that the GnRH antagonist protocol took the shortest HMG duration of stimulation when compared with the rest of the GnRH agonist protocol ($p<0.001$). The antagonist protocol of ovarian stimulation required the smallest HMG dose reaching a statistical significance dose compared with the long and short GnRH agonist protocol ($p<0.001$). The superiority of the GnRH antagonist protocol in regard to HMG dose and duration has been demonstrated by a number of studies [15–17].

Our study had some limitations, as the patients' acceptance to the use of growth hormone was a major obstacle. Many questions were raised about its safety, recommended dosage and its high cost.

Further studies to compare the four different protocols with and without growth hormone in poor responders, to reach the best protocol to be used with growth hormone in poor responders, are recommended (Three studies are already registered in this field by our team: NCT02185326, NCT02338206 and NCT02195947).

In this study we concluded that the long protocol, with GH co-treatment, gave higher number of oocytes retrieved and fertilized but required significantly higher HMG dose and longer duration of stimulation. GH is still not recommended for routine use, due its cost and the absence of impact on pregnancy rates.

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Declaration of interest

The authors report no declarations of interest. There was no source of funding for this study.

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