Growth hormone deficiency and supplementation at in-vitro fertilisation

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ABSTRACT

__Introduction:__ This study aims to evaluate the differences in oocyte stimulation, endometrial thickness, fertilisation rate and embryo quality at in-vitro fertilisation (IVF) in patients with documented growth hormone (GH) deficiency, after GH supplementation.

__Methods:__ This was a retrospective analysis of 20 cases of patients who were pregnant and had GH supplementation during IVF at the Singapore General Hospital between 1993 and 2003. All these patients had previously failed IVF due to poor stimulation, poor egg quality, poor fertilisation at intra-cytoplasmic sperm injection (ICSI) or failed implantation, and they had documented GH deficiency. These initial cycles were compared with their subsequent IVF cycles with GH supplementation. A non-parametric test was used for statistical analysis.

__Results:__ Embryo quality, determined by scoring the embryos on Day two using morphology, improved significantly after supplementation of GH (p-value is less than 0.001, median embryo score increased from 10.7 to 16). There was also a statistically significant increase in the fertilisation rate for those patients who had ICSI. There was no statistical difference in the number of oocytes retrieved or in the mean endometrial thickness with GH.

__Conclusion:__ This study implies that GH supplementation may improve embryo quality in selected patients with GH deficiency. Its role in improving fertilisation rate at ICSI merits further research and evaluation.

Keywords: embryo quality, growth hormone deficiency, growth hormone supple-mentation, in-vitro fertilisation

INTRODUCTION

The role of growth hormones (GH) at in-vitro fertilisation (IVF) has been investigated in the constant search for better pregnancy outcomes. The most recent Cochrane review of the role of GH suggested that there was a just significant improvement in the live-birth rate in previous poor responders. We have been using GH evaluation and supplementation for a period of more than ten years in our department. This study aims to evaluate the differences in oocyte stimulation, endometrial thickness, fertilisation rate and embryo quality at IVF in patients with documented GH deficiency, after GH supplementation.

METHODS

This is a retrospective analysis of GH pregnancies between 1993 and 2003 at the Singapore General Hospital. The study population was restricted to the patients with documented GH deficiency based on the clonidine test. Only patients who had a previous control cycle with us without GH and were later pregnant with GH in a subsequent cycle were included in the study. Therefore, in effect, the patients served as their own controls. Patients with panhypopituitarism were excluded. Patients who were identified to be GH deficient at our hospital with previous cycles done at other institutions were also excluded from the study.

All patients who had a poor response at stimulation, poor fertilisation at intracytoplasmic sperm injection (ICSI), poor egg quality and failed implantation in the absence of other discernible causes, were subjected to a clonidine test. A patient with poor follicular response and less than or equal to three eggs at oocyte recovery was classified as a poor responder. We took a fertilisation rate of < 50% at ICSI to indicate poor fertilisation. Poor egg quality was a subjective description by our embryologists of eggs that were dark, grainy and vacuolated. Failed implantation was recognised when there was no pregnancy after transfer of two or more embryos (at least grade 2) of a cell number appropriate for that day and an easy transfer with an adequately grown endometrium.

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Table I. Net embryo scoring on Day 2.

Embryo morphology	Embryo score	Embryo morphology	Embryo score
2 Gr1 cells	12	5 Gr2 cells	16
3 Gr 1 cells	12	6 Gr2 cells	16
4 Gr1 cells	24	2 Gr3 cells	4
5 Gr1 cells	24	3 Gr3 cells	4
6 Gr1 cells	24	4 Gr3 cells	8
2 Gr2 cells	8	5 Gr3 cells	8
3 Gr2 cells	8	6 Gr3 cells	8
4 Gr2 cells	16		

The patient's fasting blood samples were drawn for baseline GH. They were administered 150 mg of clonidine and their blood samples were taken once again for GH two hours later. A GH value of less than 4 miu/L at fasting or at two hours was taken as evidence of GH deficiency. These patients were supplemented with GH for the next IVF cycle. They had 12 iu of GH every third day, starting on the day of gonadotropin stimulation, till the administration of human chorionic gonadotropin (HCG). In all, we had 20 patients who fulfilled the above criteria. The long protocol was the standard mode of stimulation in 17 patients. Two patients had a short cycle and one had an antagonist cycle initially, which was converted to the long cycle at the second attempt. We used leuprolide acetate for suppression and recombinant follicular stimulating hormone (FSH) for stimulation (Gonal-F or Puregon). All the resulting pregnancies were included in the study if they had a documented foetal heart beat on ultrasonography at seven weeks of gestation.

A team of three embryologists, who worked in a pair

to countercheck their grading and provide consistency, graded all the embryos. The grading system is shown in Table I. On Day 2 (39–44 hours after insemination), each embryo was evaluated using standard embryo morphology criteria (uniformity of blastomeres, percentage of fragmentation, rate of cleavage) and assigned a grade. Embryos, which showed 4, 5 or 6 cells on Day 2 were given 8 points while the slow developers were assigned 4 points. Grade 1 embryos had their cell scores multiplied by 3, while grade 2 and grade 3 embryos had their scores multiplied by 2 and 1, respectively, to assign them their global score. This system was routinely followed at our hospital to decide upon the choice of embryo for transfer. All embryo grading was done at the time of the fresh cycle prior to storage. Only the embryos which were transferred, were incorporated in the study. By averaging the individual scores of the transferred embryos, we derived the final embryo score. We compared the number of oocytes, the fertilisation rates, the endometrial thickness and the embryo quality of the patients at the actual GH cycle with the previous control cycle.

RESULTS

The mean age of the patients at the time of IVF was 32.9 (median 34.5) years. Due to the small size of the study (20 patients), a non-parametric test (Wilcoxon signed ranks test) was used to test the difference between the two cycles, with and without GH. Results are listed in Tables II and III. Endometrial thickness could only be compared for 19 patients as the data was lacking in one patient. The embryo scores improved significantly after taking GH (Wilcoxon signed ranks test p-value < 0.001, median embryo score increases from 10.7 to 16.0). There were

Table II. Comparison of parameters before and after growth hormone administration.

Parameters	Median	Mean	Minimum	Maximum	SD	Valid number	p-value
Embryo score (before GH)	10.70	10.60	4	16	3.51	20	< 0.001
Embryo score (after GH)	16.0	15.78	8	24	4.5	20	
Number of eggs (before GH)	8.50	10.45	2	21	5.38	20	0.773
Number of eggs (after GH)	11.00	10.60	2	21	6.33	20	
Endometrial thickness (before GH)	10.00	10.26	7	13	1.45	19	0.108
Endometrial thickness (after GH)	11.00	11.37	7	15	1.94	19	

Table III. Mature eggs retrieved.

Mature eggs	Median	Mean	Minimum	Maximum	SD	Valid number	p-value
Before GH	6.00	7.23	2	14	3.49	13	0.253
After GH	8.00	8.38	2	14	4.21	13	

Table IV. Fertilisation rate at IVF and ICSI.

Fertilisation rate	Median	Mean	Minimum	Maximum	SD	Number	p-value
Before GH (IVF)	3.5	6.0	2.0	15.0	6.16	4	1
After GH (IVF)	5.5	6.25	1.0	13.0	5.74	4	
Before GH (ICSI)	4.0	3.85	1.0	6.0	1.34	13	0.014
After GH (ICSI)	5.0	5.77	2.0	10.0	2.71	13	

no statistically significant differences between the number of eggs, number of mature eggs and the endometrial thickness after supplementation with GH (Wilcoxon signed ranks test p-value = 0.773 for number of eggs, 0.253 for number of ICSI eggs and 0.108 for endometrial thickness, respectively).

For the comparison of fertilisation, patients were grouped into those who had only IVF and those who had only ICSI. Three of them had one cycle of IVF and another cycle of ICSI, and hence could not be compared. There was no significant difference in the fertilisation rate for those who had only IVF. However, there was a significant increase in the fertilisation rate for those who had ICSI (Table IV, Wilcoxon signed ranks test, p-value = 1 [IVF], p-value = 0.014 [ICSI]).

DISCUSSION

Recurrent IVF failure is an emotional drain both to a patient and to her physician. It is difficult to predict the prognosis of a failed IVF cycle due to poor response, poor embryo quality, poor fertilisation or failed implantation. The incidence of poor ovarian response to gonadotropin stimulation is between 5% and 18% of the IVF cycles.[1,2] This may be due to diminished ovarian reserves, advanced age, prior ovarian surgery, endometriosis or genetic factors. Endometriosis[3] and pelvic infections[4] can impair ovarian function directly (damage to the ovaries or the oocyte) or indirectly. Correction of these factors is at the molecular level and no standard treatment has been identified. The aetiology of implantation failure is often not clear and treatment options are vague. Evaluation of morphological characteristics, has been the most widely accepted method for the selection of embryos with good developmental ability. Abnormal embryo-endometrium dialogue may also play a role in failed implantation.

Although the pivotal role of gonadotropins in the modulation of granulosa cell ontogeny is well-established, the variable fate of ovarian follicles subjected to gonadotropic stimulation suggests that there are intraovarian regulatory mechanisms.[5,6] Insulin-like growth factor-1 (IGF-I) is involved in the regulation of follicular development and potentiates the FSH responsiveness of granulosa cells from preantral follicles. In a recent evaluation of the effect of IGF-1 on steroidogenesis, oocyte maturation, fertilisation, and embryo development in mice, it was found that oestradiol secretion rates were increased by IGF-1, though the oocyte fertilisation rates were comparable. The blastocyst development rate was also enhanced in the presence of IGF-1. The total cell number of the blastocysts was higher when the follicles were cultured or matured in the presence of IGF-1.[7] The addition of IGF-1 to gonadotropins in granulosa cell cultures increases gonadotropin action on the ovary by several mechanisms, including augmentation of aromatase activity, 17-beta oestradiol and progesterone production and lutenising hormone receptor formation.[8,9] IGF-1 displays GH dependence both in vivo and in vitro.[10] Insulin-like growth factor 2 (IGF-II) has been found to be the primary IGF in the human ovary. In-vitro studies confirm that IGF-II is capable of stimulating steroidogenesis and proliferation in the human theca and granulosa cells.[11-13] These actions are augmented by GH, which increases IGF production and thus, indirectly enhances gonadotropin stimulation of ovarian follicles.[14] Further, in-vivo administration of GH enhanced in vitro maturation and fertilisation of human germinal vesicle (GV) oocytes retrieved from small antral follicles.[15]

GH has also been shown to act on the ovaries independently from IGF-I.[16] Bachelot et al showed that the number of follicles per ovary was markedly reduced in mice lacking the GH receptor and GH-binding protein, despite adequate exogenous gonadotrophin treatment.[16] In view of this, we decided to look into the effect of GH on oocyte number, embryo quality, endometrial thickness and implantation.

The identification of GH deficiency in adults has been very controversial. The insulin tolerance test is considered the gold standard; however, it needs to be performed in special endocrine units, with a physician and a nurse in attendance. The clonidine stimulation test has been shown to stimulate GH in eight out of ten young adults.[17] In recent years, oral clonidine has proven to be unimpressive in stimulating GH release and better secretagogues are available. Hence, it is likely that we have under-identified GH deficiency. However, we have selected this in view of its convenience, simplicity, low cost and well-documented side-effects.

An adequate endometrial bed is essential for

implantation. Endometrial thickness is regarded as a reflection of the degree of endometrial proliferation in the absence of intrauterine pathology. Heterogeneity of the various protocols and controls has made it difficult to compare endometrial thickness in study cohorts. Potashnik et al suggested that the occurrence of pregnancies among patients who respond to ovarian stimulation by a concurrent elevation of GH may be related to the positive local effect of GH or its mediators on uterine receptivity at the time of nidation.[18] We could find no published data on the relationship of GH to endometrial thickness and hence wanted to establish a baseline in this area. The endometrial thickness in the cycles with GH and without GH were comparable (median 11 versus 10 mm, p = 0.108). There seemed to be no effect whatsoever of GH on measurable endometrial thickness.

Three trials (Bergh et al, Howles et al, Suikkari et al) reported data on the number of couples with at least one oocyte retrieved out of the total number of couples. There was no statistically significant difference between the two groups for this outcome (odds ratio 1.04, 95% confidence interval 0.53–2.04).[19-21] Bergh et al found no significant increase in the number of oocytes retrieved from the follicles in the cycles with GH compared to the cycles without it.[19] In a randomised double-blind study involving 196 patients, Howles et al concluded that growth hormone releasing factor had no significant effect on the mean number of follicles with a diameter ≥ 16 mm.[20] In our study, the total number of oocytes recovered was comparable between the cycles with and without GH. We further evaluated the number of mature eggs, in order to eliminate bias due to an early decision for HCG. No statistically significant differences existed in both groups between the number of eggs (p = 0.77) or the number of mature eggs (p = 0.25).

In poor responders, Bergh et al found that the fertilisation rate at IVF increased in patients who had received GH (81% in the GH group versus 56% in the placebo group).[19] Only four of our patients had IVF and they did not experience a significant difference upon fertilisation. However, there was a statistically significant increase in fertilisation in patients with ICSI. GH causes a significant increase in serum and follicular fluid levels of IGF-I. An increase in serum insulin-like growth factor binding protein-3 could also be recorded in patients who had received GH.[19] In-vivo administration of GH has also been shown to enhance in vitro maturation and fertilisation of human GV oocytes (51.9% with GH and 18.8% without GH) retrieved from small antral follicles.[15] The mechanism by which GH increases fertilisation is not clear, though this has been attributed to the increase in IGF-1 in the follicular fluid.[19] Subsequent to our findings, this is an important area for further evaluation.

Bergh et al also stated that there was no significant difference in early embryo quality as determined by the number of blastomeres observed two days after insemination.[19] Similar embryo morphology and rate of cleavage were also documented in patients with and without GH by Younis et al.[22] However, both these authors have not documented GH deficiency in their group of patients.

Tesarik et al noted that improvement of live birth rates in women aged over 40 years, on assisted reproduction treatment with GH, is probably due to an improvement of oocyte developmental potential, though its action on the uterus cannot be excluded.[23] They suggested that the increased intrafollicular oestradiol due to GH improved the oocyte quality. They did not find any significant difference in fertilisation or embryo morphology.

Shaker et al, however, in an evaluation of poor responders, did not find an improved oocyte quality.[24] Younis et al showed that there was no improvement of embryo morphology in normal ovulatory women who had GH supplementation.[22] Our results showed a significant increase in the embryo score. However, we will not be able to extrapolate data, as all these studies did not evaluate GH deficiency in their patients. Since our study had documented a deficiency and a subsequent supplementation, we think that this could be the reason for an improvement in the embryo scores. The Cochrane analysis has documented a just significant improvement in live births in previous poor responders treated with GH.[25] However there is no analysis on embryo quality. The increase in live births signifies that the embryo quality must have been reasonable.

This study is unique in that we tried to evaluate the GH status of the individual, and document its deficiency, prior to supplementation. To date, there are no known studies documenting GH deficiencies and the role of supplementation in relatively normal adults at assisted reproduction (excluding pan hypopituitarism). Our preliminary statistics imply that GH supplementation may improve embryo quality in selected patients with GH deficiency. Though the numbers are few, it opens up the possibility of a new tool at patient evaluation in IVF. Surprisingly, there is also an increase in the fertilisation rate at ICSI. Better tests to determine GH insufficiency may identify subnormal GH responses that may not fulfil the criteria for GH deficiency, but will help us to evaluate the need for supplementation of GH. Hence, we believe that this area merits further research and evaluation, probably a randomised control trial, with a larger cohort of patients, to quantify the exact benefits of GH at IVF.

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