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CLINICAL ARTICLE

Addition of growth hormone to the microflare stimulation protocol among women with poor ovarian response

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ABSTRACT

Objective: To assess the efficacy of adding growth hormone (GH) to the microflare stimulation protocol among women with poor ovarian response. *Methods:* A parallel, open-label, randomized controlled trial was conducted among patients with poor ovarian response who attended a center in Cairo, Egypt, between July 10 and December 31, 2014. Participants were randomly assigned using a computer program (random block size of 4–8) to undergo the microflare protocol with or without GH. Primary outcomes were the mean numbers of mature oocytes retrieved and fertilized. Analyses were done per protocol: women with cycle cancellations were excluded. *Results:* The analysis included 72 women in the GH group and 73 in the microflare only group. The mean number of oocytes collected was 7.2 ± 1.5 in the GH group versus 4.7 ± 1.2 in the microflare only group ($P < 0.001$). The mean number of metaphase II oocytes was 5.2 ± 1.2 in the GH group and 2.8 ± 1.0 in the microflare only group ($P < 0.001$). The mean number of fertilized oocytes was higher in the GH group (4.2 ± 1.1) than in the microflare only group (2.5 ± 0.7; $P < 0.001$). *Conclusion:* Addition of GH to the microflare stimulation protocol provided some potential benefits to women with poor ovarian response. However, further studies are required before it could be recommended for routine clinical use.

ClinicalTrials.gov: NCT02185326.

1. Introduction

Many women presenting for infertility treatment have poor ovarian response (POR) [1,2], with the reported prevalence varying from 9% to 24% among different studies [3,4]. At present, there is no globally accepted definition for POR, although the European Society of Human Reproduction and Embryology (ESHRE) published a consensus statement in 2011 [5]. The ESHRE definition of POR requires any two of three criteria to be met: age of at least 40 years; a previous treatment cycle that resulted in the collection of three or fewer oocytes using a traditional stimulation protocol; or an abnormal ovarian reserve test result, defined as an antral follicle count (AFC) of less than five to seven follicles or a serum anti-Müllerian hormone level of less than 0.5–1.1 ng/mL [5].

The management of patients with POR is highly controversial. No consensus exists regarding the ideal protocol and, to date, no one treatment strategy has proven optimal among this population. Approaches taken to improve oocyte yield include increasing the dose of gonadotropins, reducing the dose of gonadotropin-releasing hormone analog (GnRHa), using estrogen priming to suppress an early rise in the levels of follicle-stimulating hormone (FSH), and enhancing the intrinsic flare effect of FSH [1]. In addition, some studies have suggested the use of adjunctive growth hormone (GH) [1,3,4] or aromatase inhibitors [6,7].

The conventional long protocol in which GnRHa is administered for approximately 10 days in the late luteal phase before administration of exogenous gonadotropins might not be the best treatment for women with POR. Starting the treatment cycle with GnRHa could reduce levels of endogenous gonadotropins, which would subsequently decrease the ovarian response. These difficulties led to the development of the microflare stimulation protocol. In this approach, an adjustment is made to the conventional long protocol by initially using oral contraceptive pills (OCPs) and decreasing GnRHa doses to microdoses (e.g. 0.05 mg). These procedural changes reduce the negative effects of the FSH flare caused by GnRHa on follicle recruitment and result in the collection of increased numbers of oocytes and a marked rise in pregnancy rates [3,8]. The use of OCPs in this scenario allows enhanced cycle scheduling and prevents endogenous surges of luteinizing hormone (LH) as efficiently as does GnRHa, which might result in mild suppression of the endogenous gonadotropins and so improve the response by synchronizing follicular growth.

Numerous biological effects have been attributed to the activity of GH on the ovary, including a positive impact on steroidogenesis, follicular growth, and oocyte maturation [9]. The addition of GH during ovarian stimulation enhanced the response of granulosa cells to gonadotropins in both animal and human studies [10–13]. Furthermore, GH acts early in the cycle by enhancing the growth of small follicles and

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preventing their atresia, as well as late in the follicular phase (in combination with gonadotropins) by enhancing late folliculogenesis, luteinization, and steroidogenesis [14–18].

The aim of the present study was to assess the effect of adding GH to the microflare stimulation protocol for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) among women with POR.

2. Material and methods

A parallel, open-label, randomized controlled trial was conducted at the Kasr Al Aini IVF Center, Cairo University, Cairo, Egypt, from July 10 to December 31, 2014. To be eligible, patients had to meet the 2011 ESHRE criteria for POR [5]. The exclusion criteria were FSH levels greater than 20 IU/L, previous ovarian surgery, causes of infertility other than POR, polycystic ovary syndrome, any endocrine disorder (e.g. diabetes mellitus or thyroid disease), and male factor infertility. The present study was approved by the institutional review board of Cairo University. Participants were asked to sign an informed written consent form at enrollment, with all details of the protocol included and verbally explained.

All participants provided a detailed history. Additionally, they underwent assessment of body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters), a day-3 hormonal assay, and vaginal ultrasonography using the Voluson 730 Pro apparatus (GE Healthcare, Little Chalfont, UK). The initial imaging test involved assessment of AFC, which was performed by one expert sonographer (D.M.R.D.) to eliminate any interobserver differences.

Participants were randomly assigned to undergo the microflare protocol with or without GH. Randomization was performed using a specific computer system (Quickcalcs [Graphpad, La Jolla, CA, USA]) and sealed envelopes, with a random block size of between four and eight. Participants, investigators, and data analysts were not masked to group assignment.

All participants underwent the microflare stimulation protocol according to the following schedule. A combined OCP (drospirenone plus ethinyl estradiol) was administered for at least 21 days before starting ovarian stimulation. After a gap of 2 days, participants then began a regimen of 0.05 mg triptorelin (GnRHa) delivered subcutaneously on a daily basis. Three days later, participants began to receive 300–450 IU human menopausal gonadotropin (HMG) intramuscularly on a daily basis. Dose was determined according to age, AFC, and anti-Müllerian hormone level. Participants assigned to the GH group also received 2.5 mg GH delivered subcutaneously on a daily basis from day 6 of HMG stimulation until ovulation could be induced with human chorionic gonadotropin (hCG). Follicular growth was monitored from the eighth day of HMG administration; ovulation was induced with 10 000 IU hCG when at least two leading follicles reached a diameter of 17 mm or greater. Cycles were cancelled if fewer than three mature follicles were detected during follow-up. Serum progesterone, LH, and estradiol levels were analyzed on the day of hCG induction.

Oocyte retrieval was performed 35 hours after administration of hCG. A maximum of three embryos was transferred on the third day after oocyte retrieval; any additional embryos were cryopreserved. As part of luteal phase support, participants received 400 mg vaginal progesterone twice a day, 75 mg oral acetylsalicylic acid once a day, and 2 mg oral estradiol valerate three times a day until the pregnancy test and if positive, until the end of the first trimester. Participants returned 12 days after transfer to undergo quantitative estimation of serum β-hCG levels.

Although initially the primary outcome was clinical pregnancy rate, this outcome did not reflect the outcome of GH on treatment cycles or the oocyte yield. Therefore, the primary outcome was changed 3 months after trial initiation to the mean number of mature oocytes retrieved and fertilized. Secondary outcomes included HMG dose and duration of stimulation (days); endometrial thickness; estradiol, LH, and progesterone levels on the day of hCG triggering; the mean number of metaphase II oocytes; fertilization rates; the mean number of embryos transferred; implantation rates; chemical and clinical pregnancy rates; and cycle cancellation rates. Fertilization rate was defined as the ratio of oocytes fertilized to the number of retrieved oocytes. Implantation rate was defined as the ratio of gestational sacs to the number of embryos transferred. Chemical pregnancy was defined as a serum β-hCG level of at least 50 IU/L at day 12 after embryo transfer. Clinical pregnancy was defined as the detection of fetal heart activity by vaginal ultrasonography 5 weeks after a positive β-hCG test result. Cycle cancellation included cases in which no embryos were transferred owing to either failed oocyte retrieval or failed fertilization.

The pre-coded data were analyzed using SPSS version 15 (SPSS Inc, Chicago, IL, USA). Analyses were done per protocol: women with cycle cancellations were excluded. Data were summarized using the mean and standard deviation for quantitative variables and the number and percentage for qualitative variables. As the final cycle outcome, clinical pregnancy rate was compared using the odds ratio (OR) and 95% confidence interval (CI). Between-group comparisons were performed using the Student t test for quantitative variables and the χ^2 test for qualitative variables. $P < 0.05$ was considered statistically significant.

3. Results

Overall, 172 women met the inclusion criteria and were enrolled (Fig. 1): 84 were randomly allocated to the GH group and 88 to the microflare only group. The final analysis included 145 participants; the remaining 27 participants were excluded from the analysis owing to cycle cancellation (Fig. 1).

The characteristics of the two groups are shown in Table 1. During the controlled ovarian stimulation cycles, significant between-group differences were detected for HMG dose and duration, serum estradiol and LH levels on the day of hCG triggering, the mean number of collected oocytes, and the mean number of metaphase II oocytes (Table 2). By contrast, no statistically significant differences were observed in endometrial thickness or serum progesterone level on the day of hCG triggering.

The outcomes of the ovarian stimulation cycles are displayed in Table 3. Statistically significant between-group differences were detected for the mean numbers of fertilized oocytes and transferred embryos, with higher values associated with the addition of GH to the microflare stimulation protocol ($P < 0.001$ for both). However, fertilization and implantation rates were not significantly different.

No significant difference was recorded in the clinical pregnancy rate, although this rate was higher in the GH group than in the microflare only group (Table 3). The OR was 1.93 (95% CI 0.91–4.09), which indicated that GH exerted a favorable effect on this measure.

4. Discussion

The present study found that the numbers of collected oocytes, metaphase II oocytes, and fertilized oocytes increased when GH was added to the microflare stimulation protocol. However, GH did not exert a statistically significant effect on the rates of chemical and clinical pregnancies.

To date, few studies have assessed the impact of GH treatment on pregnancy rates among women with POR who are undergoing IVF or ICSI. A systematic review [19] found that there is potentially great benefit to be derived from adding GH; nevertheless, its routine use in clinical practice was not encouraged by the authors. To the best of our knowledge, the present study is the first clinical trial studying the effect of addition of GH to the microflare agonist stimulation protocol among women with POR.

The total duration and dose of HMG used in the present study were lower in the GH group than in the microflare only group. This finding is in line with that of a large study conducted by Kucuk et al. [9], in which the GH effect was studied using the conventional long GnRHa protocol.