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ARTICLE

Growth hormone supplementation improves implantation and pregnancy productivity rates for poor-prognosis patients undertaking IVF

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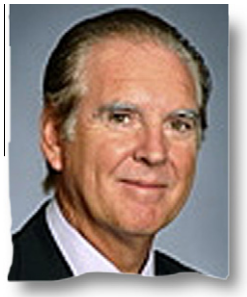

Dr Yovich presented his MD thesis 'Human Pregnancies Achieved by In-Vitro Fertilisation' following research and clinical work undertaken with Professor Ian Craft at the Royal Free Hospital in London (1976–1980). He returned to his home town of Perth, Western Australia and established PIVET Medical Centre, the first private independent fertility management facility in Australia. In 1982, the first child was born as a result of treatment at PIVET and this child has now become a father himself.

Abstract In a sequential crossover study of IVF conducted from 2002 to 2006, growth hormone (GH) supplementation was assessed in poor-prognosis patients, categorized on the basis of past failure to conceive (mean 3.05 cycles) due to low response to high-dose stimulation (<3 metaphase II oocytes) or poor-quality embryos. Pregnancy rates in both fresh and frozen transfer cycles and the total productivity rates (fresh and frozen pregnancies per egg collection) were compared. In all, 159 patients had 488 treatment cycles: 221 with GH and 241 without GH. These cycles were also compared with 1572 uncategorized cycles from the same period. GH co-treatment significantly improved the clinical pregnancy rate per fresh transfer ($P < 0.001$) as well as per frozen–thawed embryo derived from GH cycles ($P < 0.05$) creating a highly significant productivity rate ($P < 0.001$). The effect was significant across all age groups, especially in younger patients, and was independent of stimulation modality or number of transfers. GH cycles resulted in significantly more babies delivered per transfer than non-GH cycles (20% versus 7%; $P < 0.001$) although less than the uncategorized cycles (53%). The data uniquely show that the effect of GH is directed at oocyte and subsequent embryo quality.

KEYWORDS: embryo quality, growth hormone, implantation, IVF, pregnancy, productivity rate

Introduction

The prognosis for treatment by IVF is highly dependent upon ovarian responsiveness and the quality of oocytes recovered, with both factors deciding the number of good-quality embryos that will be generated. Most IVF programmes utilize ovarian stimulation schedules which all focus on capturing secondary follicles that have reached the early antral stage in the early follicular phase. At this point the follicles become FSH sensitive and can be selected for growth in the cyclic recruitment process.

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Fertility treatment by assisted reproductive techniques, particularly for IVF, has relied upon increasing the amount of endogenous FSH either by oral agents such as clomiphene citrate and, more recently, aromatase inhibitors or by giving exogenous FSH in a concentrated purified form (either urinary-derived or produced by recombinant technology). Exogenous FSH administration, particularly when supported by gonadotrophin-releasing hormone analogues to prevent early luteinization, has been very effective in follicle recruitment and the generation of pregnancies at satisfactory rates (Homburg et al., 1990a). However there is wide variability to its responsiveness, such that some patients will show excessive response with risk of ovarian hyperstimulation syndrome whilst others demonstrate relative degrees of ovarian resistance leading to the recovery of few oocytes. The number of oocytes is quite important given the age-related phenomenon, which dictates that only a proportion of oocytes recovered will have the maturational integrity for generating good-quality embryos with subsequent successful implantations and ensuing pregnancies; e.g. this level might be 20% of oocytes in younger women (<35 years) but less than 10% of oocytes for older women (>40 years).

Menstrual cycle dynamics were sufficiently well understood by the 1960s to enable the evolution of successful ovarian stimulation regimens for fertility treatments including the inevitable progression into IVF. However, all stimulation regimens have relied solely upon the importance of FSH as the key hormone for follicle recruitment and maturation. The last 20 years has seen an intense focus examining ovarian endocrinology and paracrinology using advances in molecular biology to better understand folliculogenesis, cycle dynamics and the age-related process underlying poorer-quality oocytes in older women. There is an increasing realization that folliculogenesis involves events prior to, and other than, FSH dependence, with the role of growth factors an obvious consideration. Recent suggestions that LH may improve embryo quality and implantation (Andersen et al., 2006) and the use of dehydroepiandrosterone (DHEA) for follicle recruitment (Barad and Gleicher, 2005, 2006) in low-responder patients are two examples where stimulants other than FSH may be needed. Critically, past FSH-only regimens have focused more on oocyte numbers rather than quality. Poor-responder patients by definition are those who fail to respond satisfactorily to standard regimens and highlight the complexity of follicle recruitment and viability and the problem of a universal stimulation regimen. Growth hormone (GH) is another adjunctive therapy reported to provide benefit to complex cases (Homburg et al., 1991, 1990b). Currently, reports of its use have been from limited studies in various patient subgroups (Tesarik et al., 2005) and as such remains outside routine clinical application.

The first interest in utilizing GH in the human followed from a series of reports from several independent groups showing an essential role in various animal species of growth factors on ovarian function, particularly the amplification of FSH action (Adashi et al., 1991). The early report, in particular, that GH could facilitate ovulation induction by menotrophins in the human setting (Jacobs, 1972, 1992), caused some highly fruitful collaborative activity under the leadership of Professor Howard Jacobs in London. However, one critical area that GH had received little attention was on poor-prognosis patients in whom, despite increased gonadotrophin dosages, pregnancies were difficult to achieve. Consequently, the lead author established pilot trials to be undertaken at PIVET Medical Centre to determine if GH might have a role in improving the outcome for patients who had poor responses in IVF. After positive implications in pilot studies over several years, this report details the positive outcome of a formalized 5-year study providing GH therapy to patients deemed to be poor-prognosis cases.

Materials and methods

Study period and participants

PIVET Medical Centre was established in 1980. The study period includes all cases managed at PIVET Medical Centre from 1 January 2002 to 31 December 2006. Patients offered the opportunity to participate in the GH study were those considered to fulfil one or more of the following criteria: (i) women who had generated less than 3 metaphase II oocytes despite having been given maximal FSH stimulation (i.e. ≥450 IU/day with absolute maximum of 600 IU/day); (ii) women who had generated embryos of which the majority (>50%) showed marked fragmentation and were graded as ≤1.5 in PIVET's long-standing embryo-grading system; or (iii) repetitive fresh or frozen embryo transfers without pregnancy where diminished egg or embryo quality was identified by the laboratory.

Although GH had been explored in several pilot studies over the previous decade, the selected study period (2002–2006 inclusive) embraces rigid inclusion criteria and a complete computer-record system that has been subjected to a rigorous, ongoing validation process.

Clinicians had individual flexibility in deciding which cases fitted the categories and could be offered inclusion in the study group. Participants were offered GH based upon experience from previous cycles or history from either PIVET or from outside clinics. The decision to utilize GH, when offered, was made by the patient and included several factors, one of which was cost (since patients were required to pay for GH). However, once selected, patients accepted the sequential crossover design and made their own decision to commence the next cycle with or without GH on the understanding that they could not receive further GH for treatment cycles undertaken within 6 months. There was an even distribution between women under 36 and over 36 years of age. The majority had undertaken several IVF attempts before GH was offered. It was only in the latter part of the study period that GH was offered pre-emptively on the first or second attempt, generally on the basis of advanced age or limited cycle opportunities. The number of previous cycles undertaken in outside clinics was not always clear and therefore the attempt may be understated.

Accreditation

The Reproductive Technology Accreditation Committee (RTAC), which was formed as a subcommittee under the Fertility Society of Australia, controls Australian IVF units. RTAC accredits IVF units to a maximum of 3 years under an evolving Code of Practice. For accreditation, IVF units

must provide their data in a continuous and complete form for central audit. During this period, PIVET has satisfied RTAC and received full 3-year accreditations continuously.

In Western Australia, there is also state legislation under the Human Reproductive Technology Act (1991) that provides a second and separate regulatory requirement. Data are provided quarterly and each unit is assessed on an annual basis. One of the requirements under state legislation is that the IVF unit must have up-to-date accreditation by RTAC as well as to be overseen by the Reproductive Technology Council (RTC), established under the state legislation.

Ethical approval

All research at PIVET requires approval from its institutional ethics committee. The use of GH in ovulation induction was first approved by the Cambridge Hospital Human Research Ethics Committee on 15 September 1993. The project was further scrutinized by the RTC and was granted final approval on 25 November 1993 (registration number I002). RTC oversight approvals were further granted on 19 November 2002 and 27 October 2006.

Experimental design

The study was designed as a sequential crossover, where patients identified as 'poor-prognosis cases' were given the option of using GH. They may or may not have elected to utilize it in the cycle when initially offered (due to costs or other concerns), but would be offered it in the next cycle if unsuccessful in the first. Patients who utilized GH in a cycle, but did not conceive, continued in a crossover process with no GH on the next cycle, believing that GH had no benefit. PIVET requirement was that a maximum of six injections were given for a patient within any 6-month period and patients who utilized GH waited at least 6 months before considering utilizing it again. Given that the PIVET clinic requirement is a minimum of only 2 months' rest between treatment cycles, this crossover arrangement presented an issue in that some treatment cycles without GH may well still have remained under its influence.

Patients who received GH on at least one cycle during the 5-year period were included in the study group. The analysis compares the outcome including pregnancy between the cycles in which GH was given (termed GH+) to those cycles where GH was not utilized (termed GH−). It also enables a comparison of the outcomes of these two groups to the treatment cycles for all other uncategorized patients managed concurrently (termed GHu). This term was used since it included a group of patients who undertook IVF and either conceived or withdrew from further treatment, some of whom may have ultimately been offered GH after the study period. Whilst a sequential crossover study has some weaknesses as a research model, it is highly practicable in a private clinical setting and the comparative assessments are believed to be valid.

Patient consent

Patients provided their informed consent using PIVET document, file number 18, Information and Consents 22.1 — Consent to an Innovative Procedure: Use of Saizen (Biosynthetic Growth Hormone) in ovulation induction. They were also provided with a patient information sheet 'What is Growth Hormone?'. The sheet highlighted potential side-effects and, particularly, asked patients to report any headaches, visual problems, nausea, vomiting or joint-swelling. The document noted a potential association between low GH and hypothyroidism and the potential precipitation of diabetes particularly for those with a family history. The patient information sheet was continuously updated to indicate information from current reported studies, the consideration of safety aspects along with biosynthetic preparation and costings. Patient consent for the use of GH was in addition to other consents for IVF treatment, intracytoplasmic sperm injection (ICSI) or other innovations, such as assisted hatching, with each having its own separate patient information sheet and requiring separate consent. Participating patients were required to pay for the use of GH over and above the IVF treatment cycle with charges levied according to the manufacturer's charge to the clinic.

Growth hormone protocol

In the initial phase of the study, the protocol for the commencement of GH was in the cycle preceding the actual IVF attempt. During 2004—2006, GH was increasingly started and maintained during the IVF cycle. Therefore the period 2002—2003 represented prior GH exposure, while 2005—2006 represented concurrent exposure: 2004 may be viewed as a transition between the two protocols. Twenty-six percent of transfers occurred in 2002—2003 while 57% of transfers occurred in 2005—2006, reflecting the increased usage of GH supplementation over time.

GH was given in the form of Saizen 10 IU (Serono, Australia) under two schedules. In the first four years (2000—2004), the schedule was based on the ideas of Homburg et al. (1990a,b), attempting to enhance the development of those secondary oocytes which were in the initial recruitment phase and would be available for FSH capture in the early follicular phase of the treatment cycle. GH was, therefore, given in the previous cycle on days 7, 14 and 21 with a final injection on day 2 of the treatment cycle.

With the emergence of other ideas (particularly those subsequently published by Tesarik et al. (2005), who used a course of GH injections during the treatment cycle) the majority of cases in 2005 and 2006 adopted a revised blend whereby patients received six injections with the first beginning on day 21 of the preceding cycle and the subsequent injections being on days 2, 6, 8, 10 and, if still progressing, a final injection on day 12.

Patients therefore received either four injections under the early protocol or a maximum of six injections under the updated protocol. These were analysed both together and separately in the results.

Clinical management

Infertility patients involved in the IVF programme at PIVET are treated in a flexible manner (sometimes dictated by clinician preference) utilizing different stimulation regimens.

These include a long down-regulation protocol (LDR; Synarel; Pfizer, Australia; or Lucrin; Abbott, Australia), a flare-stimulation regimen (FSR; Lucrin) or, increasingly in recent years, an antagonist protocol (AP; Cetrotide; Serono, Australia; or Orgalutran; Shering Plough, Australia). All patients were treated with recombinant FSH (mostly Gonal-F; Serono, occasionally Puregon; Schering-Plough, previously known as Organon), Gonal-F being the preferred agent when dosages of $\geq$300 IU are required.

In general, young women ($<$35 years) undergoing first treatment utilize LDR progressing to FSR and then AP if demonstrating ovarian resistance or poor outcomes. Often older women ($>$35 years) will commence on FSR, progressing to AP if required. Annualized data at PIVET shows no differences in the pregnancy rates or likelihood of a live-born baby from any of these stimulation protocols in the overall data.

Recombinant FSH dosage was prescribed in a set formula for first cycles, based on patient's age, day-2 FSH and antral follicle count graded A for high to E for low. Ovulation was triggered by 10,000 IU human chorionic gonadotrophin (HCG; Schering-Plough) in all cases included in the GH study group when the leading follicles reached 18 mm and the cohort was matched by a serum oestradiol of around 800–1000 pmol/follicle $\geq$14 mm.

As per long-standing practice, the patients were monitored during the follicular phase, initially with a basal day-2 concentrations of oestradiol, progesterone and LH, thereafter from day 7 on alternate days with oestradiol, progesterone, LH and transvaginal ultrasound for follicle dimensions. Transvaginal oocyte recovery was undertaken 35 h post trigger under IV sedation using a PIVET-Cook double-lumen flushing/aspiration needle (Cook, Australia). Each follicle was aspirated and flushed to ensure maximum potential for oocyte recovery.

The luteal phase was managed in all cases under PIVET's long-standing protocol of HCG support (1000 units on days 4, 7, 10 and 13 where day 0 is the day of oocyte retrieval. Mid-luteal hormone check (oestradiol and progesterone) signified whether additional support hormones may be given (oestradiol, progesterone or combined oestradiol/progesterone pessary; compounded PIVET products). Where $\geq$12 oocytes were recovered, progesterone pessaries replaced HCG injections.

Embryo culture and assessment

All embryo culture was conducted using Sage Biopharma culture media (Gytech, Melbourne, Australia) with 5 mg/ml human serum albumin (Gytech) or occasionally patient's serum (10%). Oocytes were cultured for 4–5 h post collection before insemination with 100,000 motile spermatozoa/ml for IVF or denuded with hyaluronidase and mature oocytes injected by ICSI.

Embryo cultures were undertaken as single embryo incubations in 10 μl drops under oil (Gytech). All cultures were in 60 mm Falcon dishes (BD, Australia) in MINC benchtop incubators (Cook) under an atmosphere of charcoal filtered 5% CO_2/5% O_2/90% N_2 medical-grade gas. Embryos were graded on day 3 under a four-point system, including half points (grade 4 = 8+ cells no fragmentation and early compaction evident; grade 3 = 7–9 cells, no fragmentation and no compaction; grade 2 = slow cleavage and/or $>$20% fragmentation; grade 1 = arrested or significantly fragmented embryos). Embryos graded $\leq$1.5 were discarded. Day 5 embryos were graded using the Gardner's scoring system (Gardner and Schoolcraft, 1999) for blastocysts.

On days 2, 3 or 5, one or two embryos were transferred to the uterus in 10–20 μl of culture media. Embryo transfers were undertaken using the Cook double-catheter system (K-JITS-2005; Cook) under transvesical ultrasound control. The embryos were deposited just short of the fundus with a clear flash identified in the fundal region and a negative check on the transfer catheter by the embryologist on completion.

Although the clinic has a strong policy of single embryo transfer for most of the study period, such cases categorized as poor prognosis can receive up to two day-3 embryos. When fertilization rates were above expectation ($\geq$5 embryos growing) the patient could elect to have blastocyst culture with a single blastocyst transferred on day 5. In the study period, there were 37 transfers at the blastocyst stage in GH+ and 34 transfers in GH− cycles. Assisted hatching was offered according to RTC approval (i.e. if three previous transfers had failed to generate a pregnancy or if the patient was $>$38 years with an elevated baseline FSH).

Residual embryos of suitable quality were cryopreserved on the day of transfer using a slow freezing, propanediol method (Testart, 1986) using 10% patient serum where possible or human serum albumin if required. The thawing of embryos followed the same protocol (Testart, 1986) and occurred on the day of embryo transfer.

Data validation and statistical analysis

PIVET has established an electronic record-keeping system that integrates demographic data and billing systems (JAM software) with a data-recording system using Filemaker Pro database. Data is transmitted electronically annually to the independent National Perinatal Statistics Unit data-recording site and quarterly to the RTC.

The main data comparison was between the GH+ and GH− treatment cycles and where relevant against the GHu cycles. The main measure used for comparison between groups was clinical pregnancy (CP) rate per embryo transfer procedure, either fresh embryo transfer or post-thaw frozen embryo transfer (FET). A productivity measure was also included which summates the total embryo transfer and FET pregnancies for the relevant group, constituting a productivity rate as the cumulative pregnancy rate per oocyte retrieval. Implantation rates are defined as the number of identifiable gestational sacs in clinical pregnancies arising as a proportion of the total number of embryos transferred. A further term, utilization rate, was used to denote the proportion of 'usable' embryos arising from the total number of two pronucleate embryos created from a single oocyte retrieval procedure. This denotes the number of embryos transferred plus the number deemed suitable for cryopreservation.

Statistical analysis was conducted by comparison of groups in 2 $\times$ 2 contingency tables using chi-squared analysis

with Pearson's correction factor where required and *t*-test for comparison between means.

Results

Patient profile

The patient profile revealed a total group of 159 women with an average age of 37.5 ± 4.1 years (**Table 1**) with similar numbers of patients in the various age groups (101 transfers below 35 years; 216 between 35 and 40 years and 78 over 40 years) indicating that age itself did not entirely constitute the prescription for GH. Infertility categories showed 21% of couples had some tubal factor, 32% had endometriosis included in their medical history, 42% had some form of abnormal semen profile and 21% had poorly explained infertility largely comprising polycystic ovaries or ovulatory disorders. A single cause was described in 49% of couples while 51% had more than one reason for infertility.

On average, couples had 3.05 cycles before being offered GH; however, this was decided on a case-by-case basis. The average number of cycles per referral case at PIVET during the study period was 1.96 cycles compared with 3.95 cycles in those patients receiving GH in one or more cycles.

Fresh and frozen transfers

During the 5-year study period, 159 patients were classified as fulfilling the criteria for consideration of GH and had 488 IVF cycles. In all, 232 cycles were started with GH and 256 cycles were not (**Table 2**). This population represented about 25% of the cycles started during the study period. Seventy-one of the 159 patients had two GH+ cycles with at least one intervening GH− cycle.

The cancellation rates and the egg collection to transfer rates were similar in both arms of the study leading to 193 transfers in GH+ and 202 transfers in GH− cycles. Despite similar numbers of fresh transfers, significantly more pregnancies arose in the GH+ group than the GH− group (49/193, 25% versus 19/202, 9%; chi-squared $P < 0.01$).

Eighty-four embryo thaw cycles were undertaken with embryos generated during GH+ cycles of which 79 resulted in a transfer, but there were twice as many thaw attempts (148) and transfers (140) undertaken from GH− cycles (partly due to the latter group recycling back more frequently for transfers as there were fewer pregnancies in the group). Importantly, there were significantly more clinical pregnancies in the GH+ thawing group (17, 20%) than in the GH− FET group (14, 9%; $P < 0.05$). The resultant pregnancy productivity rate (sum of clinical pregnancies from fresh and frozen transfers per egg collection) in the GH+ group was significantly higher (30% versus 14%; chi-squared $P < 0.001$). The improved pregnancy rate in the GH+ thaw cycles was noted as a strong trend across all age groups, but particularly the younger (24% versus 10% for <35 years) rather than the older (15% versus 11% for >40 years) age group.

Of the 1686 IVF cycles undertaken in the GHu group of patients (**Table 2**), 1572 had oocytes retrieved and 1311 proceeded to transfer which generated 499 clinical pregnancies (38%) and 1528 had thaw cycles which generated a further 494 clinical pregnancies (32%). The pregnancy productivity rate for the non-treatment group was 63% (993 clinical pregnancies from a total of 1572 egg collections). When the GH treatment groups are compared against this background population, the pregnancy productivity rate for the GH− group of 9% for fresh transfers and 9% for frozen transfers clearly justifies the classification of these patients as poor-prognosis cases. Supplementation with GH in the fresh treatment cycles of this population provided a pregnancy rate that approached but remained less than the background uncategorized population (fresh transfers 25% versus 38%; $P < 0.01$). This is reinforced by the productivity rate comparisons for frozen transfers that showed the GH+ group (30%) was significantly improved to a level higher than

Table 1 Basic demographics for patients receiving growth hormone co-treatment.

Parameter	*Value*
No. of patients	159
Mean age ± SD (years)	37.5 ± 4.1
Type of infertility (%)	
Tubal	21
Endometriosis	32
Unexplained	21
Male	42
Additional factors, e.g. fibroid/adhesions	34
Single cause	49
Multiple causes	51

Table 2 Summary of fresh and frozen treatment cycles with or without growth hormone (GH) co-treatment.

Parameter	*GH+*	*GH−*	*GHu*	*Total*
Cycles started	232	256	1686	2174
Oocyte retrievals	221	241	1572	2034
Fresh embryo transfers	193	202	1311	1706
Clinical pregnancies	49	19	499	567
Clinical pregnancy rate per fresh embryo transfer (%)	25[a,c]	9[e]	38	33
Thawing cycles	84	148	1528	1760
Clinical pregnancies	17	14	494	525
Clinical pregnancy rate per thawing cycle (%)	20[b,d]	9[e]	32	30
Total pregnancies	66	33	993	1092
Clinical pregnancy rate/ oocyte retrieval	30[a,c]	14[e]	64	54

Values are number or percentage.
GH+ = cycles managed with growth hormone; GH− = cycles managed without growth hormone; GHu = uncategorized cycles managed concurrently without growth hormone consideration.
[a]GH+ vs GH− $P < 0.001$.
[b]GH+ vs GH− $P < 0.05$.
[c]GH+ vs GHu $P < 0.01$.
[d]GH+ vs GHu $P < 0.05$.
[e]GH− vs GHu $P < 0.001$.

Table 3 Relationships between clinical pregnancies per transfer, patient age and growth hormone (GH) co-treatment.

Treatment	<35 years		35–40 years		>40 years		Total	
	n	Pregnant (%)	n	Pregnant (%)	n	Pregnant (%)	n	Pregnant (%)
GH+	41	38[a,e]	103	21[b,c]	49	24[b,e]	193	25[a]
GH−	60	9[d]	113	11[d]	29	3[d]	202	9[d]
GHu	653	46	527	34	131	16	1311	38
Total	754	43	743	29	209	16	1706	33

GH+ = cycles managed with growth hormone; GH− = cycles managed without growth hormone; GHu = uncategorized cycles managed concurrently.
[a]GH+ vs GH− $P < 0.001$.
[b]GH+ vs GH− $P < 0.05$.
[c]GH+ vs GHu $P < 0.05$.
[d]GH− vs GHu $P < 0.001$.
[e]GH vs GHu not statistically significant.

the GH− (14%) and but still less than GHu group (64%) ($P < 0.001$ and $P < 0.01$, respectively).

Influence of age

Twenty-six percent of the 395 transfers in the GH study group were for women <35 years of age, 55% for women aged 35–40 years and 20% for women over 40 years (Table 3). While the number of transfers with or without GH was similar in each age group, the pregnancy rate was significantly higher in those cycles that included GH supplementation ($P < 0.001$). This effect of GH was most significant for women under 35 years of age who displayed a four-fold improvement (38% versus 9%; $P < 0.001$) but also significant to a moderate level for those aged 35–40 who displayed a two-fold improvement (21% versus 11%; $P < 0.05$) and for those over 40 who displayed an eight-fold improvement on smaller numbers (24% versus 3%; $P < 0.05$). Women over 40 years generated 29 pregnancies in the GH− group and 131 in the GHu group, providing a rate when not exposed to GH of 160/1513 (10.6%) rising significantly when exposed to GH to 50/193 (24%; $P < 0.001$). Given that the GHu group can also be categorized as poor prognosis, the true benefit of GH in the over-40 years group is therefore more likely a 2.5-fold improvement.

Utilization rate and implantation rate

There was no significant difference in mean FSH concentration, the number of oocytes recovered nor in their fertilization rate or utilization rate between GH+ and GH− cycles (Table 4). The difference in serum FSH at the start of each cycle was slightly elevated in the GH+ and GH− compared with the GHu control group but this difference was not significant. In this study, the utilization rate was used as a measure of embryo quality rather than applying an embryo score and reflects the proportion of embryos deemed suitable for transfer or freezing. The average number of embryos transferred was the same in all groups (GH+ 1.78; GH− 1.77; GHu 1.64); however, there was a significant increase in the implantation rate in GH+ cycles compared with GH− cycles (Table 4; 15.2% versus 5.1%; $P < 0.01$). The low implantation rate in GH− cycles reinforced the patient's definition as poor prognosis and their inclusion in the GH study. Although the implantation rate was increased under GH+, the rate remained significantly less than observed in the GHu group (27.4%; $P < 0.05$).

The improved effect was present in all three age groups defined in the study. However, when compared with the non-treatment GHu group, it was the older women (>35 years) who demonstrated the most significant improvement with the use of GH, demonstrating implantation rates which were equivalent to that of the uncategorized non-treatment group (i.e. no significant differences; 12.8% versus 22.0% in 35–40 year group and 13.0% versus 11.0% in >40 year group). Younger women (<35 years) receiving GH showed an improved implantation rate (20.6% versus 4.7%; $P < 0.01$) but this rate still fell significantly short of the GHu young group (34.3%; $P < 0.05$).

Stimulation regimens

In both the AP and FSR regimens, GH+ cycles had significantly higher pregnancy rates than GH− cycles (both $P < 0.05$) but there were no significant differences in pregnancy rates among any of the stimulation regimens which received GH augmentation (Table 5). Whilst overall, the pregnancy rate was similar regardless of whether the stimulation was AP, FSR or LDR, there were more AP cycles ($n = 205$) than FSR ($n = 147$) and LDR cycles ($n = 43$) in the GH study group ($n = 395$). This reflects the clinic's policy at that time that poor responders were increasingly managed by an AP protocol. LDR protocols were infrequently used in the patients who received GH.

Attempt number

There was a significantly better pregnancy rate with GH on the second ($P < 0.05$) or third transfer ($P < 0.01$; Figure 1) than without GH. Individually, there was no better outcome with GH when given for any transfer after the third but collectively the combined pregnancy rate was significantly higher with GH. The pregnancy rate for combined one–three cycles was significantly higher for GH+ treatment cycles (33% versus 11%; $P < 0.001$) as well as for the combined

Table 4 Embryology information by age and growth hormone (GH) co-treatment.

	GH+	*GH−*	*GHu*	*Total*
<35 years				
Oocyte retrievals	41	71	804	916
Oocytes/retrieval	10.7	9.8	12.2	11.7
Fertilization rate (%)	58.3	58.0	61.9	61.4
Utilization rate (%)	64.4	64.7	84.7	82.2
Implantation rate (%)	20.6[a,c]	4.7[b]	34.3	29.9
Mean FSH ± SD (IU/l)	7.4 ± 1.6	7.3 ± 1.9	5.3 ± 2.5	5.9 ± 2.4
35—40 years				
Oocyte retrievals	126	134	601	861
Oocytes/retrieval	9.3	8.4	9.8	9.5
Fertilization rate (%)	52.7	48.4	61.7	58.0
Utilization rate (%)	68.8	81.0	76.2	75.0
Implantation rate (%)	12.8[a]	7.2[b]	22.0	18.9
Mean FSH ± SD (IU/l)	8.0 ± 3.1	7.8 ± 3.8	5.6 ± 1.9	6.6 ± 2.9
>40 years				
Oocyte retrievals	54	36	167	257
Oocytes/retrieval	7.2	7.9	6.9	7.1
Fertilization rate (%)	57.0	55.0	59.0	58.40
Utilization rate (%)	67.0	77.0	26.0	43.0
Implantation rate (%)	13.0[a]	1.3[b]	11.0	10.0
Mean FSH ± SD (IU/l)	6.6 ± 3.0	6.5 ± 2.4	6.4 ± 3.7	6.5 ± 3.2
Total				
Oocyte retrievals	221	241	1572	2034
Oocytes/retrieval	8.0	8.9	10.7	10.3
Fertilization rate (%)	55.0	53.0	61.0	60.0
Utilization rate (%)	67.10	72.0	78.0	75.0
Implantation rate (%)	15.2[a,c]	5.1[b]	27.40	22.0
Mean FSH ± SD (IU/l)	7.4 ± 2.7	7.4 ± 2.9	5.4 ± 2.4	6.5 ± 2.7

Utilization rate = percentage of embryos suitable for transfer or freezing.
GH+ = cycles managed with growth hormone; GH− = cycles managed without growth hormone; GHu = uncategorized cycles managed concurrently.
[a]GH+ × GH− $P < 0.01$.
[b]GH− × GHu $P < 0.05$.
[c]GH+ × GHu $P < 0.05$.

Table 5 Relationships between clinical pregnancies per transfer, method of ovarian stimulation and growth hormone (GH) co-treatment.

Group	*Antagonist*		*Flare stimulation*		*Long down-regulation*	
	n	*Pregnant (%)*	n	*Pregnant (%)*	n	*Pregnant (%)*
GH+	138	30[a]	38	21[a,b]	17	17[c]
GH−	67	15	109	7	26	4
GHu	251	39	686	39	374	38

n = no. of transfers; Pregnant = percentage clinical pregnancy per embryo transfer.
GH+ = cycles managed with growth hormone; GH− = cycles managed without growth hormone; GHu = uncategorized cycles managed concurrently.
[a]GH+ × GH− $P < 0.05$.
[b]GH+ × GHu $P < 0.05$.
[c]GH+ × GH− not statistically significant.

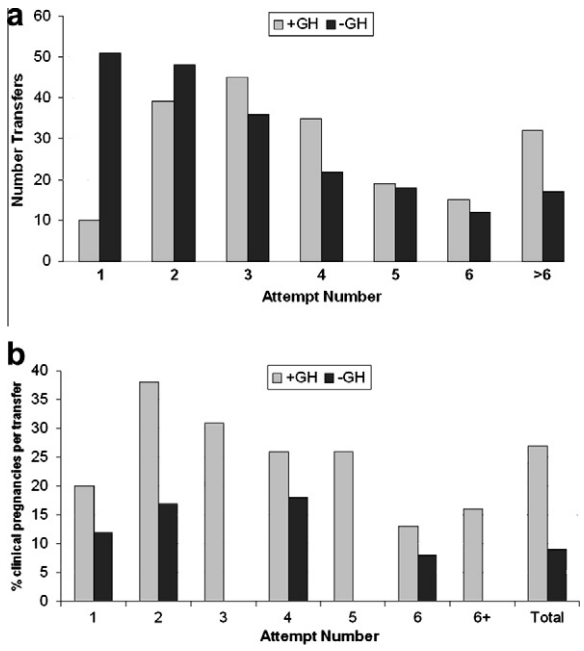

Figure 1 The number of transfers by attempt number (a) and the attempt number in which pregnancy ensued (b) for poor prognosis patients receiving growth hormone.

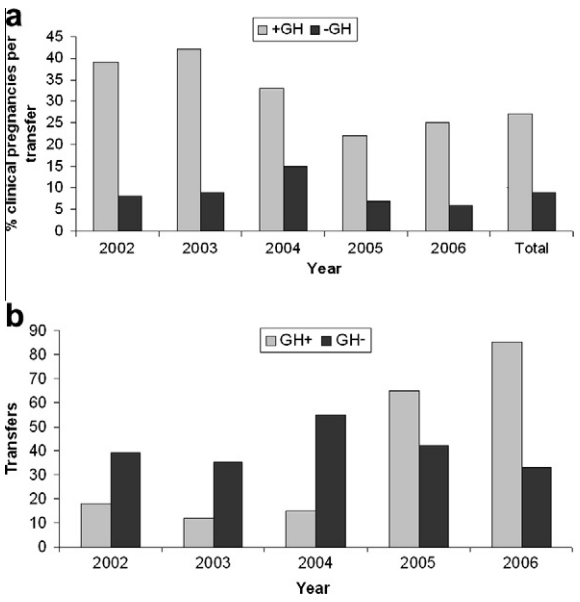

Figure 2 Clinical pregnancy rates (a) and number of transfers (b) in poor prognosis cases with or without GH supplement during individual years of the study period.

four—nine cycles (20% versus 7%; $P < 0.05$). Although the number of patients who underwent a high number of transfers was low, pregnancies were recorded in all transfer series, even after nine or 10 previous unsuccessful attempts with more pregnancies following the use of GH.

It is worth pointing out that all but ten (Figure 1a) of the GH+ transfers had at least one prior transfer without GH. All six of the patients who did have GH+ transfers in their first IVF cycles were over 40 years of age. The majority of the GH+ transfers were either the second or third transfer; however, where GH was administered after high-order multiple transfers, most were patients referred to PIVET after multiple unsuccessful transfers elsewhere, where GH was not offered. In all, 24% of GH+ transfers were associated with prior poor outcomes outside of PIVET. This would have the effect of decreasing the pregnancy rate of the GH— cycles if it were factored into this analysis and further enhance the differences in the implantation and pregnancy rates between GH+ and GH— cycles.

Year of treatment

GH+ significantly increased pregnancy rates compared with GH— transfers in the same year in both the 2002—2003 and 2005—2006 intervals (Figure 2a). In 2004, the pregnancy rate between GH+ and GH— cycles was not significant although the same trend existed. During 2002—2003, there were 12 clinical pregnancies from 30 transfers in GH+ cycles compared with only 8% in GH— cycles (40% versus 8%; $P < 0.001$) while in 2005—2006, there were slightly less clinical pregnancies with GH+ (24% versus 7%; $P < 0.01$). The pregnancy rate in GH— cycles was the same in both periods, indicating the referral of patients was similar over the review period, although the proportion of cases taking up GH treatment was higher in the years 2005—2006 (Figure 2b).

Side effects

No case of diabetes or hypothyroidism emerged during GH administration. Two patients described joint swellings of the hands — one after a single GH injection, another after a series of four injections. Both ceased further GH treatment and their symptoms resolved spontaneously over 1 or 2 weeks. There were no cases of clinically significant ovarian hyperstimulation syndrome, although for two patients receiving GH, all embryos were frozen as per PIVET protocol as they had 12 oocytes recovered at oocyte retrieval. They were monitored during the luteal phase and did not develop significant symptoms or other features of ovarian hyperstimulation syndrome.

Pregnancy outcome

The analysis of the clinical outcomes combined the pregnancies from both fresh and frozen transfers (Table 6). There was no difference in the number of clinical pregnancies per positive HCG pregnancy test (66/73, 90.4% versus 33/37, 89.2%) or live births per clinical pregnancy (43/66, 65.2% versus 17/33, 51.5%) between GH+ and GH— cycles. In this study, the multiple pregnancy rate (two twin pregnancies after GH+ and one after GH— stimulation) was low regardless of treatment but there was no difference in the pregnancy loss rate between GH+ and GH— cycles (35% versus 48% miscarriage rate per clinical pregnancy) both higher than the rate in the GHu group. The multiple pregnancy rate from the patient population utilizing GH on some cycles (two sets of twins from the GH+ group and one set from the GH— group) was lower than the non-treatment group

Table 6 Summary of pregnancy outcomes between growth hormone (GH) co-treatment groups.

Parameter	*GH+*	*GH−*	*GHu*
Cycles started	232	256	1686
Cancelled cycles	11	15	114
Oocyte retrievals	221	*241*	*1572*
Nil oocytes retrieved	4	4	28
Nil fertilization	17	17	80
Deferred transfer	4	5	175
Fresh transfer cycles with embryos frozen	95	103	879
Cycles with freezing (%)	49	50	56
Fresh transfers	193	202	1311
Frozen transfers	73	145	1528
Total transfers	266	347	2839
Total biochemical pregnancies	7	4	33
Total clinical pregnancies	66	33	993
Failed pregnancies	23	16	228
Live births from fresh embryo transfer	33	11	387
Live births from frozen embryo transfer	10	6	378
Total live births	43	17	765
Multiple pregnancies	2	1	70
Total babies delivered	45	18	836
Miscarriage rate/positive HCG (%)	30/73 (41)	20/37 (54)	261/1026 (25)
Miscarriage rate/clinical pregnancies (%)	23/66 (35)	16/33 (48)	228/993 (23)
Babies/oocyte retrieval (%)	20[a]	7	53
Mean birthweight ± SD (g)	3111 ± 738	3271.3 ± 446	3043 ± 732[b]
Mean gestation ± SD (weeks)	38.0 ± 3.2	38.9 ± 0.91	37.6[c]
Males:females	20:25	7:11	431:405

GH+ = cycles managed with growth hormone; GH− = cycles managed without growth hormone; GHu = uncategorized cycles managed concurrently.
[a]GH+ × GH− $P < 0.001$.
[b]Mean birthweight for all births between 2002 and 2008.
[c]National Perinatal Statistics Unit data for 2006: average gestational age for all births from all transfers.

(5.0% versus 9.2%) over the same period reflecting, once again, the group's lower implantation potential. In all, significantly more babies were delivered in GH+ cycles than in GH− cycles ($P < 0.05$).

There were three abnormalities identified from the GH+ derived pregnancies (one anencephaly terminated in the first trimester, one complex congenital heart anomaly, one diamniotic twin/twin transfusion syndrome and one case of fetal growth retardation). There were two abnormalities from the GH− pregnancies (one tetraploidy and one trisomy 15, both terminated in the second trimester). These cases represented a similar profile to the background of subfertility pregnancies managed at PIVET although the 'highish' aneuploidy rate and pregnancy loss rates might well reflect the poorer prognosis status of those cases selected for consideration of GH treatment.

Discussion

This report represents the application of GH as an adjuvant to ovarian stimulation and follicle recruitment in highly selected, poor-prognosis cases deemed to be poor responders or patients with suboptimal embryo development. The clear observation arising from this study is that implantation rates and resultant pregnancy rates along with the numbers of healthy babies delivered are significantly higher when GH co-treatment is given to the defined categories of poor-prognosis cases. The results confirm a role for GH treatment during an IVF stimulation cycle and the benefits apply to women categorized as poor prognosis regardless of age, stimulation regimen or attempt number. The results not only confirm a benefit in fresh transfer cycles involving GH co-treatment but reveals an extension to subsequent frozen embryo transfers. This appears therefore to arise as a consequence of improved oocyte quality rather than numbers of oocytes and embryos, which were not influenced. The report is the first to span such a long time period reinforcing its efficacy and is the first to include aspects of a whole-of-cohort analysis that views the total productivity from an egg collection incorporating both fresh and frozen transfers. The information reported in this study was collected over a 5-year period and applied to patients in a private clinical environment who have demonstrated via past performance a reduced prognosis due to poor embryo numbers and/or quality and to a lesser extent response to FSH.

There are several reports of small, limited randomized studies involving GH in ovulation induction and IVF. An early study with GH on anovulatory or amenorrhoeic women showed less gonadotrophin was required in the presence

of GH (Homburg et al., 1991,1990b), a result confirmed by Owen et al. (1991) for polycystic ovary patients. These studies argued that GH was acting in an augmentation role to FSH in follicle development via increased insulin-like growth factor (IGF)-I activity. Younis et al. (1992), in a limited randomized study on 42 young, tubal patients, found no difference in the FSH required nor in the number of oocytes or embryos recovered. The implantation rate and pregnancy rate differed slightly but was not significant. Bergh et al. (1994) also found similar oocyte numbers with GH but improved fertilization rates in 40 'poor responders' in a randomized, placebo-controlled study. Schoolcraft et al. (1997) suggested that a protocol utilizing a flare stimulation regimen with GH whilst using similar amounts of FSH appeared to deliver a better pregnancy outcome. More recently, Sugaya et al. (2003) confirmed in nine poor-responder patients increased oocyte recovery and embryo quality than in their previous cycles and that, while IGF-I concentrations were higher after GH, there were no adverse effects noted. Finally, Hazout et al. (2009) observed an improved response to GH co-treatment in a large sample of 245 'poor' responder patients with increased oocyte and embryo numbers and increased pregnancy rate when compared with other similar patients.

Rajesh et al. (2007) reported a similar outcome in 20 poor-prognosis patients. Kolibianakis et al. (2009), in a meta-analysis study, argued that GH may improve pregnancy rate in poor-responder patients but there remains insufficient information to confirm these observations. This meta-analysis supported a previous study in 2003 from the Cochrane database (Harper et al., 2003) and a recent review (Kyrou et al., 2009). Together, these reports suggest that while oocyte recovery is a variable outcome measure, most studies have found that more pregnancies occur with GH co-treatment.

These data also do not confirm an increase in oocyte recovery or fertilization rate as reported elsewhere (Bergh et al., 1994). There may be several reasons for the lack of difference in oocyte numbers; the one most likely is that the poor-responder groups are normally on high dose FSH regimens. If one of the roles of GH is to facilitate the actions of FSH at normal serum concentrations (Volpe et al., 1992), then the prescription of maximal doses may override this role (Kyrou et al., 2009). Alternatively, this result was not surprising given the similar starting FSH concentrations, reinforcing the view that these patients were not poor responders relative to other patients in the same age group. Future studies may need to elucidate the interaction between FSH dose and GH exposure.

In all age groups, the implantation rate in GH− cycles was highly significantly poorer compared with the implantation rate in GHu cycles. This observation supports the patient categorization of poor prognosis. This report did find that within the study group, co-treatment of GH significantly improved the chance of pregnancy and specifically the implantation rate compared with the cycles without GH. Importantly, the implantation rate with GH+ cycles increased to levels only marginally less than the uncategorized group, especially so for women over 40 years.

A key observation was that in the thaw cycles with embryos originating from the GH+ cycles, the pregnancy rate per thaw cycle was significantly higher compared with the cycles where the frozen embryos arose from the GH− group. This was evident across all ages implying better-quality embryos arise following GH co-treatment in poor-prognosis cases. The best measure of GH effectiveness is the cumulative number of pregnancies expressed against the number of collections since it is the cohort of embryos that is important, not just the fresh transfer. In this study, the cumulative pregnancy rate per collection (productivity rate) was significantly higher after GH augmentation than without GH, although this was still less than the rate of 64% in the uncategorized group, which, of course, benefited by exclusion of those poor-prognosis cases selected out for the GH study. This analysis again supports the argument that the patients referred for GH indeed have a significantly poorer prognosis. In the cycles without growth hormone, only 13.7 clinical pregnancies were achieved per 100 collections. It may be surmised from this data that GH significantly improves the outcome in the poor-responder and other poor-prognosis groups but its application does not restore these patients to a 'normal' responder. The higher implantation rate with GH and the higher productivity over fresh and frozen cycles argues the effect of GH augmentation was on oocyte competency rather than on uterine interactions.

The beneficial effect of GH was apparent over all attempt numbers and over time, with the observation that the provision of GH in 2002–2003 in the cycle preceding the IVF cycle generated better outcomes compared with the GH− cycles than in the later part of the study in 2004–2005 when GH was given during the treatment cycle. Most of the published studies administered GH after the start of ovarian stimulation and continued exposure until ovulation induction trigger. The two differing exposure periods in this study reflect this study's endeavours to explore whether one regimen was superior and the results suggest that both pre- or peri-treatment cycle administration provided a benefit. It does not, however, imply that the mode of action is the same. There have been no reports comparing the timing of GH exposure, yet there are two proposed modes of action: either a supportive effect on FSH stimulation on follicle recruitment and development or a role in oocyte maturation and maturity.

The requirement of GH in follicle development can be demonstrated by the management of patients who have either GH deficiency (Giampietro et al., 2009) or GH excess (Esfandiari et al., 2005). In the report of four eugonadotrophic women, GH replacement therapy of 4–6 months results in natural conceptions without the addition of any other stimulatory means. There have been many reports documenting that GH supplementation increases IGF-I serum and follicle concentrations in normal ovulating women (Carson et al., 1989; Volpe et al., 1992) and that its action was largely to promote FSH activity by IGF-1 (Homburg et al., 1988). As such, the early studies focused on whether GH supplementation increased follicle recruitment and oocyte numbers mainly for poor-responder patients. Whether this applies to the poor-prognosis patients in this study who are already receiving high dose gonadotrophin stimulation is unclear. Sugaya et al. (2003) did find, even at 450 IU/day, that GH treatments resulted in more oocytes recovered but only in patients with low IGF-I binding protein-3. This observation suggests that where GH involves an action via IGF-I, it is mediated by associated binding proteins. Recent

articles demonstrating that DHEA may augment the ovarian action of FSH in poor responders by increasing oocyte recovery rates and embryo quality (Barad and Gleicher, 2006; Casson et al., 2000) appear to be different observations made in this study and suggest that DHEA and GH may differ in some modes of action. If so, combining both DHEA and GH may warrant specific investigation.

An alternative mode of action may be a direct effect of GH on oocyte maturation. In the bovine model for instance, GH has been shown to promote nuclear maturation of oocytes *in vitro* via cumulus cell interaction (Izadyar et al., 1996) in a manner that is independent of IGF-1 (Izadyar et al., 1997). In this model, GH receptors have been identified on both the cumulus cells and the oocyte along with GH mRNA expression in the oocyte (Bevers and Izadyar, 2002). In-vitro co-culture of GH with bovine oocytes promoted embryo development (Izadyar et al., 2000), possibly by improving cytoplasmic aspects of the oocyte during maturation. Izadyar et al. (1998a,b) further demonstrated that GH and FSH acted via separate pathways since the FSH effect on oocyte maturation was cAMP dependent, unlike the GH effects that were not. Together, these articles argue that GH has a specific stimulatory role in oocyte maturation via GH receptors to activate transcription and that this may be in part mediated via the oocyte. There are no reports of GH receptor activity on human oocytes and species variations make extrapolation difficult, since oocyte-specific factors may promote granulosa cell proliferation in the mouse, but in the bovine and porcine models this effect involves IGF-1 (Gilchrist et al., 2008). This study's data, demonstrating improved implantation rates for both fresh and frozen embryos, suggest that GH has an active role in human embryo quality and this would most likely reflect a role in oocyte maturation rather than oocyte numbers. There are other reports of an association between GH concentrations in follicular fluid and IVF outcomes. Mendoza et al. (1999) reported elevated GH concentrations in the follicular fluid of women were associated with improved oocyte maturity, fertilization and embryo quality. A follow-up study by Mendoza et al. (2002) confirmed that elevated GH concentrations were also found within the follicle fluid of oocytes that were selected for transfer and that gave rise to pregnancy. In other studies, the concentrations were at the low end of the normal range in females and the concentration of serum GH was not described (Mendoza et al., 2002). Mendoza et al. (1999) suggested that the differing concentration of GH in follicular fluid may arise from the local action of cytokines on the permeability of the follicle wall to serum concentrations. Little is known about the relative concentrations of GH in serum after co-treatment and whether the concentrations are raised significantly near the time of ovulation induction to influence oocyte maturation. The phase of GH administration may therefore produce different outcomes, depending on whether GH was prescribed before treatment, and therefore acts on follicle selection and response to FSH, or later in treatment where the effects may be more to do with oocyte competency.

There are three studies in humans that support the observations presented here. Tesarik et al. (2005) found GH administration in women over 40 years of age significantly increased the pregnancy rate. The clinical pregnancy rate for women greater than 40 years in the current study was 12/49 and 1/29 for GH+ and GH−, respectively, and the combined results showed a 24% pregnancy rate in GH-treated older women compared with 3% for all women over 40 years treated without GH during the 5-year study period. These results are consistent with the observations by Tesarik et al. (2005) who reported clinical pregnancy rates of 32/50 and 6/50, respectively. These observations support the nature of this study's sequential crossover design and confirm that GH administration in older women can be beneficial. Rajesh et al. (2007) reported that GH increased embryo numbers and pregnancy in poor-prognosis patients and Hazout et al. (2009) also demonstrated a better outcome with GH in poor-prognosis groups (more than three transfers) when compared against other patients of similar background. Sugaya et al. (2003) identified a subgroup of patents who did not have elevated concentrations of IGF-I binding protein-3 that appeared to produce more oocytes with GH treatment.

The type of patient who may benefit from GH augmentation remains unclear. While this study has demonstrated a positive role that in patients who clinically present with reduced prognosis, usually after three or more IVF attempts, clarification on how it may improve outcomes may better elucidate those patients who may benefit. The better outcomes in younger women argue their poor reproductive potential may be due to processes that depend on GH. Addition of GH appears to improve their prognosis but does not fully restore it to levels seen in the uncategorized GHu group. In other words, GH deficiency is only part of the problem. In older women, while the need for GH remains, other effects of oocyte ageing may make GH supplementation harder to demonstrate if given universally. This study has shown that GH improves clinical outcomes in both younger women (<35 years) and older women. It would, however, seem logical to separate the aetiologies of these two groups since women over 40 years have lower ovarian reserve and decreased oocyte quality. In contrast, poor responders under 35 years of age represent women with either premature reduction of ovarian reserve or elevated FSH threshold.

This paper also is the first to report on the outcome of children conceived from a treatment cycle involving GH during IVF. The results suggest a similar miscarriage rate consistent with the poor outcome feature of this group of patients, a rate still higher than in the GHu group suggesting that GH may not address some key reproductive failings in such patients. However, a higher clinical pregnancy rate and live birth rate per egg collection has been demonstrated in the GHu group. Birthweights and sex ratio were normal. One report (Esfandiari et al., 2005) documents healthy triplets after GH treatment for acromegaly and another (Salle et al., 2000) documents a normal birth after GH treatment for panhypopituitarism. The similar miscarriage rate differs from a recent observation that DHEA may reduce the miscarriage rates in poor responders (Gleicher et al., 2008) again suggesting DHEA and GH may not act in the same manner.

In summary, this 5-year comprehensive study of IVF/ICSI treatment in a large group of patients categorized as having a poor prognosis, co-treatment with GH was shown to significantly increase the clinical pregnancy and live birth rate over treatment cycles without GH, albeit to rates lower than observed with all other patients not categorized as

poor responders. The effect was apparent across time periods, in all age groups, regardless of previous attempts and independent of stimulation protocols. The benefit of GH was also expressed in subsequent frozen embryo transfers implying improved qualitative aspects for oocytes. There appeared to be no adverse effects in the children born after GH exposure. The study does support other reports for a role for GH supplementation for various poor-prognosis cases and further research is required to ascertain whether the effects observed are due to improved follicle health or improvements in the peri-ovulatory environment.

References

Adashi, E.Y., Resnick, C.E., Hurwitz, A., Ricciarelli, E., Hernandez, E.R., Roberts, C.T., et al., 1991. Insulin-like growth factors: the ovarian connection. Hum. Reprod. 6, 1213—1219.

Andersen, A.N., Devroey, P., Arce, J.C., 2006. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Hum. Reprod. 21, 3217—3227.

Barad, D., Gleicher, N., 2006. Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. Hum. Reprod. 21, 2845—2849.

Barad, D.H., Gleicher, N., 2005. Increased oocyte production after treatment with dehydroepiandrosterone. Fertil. Steril. 84, 756.

Bergh, C., Hillensjo, T., Wikland, M., Nilsson, L., Borg, G., Hamberger, L., 1994. Adjuvant growth hormone treatment during in vitro fertilization: a randomized, placebo-controlled study. Fertil. Steril. 62, 113—120.

Bevers, M.M., Izadyar, F., 2002. Role of growth hormone and growth hormone receptor in oocyte maturation. Mol. Cell. Endocrinol. 197, 173—178.

Carson, R.S., Zhang, Z., Hutchinson, L.A., Herington, A.C., Findlay, J.K., 1989. Growth factors in ovarian function. J. Reprod. Fertil. 85, 735—746.

Casson, P.R., Lindsay, M.S., Pisarska, M.D., Carson, S.A., Buster, J.E., 2000. Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders: a case series. Hum. Reprod. 15, 2129—2132.

Esfandiari, N., Gotlieb, L., Casper, R.F., 2005. Live birth of healthy triplets after in vitro fertilization and embryo transfer in an acromegalic woman with elevated growth hormone. Fertil. Steril. 83, 1041.

Giampietro, A., Milardi, D., Bianchi, A., Fusco, A., Cimino, V., Valle, D., et al., 2009. The effect of treatment with growth hormone on fertility outcome in eugonadal women with growth hormone deficiency: report of four cases and review of the literature. Fertil. Steril. 91, 930.e7-930.e11.

Gilchrist, R.B., Lane, M., Thompson, J.G., 2008. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum. Reprod. Update 14, 159—177.

Gleicher, N., Ryan, E., Weghofer, A., Oktay, K., Blanco-Mejia, S., Barad, D.H., 2008. Dehydroepiandrosterone (DHEA) reduces miscarriage rates in women with diminished ovarian reserve: a multicenter study. Fertil. Steril. 90, S258—S259.

Harper, K., Proctor, M., Hughes, E., 2003. Growth hormone for in vitro fertilization. Cochrane Database Syst. Rev. Article No. CD000099. doi:10.1002/14651858.CD000099.

Hazout, A., Junca, A., Menezo, Y., Demouzon, J., Cohen-Bacrie, P., 2009. Effect of growth hormone on oocyte competence in patients with multiple IVF failures. Reprod. Biomed. Online 18, 664—670.

Homburg, R., Eshel, A., Abdalla, H.I., Jacobs, H.S., 1988. Growth hormone facilitates ovulation induction by gonadotrophins. Clin. Endocrinol. (Oxf.) 29, 113—117.

Homburg, R., Eshel, A., Kilborn, J., Adams, J., Jacobs, H.S., 1990a. Combined luteinizing hormone releasing hormone analogue and exogenous gonadotrophins for the treatment of infertility associated with polycystic ovaries. Hum. Reprod. 5, 32—35.

Homburg, R., West, C., Ostergaard, H., Jacobs, H.S., 1991. Combined growth hormone and gonadotropin treatment for ovulation induction in patients with non-responsive ovaries. Gynecol. Endocrinol. 5, 33—36.

Homburg, R., West, C., Torresani, T., Jacobs, H.S., 1990b. Cotreatment with human growth hormone and gonadotropins for induction of ovulation: a controlled clinical trial. Fertil. Steril. 53, 254—260.

Izadyar, F., Colenbrander, B., Bevers, M.M., 1997. Stimulatory effect of growth hormone on in vitro maturation of bovine oocytes is exerted through the cyclic adenosine 3′,5′-monophosphate signaling pathway. Biol. Reprod. 57, 1484—1489.

Izadyar, F., Hage, W.J., Colenbrander, B., Bevers, M.M., 1998a. The promotory effect of growth hormone on the developmental competence of in vitro matured bovine oocytes is due to improved cytoplasmic maturation. Mol. Reprod. Dev. 49, 444—453.

Izadyar, F., Van Tol, H.T., Hage, W.G., Bevers, M.M., 2000. Preimplantation bovine embryos express mRNA of growth hormone receptor and respond to growth hormone addition during in vitro development. Mol. Reprod. Dev. 57, 247—255.

Izadyar, F., Zeinstra, E., Bevers, M.M., 1998b. Follicle-stimulating hormone and growth hormone act differently on nuclear maturation while both enhance developmental competence of in vitro matured bovine oocytes. Mol. Reprod. Dev. 51, 339—345.

Izadyar, F., Zeinstra, E., Colenbrander, B., Vanderstichele, H.M., Bevers, M.M., 1996. In vitro maturation of bovine oocytes in the presence of bovine activin A does not affect the number of embryos. Anim. Reprod. Sci. 45, 37—45.

Jacobs, H.S., 1972. The clinical application of the measurement of human growth hormone. J. Endocrinol. 54, xxvi—xxvii.

Jacobs, H.S., 1992. Growth hormone and ovulation: is there an indication for treatment of infertile women with growth hormone? Horm. Res. 38 (Suppl. 1), 14—21.

Kolibianakis, E.M., Venetis, C.A., Diedrich, K., Tarlatzis, B.C., Griesinger, G., 2009. Addition of growth hormone to gonadotrophins in ovarian stimulation of poor responders treated by in-vitro fertilization: a systematic review and meta-analysis. Hum. Reprod. Update 15, 613—622.

Kyrou, D., Kolibianakis, E.M., Venetis, C.A., Papanikolaou, E.G., Bontis, J., Tarlatzis, B.C., 2009. How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Fertil. Steril. 91, 749—766.

Mendoza, C., Cremades, N., Ruiz-Requena, E., Martinez, F., Ortega, E., Bernabeu, S., et al., 1999. Relationship between fertilization results after intracytoplasmic sperm injection, and intrafollicular steroid, pituitary hormone and cytokine concentrations. Hum. Reprod. 14, 628—635.

Mendoza, C., Ruiz-Requena, E., Ortega, E., Cremades, N., Martinez, F., Bernabeu, R., et al., 2002. Follicular fluid markers of oocyte developmental potential. Hum. Reprod. 17, 1017—1022.

Owen, E.J., Shoham, Z., Mason, B.A., Ostergaard, H., Jacobs, H.S., 1991. Cotreatment with growth hormone, after pituitary suppression, for ovarian stimulation in in vitro fertilization: a randomized, double-blind, placebo-control trial. Fertil. Steril. 56, 1104—1110.

Rajesh, H., Yong, Y.Y., Zhu, M., Chia, D., Yu, S.L., 2007. Growth hormone deficiency and supplementation at in-vitro fertilisation. Singapore Med. J. 48, 514—518.

Salle, A., Klein, M., Pascal-Vigneron, V., Dousset, B., Leclere, J., Weryha, G., 2000. Successful pregnancy and birth after sequential cotreatment with growth hormone and gonadotropins in a woman with panhypopituitarism: a new treatment protocol. Fertil. Steril. 74, 1248—1250.

Schoolcraft, W., Schlenker, T., Gee, M., Stevens, J., Wagley, L., 1997. Improved controlled ovarian hyperstimulation in poor responder in vitro fertilization patients with a microdose follicle-stimulating hormone flare, growth hormone protocol. Fertil. Steril. 67, 93—97.

Sugaya, S., Suzuki, M., Fujita, K., Kurabayashi, T., Tanaka, K., 2003. Effect of cotreatment with growth hormone on ovarian stimulation in poor responders to in vitro fertilization. Fertil. Steril. 79, 1251—1253.

Tesarik, J., Hazout, A., Mendoza, C., 2005. Improvement of delivery and live birth rates after ICSI in women aged >40 years by ovarian co-stimulation with growth hormone. Hum. Reprod. 20, 2536—2541.

Testart, J., 1986. Cleavage stage of human embryos two days after fertilization in vitro and their developmental ability after transfer into the uterus. Hum. Reprod. 1, 29—31.

Volpe, A., Artini, P.G., Barreca, A., Minuto, F., Coukos, G., Genazzani, A.R., 1992. Effects of growth hormone administration in addition to gonadotrophins in normally ovulating women and polycystic ovary syndrome (PCO) patients. Hum. Reprod. 7, 1347—1352.

Younis, J.S., Simon, A., Koren, R., Dorembus, D., Schenker, J.G., Laufer, N., 1992. The effect of growth hormone supplementation on in vitro fertilization outcome: a prospective randomized placebo-controlled double-blind study. Fertil. Steril. 58, 575—580.

Gardner, D.K., Schoolcraft, W., 1999. In-vitro culture of human blastocysts. In: Jansen, R., Mortimer, D. (Eds.), Towards Reproductive Certainty: Fertility and Genetics Beyond. Parthenon Press, pp. 378—388.

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