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Full length article

The addition of growth hormone adjuvant therapy to the long down regulation protocol in poor responders undergoing in vitro fertilization: Randomized control trial

Dina M.R. Dakhly*, Yasmin A. Bassiouny, Yomna A. Bayoumi, Mohamed A. Hassan, Hisham M. Gouda, Ayman A. Hassan

Department of Obstetrics and Gynecology, Cairo University, Cairo, Egypt

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ABSTRACT

Objective: to detect the impact of growth hormone (GH) co-treatment to the long down regulation protocol, on the outcomes of IVF/ICSI cycles in poor responders.
Study Design: this parallel open label randomized control trial was conducted in a university hospital. It included 240 females satisfying the bologna criteria for poor responders. The enrolled females were randomized into 2 groups: A (long/GH) receiving GH adjuvant therapy to the long protocol and group B (control) receiving the long protocol alone. The main outcome measure was the live birth rate (fresh, frozen and cumulative).
Results: GH supplementation improved the number of collected oocytes (5.4 ± 1.7 vs. 4.3 ± 2.1), MII oocytes (4.1 ± 2.1 vs. 2.1 ± 1.4), fertilized oocytes (4.0 ± 2.2 vs. 2.0 ± 1.2), transferred embryos (2.4 ± 0.9 vs. 1.6 ± 1.1) and cryopreserved (0.5 ± 0.7 vs. 0.2 ± 0.5). There was no significant difference in the live birth rate whether fresh (17.5% vs. 14.1%) or cumulative (18.3% vs. 14.7%).
Conclusions: Further studies are needed to know the true impact of adding GH to the induction protocols in poor responders, as there was no difference in the live birth rates between the study groups, indicating a lack of trend toward benefit from GH supplementation in poor responders.
Clinical Trial Registration: NCT02338206.

Introduction

The term 'poor responder', in assisted reproduction, refers to a subpopulation of patients, with diminished ovarian reserve, and major problems in conceiving using assisted reproductive techniques. Although, there is no standard definition of a 'poor responder' [1], the Bologna criteria suggested that classification of a poor responder requires two of the following features: (i) old age (≥40 years) or other factor for poor ovarian response, (ii) previous poor ovarian response (≤3 oocytes on ovulation induction), and (iii) low ovarian reserve test (antral follicle count <5–7 or anti-Müllerian hormone <0.5–1.1 ng/ml) [1].

However, several published studies suggested a variety of alternative criteria to define poor responders. Therefore the criteria, which define poor responders and their management options, are still debatable [2–5].

Many different ovarian hyperstimulation protocols have been tried to optimize the outcome for poor responders including increasing the gonadotrophins dose, short agonist protocol, microflare, antagonist protocol, minidose long protocol [6,7]. But the ideal protocol is not yet recognized.

Comparing the long protocol versus the short agonist protocol. Studies have shown that the long protocol gives better results when compared with the short protocol. On the other hand, the long protocol has a major adverse effect which is the long ovarian suppression, which is, in poor responders especially, will require the use of higher gonadotrophin doses and subsequently a modest ovarian response. Different modalities have been proposed to improve the outcome as the use of mini- dose long protocol as well as the addition of adjuvant treatment such as growth hormone (GH) [8].

Growth hormone, have long been studied as a co-treatment to the various ovarian stimulation protocols. GH can act directly or indirectly by releasing insulin-like growth factor 1 (IGF-1), as well as regulating oocyte maturation by increasing the ovarian sensitivity to gonadotrophins and enhancing early follicular

* Corresponding author at: Kasr Al Aini hospitals, Obstetrics and Gynecology Department, Manial, Cairo, Egypt.
E-mail address: dinadakhly@gmail.com (D.M.R. Dakhly).

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development [9]. However, its effect on IVF cycle outcomes' is still controversial as some studies demonstrated its positive impact on GH oocyte, endometrium and embryo related outcomes, and others failed to reach the same result [10–13].

The aim of this study was to detect the impact of adding GH, to the long down regulation protocol, as adjuvant therapy on the outcomes of IVF/ICSI cycles in poor responders.

Material and methods

This open label randomized control trial was carried out in Cairo University hospital, Kasr Al-Aini, Egypt, from April 2015 to November 2017. Before commencing the trial, it was approved by the university ethical committee "institutional review board".

The study included poor responder females who satisfied the bologna criteria [1]. Females above 45 years, or having FSH > 20 IU/L, and those with other causes of infertility as tubal occlusion or severe male factor as severe azospermia or teratospermia, as well as couples who refused to participate were excluded from the trial.

Prior to starting the study, detailed explanation of the protocol and intervention to all the enrolled couples was done and a signed consent was obtained.

The participating females underwent full history taking, medical and gynecological examination. Transvaginal sonographic (TVS) evaluation by Voluson 730 Pro ultrasound machine (GE, Fairfield, CT) was done.

The participants were then randomized into two groups; group A: (Long/GH) undergoing ovarian stimulation the long down regulation protocol with the addition of GH, and group B (Control group): ovarian stimulation was done by the long protocol only.

Randomization was performed using specific computer programs and the results were placed in opaque sealed envelopes with the patients' number written outside (and after opening the envelope, it would reveal which group the patient belonged to (A or B).There was no blinding to either the participants or study conductors.

The treatment protocol was as following:

Group A and B: received down-regulation with triptorelin (Decapeptyl; Ferring, Switzerland) 0.1 mg/day from day 21 of the previous cycle. Reducing it to 0.05 mg/day from the start of the following cycle and continued till HCG administration.

Gonadotropins therapy started at day 2-3 of the menses by a dose of 300 IU of recombinant human FSH (Gonal-F, Serono, Switzerland) after confirming that proper down regulation was achieved. The gonadotropin dose was adjusted from day 6 of stimulation according to the ovarian response monitored by serial TVS until the day of HCG administration. 10000 IU of HCG (Choriomon, IBSA) were given IM, when at least two follicles had reached a diameter of 18 mm or more.

Group A patients (long/GH) received adjuvant Growth hormone co-treatment 2.5 mg subcutaneous injection of GH (equivalent to 7.5 IU) (Norditropin pen, Novo Nordisk, Denmark) from day 21 of the previous cycle along with GnRHa, until the day of HCG. Analysis of the serum progesterone, LH and E2 was performed on the day of HCG administration.

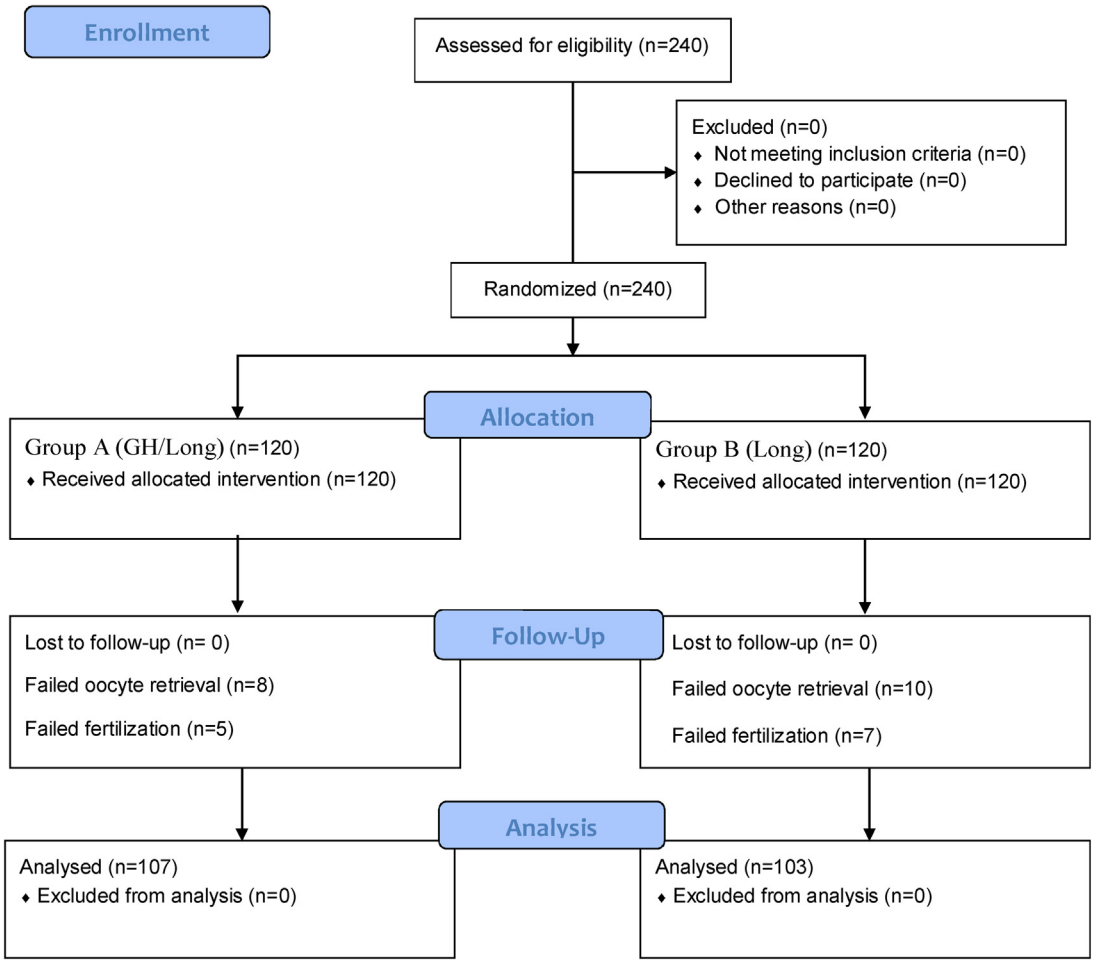

Fig. 1. CONSORT 2010 Flow Diagram.

Oocyte retrieval was performed 35 hours following HCG administration under TVS guidance. Our protocol included the transfer of a maximum of three embryos on day 3 of oocyte retrieval. Any additional embryos were cryopreserved. Luteal phase support was maintained by Cyclogest 400 mg (Alpharma, UK) vaginal suppositories twice daily.

Follow up continued through out the luteal phase, quantitative β HCG was performed 14 days following embryo transfer and was considered positive if ≥ 50 IU/L. In cases with confirmed pregnancy TVS was performed two weeks later, confirming the presence of an intrauterine sac with positive fetal pulsations. Pregnant cases were followed up till delivery.

The main outcome of the study was live birth rate (fresh, frozen and cumulative), while the secondary outcomes of the study included comparing the two groups regarding the duration of gonadotrophin stimulation (days), the total units gonadotrophins given, the number of oocytes retrieved, the number of MII oocytes, the number of fertilized oocytes, the number of embryos transferred and frozen, the fertilization rate, the implantation rate, the chemical, clinical and ongoing pregnancy rates.

Chemical pregnancy was diagnosed by serum βHCG ≥ 50 IU/L, 14 days after the embryo transfer. Clinical pregnancy was diagnosed by the presence of viable heartbeats in a gestational sac, 5 weeks after positive βHCG. The implantation rate was calculated as the number of intrauterine sacs divided by the number of embryos transferred. Early miscarriage was defined as pregnancy loss before 12 weeks of gestation. Ongoing pregnancy was defined as pregnancies continuing beyond 12 weeks of gestation. Live birth rate was defined as the number of achieved live birth after 28 weeks of gestation.

Pre-coded data was entered into the Statistical Package of Social Science Software program, version 15 (SPSS) to be statistically analyzed. Data was described using mean, and standard deviation for quantitative variables and frequency and percentage for qualitative ones. The odds ratio was calculated for the clinical pregnancy rate and the live birth rate (OR and the 95% confidence interval (95%CI). Comparison between groups was performed using Student *t* test for quantitative variables and Chi square (c [2]) test for qualitative ones. *P* values less than 0.05 was considered statistically significant. Post-hoc power analysis was done for some of the variables.

Results

Fig. 1 shows the flow chart for recruiting patients in this study. The patients were randomized into two groups; each group consisted of 120 patients. Group A (long/GH) received GH along their induction with the long protocol, while Group B (control) did not take GH supplementation. All patients were counseled regarding their poor reproductive outcomes, having a smaller yield of oocytes, 18 patients from the 2 groups had no oocytes collected on the day of ovum pickup and failure of fertilization of the collected oocytes occurred in 12 patients. Thus 13 patients in group A had their cycles cancelled versus 17 in group B.

There was no difference in the base line characteristics for the patients in the 2 groups, as shown in Table 1.

Table 2 shows the cycle characteristics for the 2 groups. The GH group showed significantly less days of stimulation, dose of gonadotropin used and mean LH levels on day of HCG. While, it showed significantly higher; mean E2 levels on the day of HCG, endometrial thickness, collected oocytes, MII oocytes, fertilized oocytes, transferred embryos, cryopreserved embryos and cycles with cryopreserved embryos.

Despite this significant difference in cycle outcomes, there was no difference in the reproductive outcomes. The results are displayed in Tables 3 and 4. This study analyzed the cumulative live births rate achieved after transferring all the cryopreserved embryos in the 2 groups and it also did not show a significantly higher preference to the GH group. The odds ratio (95% confidence interval) between the 2 groups for clinical pregnancy was 1.29 (2.43 – 0.68) and for live births (fresh cycles) was 1.28 (2.57 – 0.64) it still showed no significant effect for GH supplementation in IVF/ICSI cycles.

Post-hoc power analysis for the number of MII oocytes, fertilized oocytes, number of embryos transferred with alpha error 0.05 was 100%. While for clinical pregnancy rate/ cycle start was 15.3%, for live birth rate/ cycle start was 10.8% and cumulative live birth rate/ cycle start was 11.3%.

Discussion

This study shows the effect of adding GH to the long down regulation protocol in IVF/ICSI cycles, in poor responder females to improve the number and quality of recruited oocytes.

The results of this work go in line with our previous studies adding the GH to the microflare and antagonist protocols and still concludes that although GH supplementation improved mean E2 levels on the day of HCG, endometrial thickness, collected oocytes, MII oocytes, fertilized oocytes, transferred embryos, cryopreserved embryos and cycles with cryopreserved embryos. It still did not improve the clinical pregnancy rate and live birth rate [10,11]. Taking it a step further the study followed up all the cryopreserved embryos replacement and assessed the cumulative live birth from all the oocytes retrieved from the initial stimulation cycle, and still it did not show a trend of benefit from GH supplementation.

In this study the long down regulation protocol, was used for ovarian stimulation. Several studies analyzed the use of different protocols for induction of ovulation in patients with diminished ovarian reserve, either due to old age or compromised ovarian reserve. Ho et al. used the long agonist protocol in 3 groups of patients; the first group was women of advanced age. The second group was with one or more previous IVF failures, and the third group was younger women with poor ovarian response. They concluded that GH did improve the number of oocytes retrieved,

Table 1
Basal characteristics of patients.

Variables	Group A GH/Long group *n* = 120	Group B Long group *n* = 120	P value
Age (years)	36.4 ± 4.4	36.2 ± 4.5	0.765
BMI (kg/m [2])	23.3 ± 3.9	23.6 ± 4.1	0.463
Duration of infertility (years)	6.2 ± 2.3	6.3 ± 2.4	0.532
Number of previous cycles with poor response	2.3 ± 1.1	2.2 ± 1.2	0.510
Basal FSH (IUI/L)	10.6 ± 1.5	10.5 ± 1.8	0.391
AntiMullerian Hormone (ng/ml)	0.4 ± 0.2	0.5 ± 0.2	0.557
Antral follicular count	5.5 ± 2.2	5.6 ± 2.1	0.953

*P value < 0.05 is considered statistically significant, all values presented as mean and standard deviation.

Table 2
Cycle characteristics.

Variables	Group A GH/Long group $n = 120$	Group B Long group $n = 120$	P value
Duration of HMG treatment (days)	11.1 ± 1.4	12.2 ± 1.5	<0.001*
Total doses of gonadotropin (IU)	3386.2 ± 1113.7	4789.3 ± 1332.2	<0.001*
E2 levels on hCG day (pg/mL)	1903.9 ± 722.1	882.7 ± 355.5	<0.001*
LH levels on hCG day (IU/L)	2.9 ± 1.1	4.9 ± 1.2	<0.001*
Progesterone levels on hCG day (ng/ml)	0.7 ± 0.3	0.8 ± 0.2	0.231
Endometrial thickness (mm)	11.8 ± 1.3	11.3 ± 1.2	<0.001*
Number of collected oocytes	5.4 ± 1.7	4.3 ± 2.1	<0.001*
Number of MII oocytes	4.1 ± 2.1	2.1 ± 1.4	<0.001*
Number of fertilized oocytes	4.0 ± 2.2	2.0 ± 1.2	<0.001*
Number of transferred embryos	2.4 ± 0.9	1.6 ± 1.1	<0.001*
Number of frozen embryos	1.1 ± 1.4	0.2 ± 0.5	<0.001*
Numbers of cycles with frozen embryos per cycle start n/n (%)	51/120, 42.5%	22/120, 18.3%	<0.001*
Number of cycles with frozen embryos per embryo transfer n/n (%)	49/120, 40.8%	20/120, 16.6%	<0.001*

* P value < 0.05 is considered statistically significant, all values presented as mean and standard deviation, unless stated otherwise.

Table 3
Reproductive outcomes.

Variables	Group A GH/Long group $n = 120$	Group B Long group $n = 120$	P value
Cancelled cycles, *n/n* (%)	13/120, 10.8 %	17/120, 14.8 %	0.435
Fertilization rate (%)	46.7 %	41.1 %	0.019*
Implantation rate (%)	10.3 %	8.8 %	0.587
Chemical pregnancy rate/cycle start, *n/n* (%)	37/120, 30.8%	32/120, 26.6%	0.475
Chemical pregnancy rate/embryo transfer, *n/n* (%)	37/107, 34.6 %	32/103, 31.1 %	0.588
Clinical pregnancy rate/cycle start, *n/n* (%)	29/120, 24.2%	23/120, 19.2%	0.347
Clinical pregnancy rate/embryo transfer, *n/n* (%)	29/107, 27.1 %	23/103, 22.3 %	0.423
Early miscarriage rate/cycle start, *n/n* (%)	8/120, 6.6%	6/120, 5.0%	0.581
Early miscarriage rate/embryo transfer, *n/n* (%)	8/107, 7.5%	6/103, 5.8%	0.631
Ongoing pregnancy rate/cycle start, *n/n* (%)	21/120, 17.5%	17/120, 14.1%	0.479
Ongoing pregnancy rate/ embryo transfer, *n/n* (%)	21/107, 19.6%	17/103, 16.5%	0.557

* P value < 0.05 is considered statistically significant.

Table 4
Live birth rates.

Variables	Group A GH/Long group $n = 120$	Group B Long group $n = 120$	P value
Live birth rate (Fresh)/cycle start, *n/n* (%)	21/120, 17.5%	17/120, 14.1%	0.479
Live birth rate (Fresh)/embryo transfer, *n/n* (%)	21/107, 19.6%	17/103, 16.5%	0.557
Live birth rate (Frozen)/embryo transfer, *n/n* (%)	7/33, 21.2%	3/16, 18.7%	0.841
Cumulative live birth rate/cycle start, *n/n* (%)	28/153, 18.3%	20/136, 14.7%	0.412
Cumulative live birth rate/embryo transfer, *n/n* (%)	28/140, 20%	20/119, 16.8%	0.509

*P value < 0.05 is considered statistically significant.

implantation and pregnancy rates in women with recurrent failure and in young poor responders, but not in women with advanced age [14].

Another study used low dose GH, in a long agonist protocol, they started GH daily from the first day of the agonist treatment until the HCG trigger. They found a larger number of oocytes and embryos but not reaching significance and reported a higher embryo quality and clinical pregnancy rate in the GH group [13].

Yovich and his group who had studied adjuvant therapy with GH in poor responders extensively, in a 5-year data report, used different protocols of induction including the long downregulation protocol, the antagonist protocol and the flare stimulation protocol. In general, they used the long down regulation protocol in younger poor responders and it was the least protocol to be used in their report, but they concluded that there were no significant differences in pregnancy rates or the likelihood of a live-born baby among any of the stimulation regimens, which received GH augmentation [15]. In a more recent report by the same group they also used antagonist as well as agonist protocols of induction in GH supplemented cycles with no different outcomes between different protocols [16] and in both reports they suggested a favorable significant outcome of GH therapy on implantation and pregnancy rates.

The number of oocytes retrieved is reflected by the mean E2 levels achieved on the day of HCG. GH supplementation has significantly increased the mean E2 levels in this study as well as others [10,11,17,18]. It might be anticipated that more estradiol was secreted from each follicle when midluteal GH was administrated in the GH group. As higher pregnancy rates were predicted with higher levels of E2 in pre-ovulatory follicular fluid [19], this observation implies that GH supplementation in the recruitment phase may be a better approach for poor responders even when combined with other induction protocols.

The endometrial thickness is one of the parameters assessing endometrial receptivity. In this study we found the endometrial thickness to be significantly higher in the GH group. The

endometrial receptivity and the effect of GH is not studied yet, this might be just a coincidental finding, and is not supported by an increase in implantation. However, it is still a matter of debate if GH improves the endometrial quality [16].

Many studies using different protocols of induction found an increase in the number of oocytes collected, as well as an increase in the number of MII oocytes [10–12,17]. The role of the GH/IGF-1 axis on folliculogenesis is improving the ovarian response to gonadotropin stimulation, IGF 1 in the follicular fluid, amplifies the action of FSH, improving granulosa cell division, aromatase activity, and inhibiting follicle apoptosis [20]. Which might explain the increase in the number and quality of oocytes especially when using GH from day 21 of the preceding cycle of stimulation.

The concentration of GH in the follicular fluid allows the human oocytes to form high-grade embryos [21]. GH is considered one of the important hormones causing faster cleavage with good morphology, and higher implantation potential [22]. In this study, in the GH group, there was significantly more fertilization and more embryos available for transfer and cryopreservation.

The best cohort of patients to benefit from GH supplementation is still mystery. It is speculated by some researchers that as fasting GH concentrations drop by age [23], older poor responders will be the ideal subgroup of patient to use adjuvant GH. This was addressed by several studies that found an increase in pregnancy rates in patients more than 40 years old [16,24].

A recent updated meta-analysis, concluded that although GH almost universally seems to shorten the days of stimulation, yield more oocytes and all the early clinical parameters appear favorable, however there is no evidence to prove an increased chance of a live birth for women receiving GH as a supplement for their induction of ovulation [25].

They highlighted one of the largest studies performed to date, the LIGHT study performed in Australia, which also showed improvement in ovarian response to stimulation, but with no benefit in live birth [26].

This contradicts the latest Cochrane review, which stated that the use of GH significantly improved the live birth rate, but they stated that the main limitation of the available studies was the small sample size [27].

Limitations of this study include, not performing a cost-effective analysis for the use of GH, especially that GH was used for a long duration in their protocol.

In conclusion, the use of GH as an adjuvant therapy in induction of ovulation in poor responders did not improve the clinical pregnancy rates, live birth rates or cumulative live birth rate, awaiting further systemic reviews and meta-analysis.

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