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ORIGINAL ARTICLE

Low-dose growth hormone supplementation increases clinical pregnancy rate in poor responders undergoing *in vitro* fertilisation

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Abstract

Poor ovarian response (POR) often means low success rates after *in vitro* fertilisation (IVF). We aim to study the impact of a low-dose growth hormone (GH) supplementation in pregnancy rates in poor responders in a prospective, self-controlled study of 64 poor responders to previous IVF cycles, who failed to achieve pregnancy and were supplemented with low-doses of GH in a subsequent cycle using the same gonadotropin dose and protocol. Our primary endpoint was the clinical pregnancy rate (CPR), considering secondary endpoints, the number of retrieved oocytes, embryos, embryo quality and the proportion of cycles with embryo transfer. CPR in the GH group was 34.4%. Significant differences were observed for the GH group both in the number of top quality embryos (0.64 ± 0.88 versus 1.03 ± 1.17, $p < 0.05$) and cryopreserved embryos (0.3 ± 0.81 versus 0.85 ± 1.49, $p < 0.05$). This is, to our knowledge, the first clinical trial to use a low dose of GH as a supplement for IVF in POR patients. Despite this low dose, we achieved excellent success rates in patients with a very poor prognosis, at a reasonable cost and without side effects, which makes this a safe and cost-effective alternative.

Keywords

Growth hormone, IVF, poor ovarian response

History

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Introduction

Poor ovarian response (POR) is defined by the Bologna criteria [1], with an incidence between 9 and 24% of all cycles of *in vitro* fertilisation (IVF) [2]. Multiple interventions have been proposed to improve outcomes in this group of patients, with limited effectiveness [3,4].

One of them is growth hormone (GH) supplementation, as its participation in follicular development has been well established. GH regulates the effect of gonadotropins in granulosa cells by regulating the synthesis of insulin growth factor-I (IGF-I), which plays an important role in the synthesis of sex steroids and oocyte maturation [5,6].

GH supplementation for IVF has been in use since 1990s [7], and the first trials in normogonadotropic patients showed no therapeutic advantage [8]. Subsequent studies showed that GH could be useful in women of advanced reproductive age [9] and is the only pharmacological intervention that might increase pregnancy and live birth rates (LBR) in patients with POR undergoing IVF [3].

Many GH supplementation protocols have been published that include different strategies of controlled ovarian hyperstimulation (COH). Long protocols of gonadotropin-releasing hormone (GnRH) agonist, flare-up and antagonist protocols have been used, with GH doses ranging from 4 to 24 IU [7–14].

Available evidence shows increased pregnancy and LBR with the use of GH in POR patients [2–4,15]. However, its high price has prevented conducting a greater number of trials, and it is not an extended strategy. Moreover, the doses commonly used in clinical trials are those used for GH-deficient patients. POR patients do not have proven GH deficiency and, perhaps, need not be supplemented with such high doses.

The objective of this study is to assess whether a low dose of GH supplementation (0.5 IU/day) increases clinical pregnancy rates (CPR) in women with a history of POR, who failed to become pregnant in two previous IVF cycles.

Materials and methods

Study population

This is a prospective, self-controlled study that recruited women with a history of POR, defined according to the Bologna criteria [1], and an absence of pregnancy in at least two previous IVF cycles, supplementing the third cycle with GH. It was conducted at CIRH – Barcelona, between July 2012 and December 2013, and was approved by a central ethics committee.

The exclusion criteria were: body mass index $\geq 30\,kg/m^2$; presence of endocrinopathies; altered karyotype in one or both partners; history of invasive ovarian surgery; history of chronic, autoimmune or metabolic diseases; altered meiosis in testicular biopsy or altered sperm-FISH, drug therapy in the male partner; and participation, within the previous 6 months, in another clinical trial with medication.

During the study period, participation was offered to 106 patients with previous IVF failures, who were offered, as an alternative to enrolment, another IVF cycle without GH supplementation or an oocyte donation cycle. Patients who agreed to participate were recruited consecutively to achieve the sample size.

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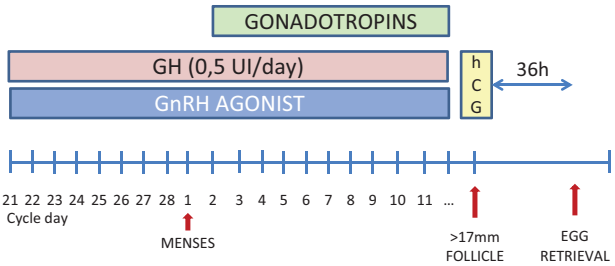

Figure 1. Controlled ovarian stimulation protocol.

Ovarian stimulation protocol

Controlled ovarian hyperstimulation was performed using a GnRH agonist long protocol from day 21–23 of the menstrual cycle (Procrin, Abbvie Pharmaceutical, Saint-Remy-sur-Avre, France) at a dose of 1 mg/d, using the same schedule and dose as in the previous cycle, so that the only difference between them was the GH supplementation (Figure 1).

After confirming pituitary inhibition, the dose of GnRH agonist was decreased to 0.5 mg/d, and COH was begun with gonadotropins at a dose of 300 UI (Gonal-F, Merck Serono, Tres Cantos, Spain; Pergoveris, Merck Serono, Tres Cantos, Spain), subsequently adjusting according to the clinical response. Human chorionic gonadotropin (hCG) was administered when follicles reached >17 mm in diameter (Ovitrelle 250 mcg, Merck Serono, Bari, Italy). A dose of 0.5 IU of GH (Saizen, Merck Serono) was administered daily from the first day of the agonist until the day of hCG administration. Oocyte retrieval was performed 36 h after hCG administration by ultrasound-guided follicle aspiration.

Sperm was capacitated using density gradients. Oocytes were cultured for 4 h post-harvest before being inseminated for IVF or decumulated for intracytoplasmic sperm injection (ICSI). The embryo culture was performed following standard protocols using the system of morphological assessment of oocytes, early embryos and human blastocysts of the Spanish Association of Reproductive Biology (ASEBIR, II Journal of Clinical Embryology, Second Edition, 2008) for classification. This classification divides embryos into four categories (A, B, C and D), with A and B considered to be top quality embryos.

Transfer of 1, 2 or 3 embryos was performed on the third day after insemination and embryos suitable for cryopreservation underwent vitrification on that same day. The luteal phase was supported with 600 mg/day of micronised progesterone.

Statistical analysis

The primary objective of this study was the CPR. Secondary endpoints were the number of retrieved oocytes and obtained embryos, embryo quality and cycle cancellation rate.

Clinical pregnancy is the presence of an intrauterine gestational sac with embryonic cardiac activity display, evaluated by ultrasound at 7 weeks of gestation. Miscarriage is a pregnancy that fails to become an ongoing pregnancy. A cancelled cycle was defined as one in which embryo transfer could not be performed due to a lack of follicular or embryonic development.

The sample size was calculated assuming an alpha risk of 0.05 and a power level of 0.80 in a bilateral contrast, assuming an increase by 15 percentage points in CPR from the historical CPR for POR patients and estimating a loss to follow-up of 10%. A value of N of 59 patients was calculated.

Data analysis was performed using the SPSS 20 (SPSS Inc., Chicago, IL) software. The chi-square test, Mann–Whitney and Student's t-test with a level of statistical significance of $p < 0.05$ were used as needed.

Table 1. Demographic characteristics for GH and non-GH cycles.

Parameter	Non-GH cycle (mean ± SD; $N = 64$)	GH-cycle (mean ± SD; $N = 64$)	p
Age (years)	37.91 ± 3.84	38.04 ± 3.82	0.69
Baseline FSH	10.83 ± 4.92	10.85 ± 4.91	0.98
Baseline Estradiol	54.61 ± 31.4	54.66 ± 31.05	0.99
AMH (ng/dl)	0.64 ± 0.5	0.63 ± 0.49	0.99
AFC	4.84 ± 2.61	4.86 ± 2.68	0.94

Results

Sixty-four patients were recruited. The age of the study population was 38.4 ± 3.82 years; and the anti-Müllerian hormone (AMH) value was 0.63 ± 0.49 ng/ml. A demographic comparison is presented in Table 1, showing no significant differences.

The characteristics of COH and the cycle comparative results are shown in Table 2. There were no differences in the duration of COH or the total dose of gonadotropins administered. A greater number of oocytes (4.67 ± 2.69 versus 5.32 ± 3.98, $p = 0.38$) and embryos (1.94 ± 1.29 versus 2.48 ± 1.93, $p = 0.08$) were obtained in the GH cycles, although it was not statistically significant. The cycle cancellation rate was lower in GH cycles, which did not reach significance (32.8 versus 18.8%, $p = 0.06$).

Significant differences were observed for the GH group both in the number of top quality (A/B) embryos (0.64 ± 0.88 versus 1.03 ± 1.17, $p < 0.05$) and cryopreserved embryos (0.3 ± 0.81 versus 0.85 ± 1.49, $p < 0.05$).

The CPR in the GH group was 34.4% per initiated cycle, with an abortion rate of 24%. There were no cases of ovarian hyperstimulation syndrome or ectopic pregnancies. No side effects associated with GH administration were observed.

Discussion

Our results show that, in POR patients, low-dose GH supplementation increases CPR. These results agree with the conclusions of several meta-analyses reporting increased pregnancy and LBR [2–4,15].

Unlike other studies, our results show no decrease in the total dose of gonadotropins used during GH cycles [11]. This decrease in FSH requirements has proposed a role for GH in optimising FSH action in follicular development through the increased IGF-I activity [7,16], which would also lead in some cases to obtaining a higher number of oocytes and embryos [10,17]. Not having observed this in our study, as in others [18], leads us to believe that this is at least partially influenced by the use of high doses of gonadotropins, which would render the effect of GH irrelevant in this regard.

GH is a polypeptide hormone that stimulates growth and cell proliferation. Its synthesis, storage and secretion are performed by the anterior hypophysis. GH exerts biological effects on most of the tissues, inducing local synthesis of IGF-I. In addition to their actions as growth factors, both GH and IGF-I have effects on tissue metabolism. In ovarian function, GH is necessary for follicular development [19,20].

The evidence for ovarian GH receptor expression in animals suggests the participation of GH in oocyte maturation [6,21–23], as there is a correlation between an increase in GH levels in follicular fluid and improved pregnancy rates [24,25]. This could explain the effect of GH on oocyte maturation and the quality of embryos obtained [6,12], especially in women of advanced age, in which decreased oocyte quality is a major cause of failure [26] that could be partially amended by GH supplementation [9]. We observed a significant difference in embryo morphology in

Table 2. Results of the controlled ovarian stimulation in GH and non-GH cycles.

Parameter	Previous (non-GH) cycle (mean ± SD)	GH cycle (mean ± SD)	*p* Value
Patients (*N*)	64	64	
Days of COH	11.2 ± 2.5	11.2 ± 2.9	0.98
Total FSH dose (UI)	2630.7 ± 721.9	2764.5 ± 730.7	0.32
Total LH dose (UI)	934.2 ± 612.3	960.5 ± 445.8	0.79
Number of oocytes retrieved	4.67 ± 2,69	5.32 ± 3.98	0.31
Number of embryos	1.94 ± 1.29	2.48 ± 1.93	0.08
Number of A/B embryos	***0.64 0.88***	***1.03 1.17***	***0.046***
Number of C/D embryos	1.2 ± 0.97	1.26 ± 1.15	0.76
Number of embryos transferred	1.22 ± 0.93	1.46 ± 0.96	0.91
Number of frozen embryos	***0.30 0.81***	***0.85 1.49***	***0.02***
Number of cancelled cycles	21 (32.8%)	12 (18.8%)	0.06
Pregnancy rate		25 (39.1%)	
Clinical pregnancy rate		22 (34.4%)	
Abortion rate		6 (24%)	

Bold/italics value signifies statistical significance.

favour of GH cycles, which should result in a better embryo quality.

Finally, there is evidence in animal models of a modulating action of GH on endometrial receptivity [27], which cannot be excluded when explaining our results. We are not aware of any studies that have analysed this in humans.

There is no consensus regarding the dose or administration regimens of GH to be used during COH [7,9,11,12,17,28,29]. Regardless, most of the studies have shown a positive effect on pregnancy rate. Our data show comparable results in terms of CPR with the administration of lower doses. The primary advantage is related to safety, as the occurrence of adverse effects is directly correlated with the dose. Moreover, there is evidence that supplementation of GH in low doses, such as used in this study, maintain IGF-1 levels within normal ranges in the absence of side-effects [30]. The second advantage is that it offers a more economic treatment strategy. Kukuc et al. [11] estimated an increase of USD 2380 in the cost of cycle medication when supplementing with 12 UI/day of GH. This essentially doubles the cost of medication, which makes this therapy less accessible. In our study, GH supplementation of 0.5 IU/day increases the medication cost per cycle by only USD 390.

As it is not a randomised trial, the primary limitation of our study is exposal to a number of biases of the design itself. In our centre, the CPR for the POR patients that declined to participate in this study and underwent another autologous IVF cycle was 17.9%, a result consistent with published data ranging from 6% [9] to 18.2% [13]. In our study, we were able to increase the CPR to 34.4%, a figure higher than that has been reported in studies using doses at least 16 times higher; Tesarik et al. [9] published pregnancy rates of 26% with an 8 IU/day supplementation. Kukuc et al. [11] also reported rates of 32.3% using 12 IU/day of GH. It has already been demonstrated that there is no significant difference in the results obtained at various doses of GH. In our opinion, this is because we are dealing with women who suffer a physiological decline in GH levels due to age, not with patients with proven GH deficiency; therefore, low-dose supplementation is sufficient to optimise their chances of achieving pregnancy.

The strength of our study is that, to our knowledge, it is the first clinical trial to use a low dose of GH (0.5 IU/day) as a supplement to COH for IVF in patients with POR, an innovative dose with respect to all the trials to date. Despite this low dose, we achieved excellent success rates in patients traditionally associated with a poor reproductive prognosis, at a reasonable cost and without side effects, which makes this procedure a safe and cost-effective therapeutic alternative. Our results allow us to raise a reasonable doubt as to the utility of supplementing high doses of GH.

Finally, our study confirms the results of systematic reviews to date, reporting an increase in pregnancy rates in women with POR supplemented with GH. We can propose an explanation of a probable increase in embryo quality mediated by the action of GH but are unable to rule out a beneficial effect on the endometrium, as has been proposed in animal models [27]. New studies evaluating these courses of treatment are needed to confirm these results.

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Declaration of interest

The authors report no declaration of interests.

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