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(RESEARCH ARTICLE)

Measuring IGF-1 and IGFBP-3 profiles in women seeking assisted reproduction; relationship to serum growth hormone levels (Study 3)

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Abstract

Extending from our first two studies examining the IGF profile (IGF-1, IGFBP-3 and the ratio of IGFBP-3/ IGF-1) in women presenting for assisted reproductive technologies (ART), this study examines its relevance to the testing of human growth hormone (hGH) levels on the same blood sample. These were taken in the morning during the early follicular phase of the woman's menstrual cycle and included 408 women who were ART-naïve, undertaking the tests as part of an assessment cycle prior to any ART treatment. The growth hormone levels were also tested on a further 945 women classified as ART-interval cases. It was shown that the vast majority of hGH levels (73%) were very low at <1.0 ng/mL and 22% are extremely low <0.1 ng/mL, close to the detection level of the chemiluminescent immunoassay (0.03 ng/mL). Only 12% of hGH levels were recorded in the range ≥3.0 ng/mL, levels which exclude adult growth hormone deficiency (AGHD). Although IGF-1 levels are regarded as a screening test for AGHD, our studies showed no correlation between hGH levels and the entire range of IGF-1 levels, neither across IGFBP-3 levels, nor across IGF Ratios, albeit there was an apparent inverse trend for the latter. Across the entire age range, the hGH levels were not statistically different among neither the ART-naïve nor the ART-interval women. Furthermore, hGH levels were not different among the clinical parameters of stature or BMI; nor for ovarian reserve parameters AMH or AFC. It is concluded that serum hGH screening probably has very limited value as a screening test for potential AGHD.

Keywords: Human growth hormone (hGH); Insulin-like growth factor-1 (IGF-1), Insulin-like growth factor binding protein-3 (IGFBP-3); IGF profile (IGF-1, IGFBP-3 and IGF Ratio); IGFBP-3/ IGF-1 ratio (IGF Ratio); Assisted reproductive technology (ART); *In vitro* fertilization (IVF).

1. Introduction

In the 42 years since successful livebirths from in vitro fertilization (IVF) the field has progressed rapidly with, according to current estimates, more than 10 million infants arising from this and related areas of assisted reproductive technology (ART) [1,2]. Nonetheless, most of the successes have been enjoyed by younger women, whilst those over age 40 years have achieved only a small component of the benefits of ART, despite investing a much higher proportion of their time, their finances, and their energies (including emotional, physical and marital energy) into IVF-related attempts [3]. The ART facilities have introduced an array of protocols, techniques and adjuvants for those women categorised as poor-prognosis, and which is due mostly to the age-related decline in fertility, but which is sometimes caused by other, poorly defined factors in younger women. The extensive array of adjuvants applied in the poor-

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prognosis category attempting to redress the outcome disparity has been introduced in an empirical manner. Therefore, it is currently difficult to know if the successes achieved in such poor-prognosis cases is fortuitous or treatment-related [4,5]. Whilst the vast majority of adjuvant therapies lack high-level evidence-based support at this stage, one such adjuvant, namely growth hormone (GH), has some supportive evidence [4,6]. Nonetheless, most GH trials have been given on an empirical basis, without any attempt to define adult growth hormone deficiency (AGHD) and this has drawn criticism [7]. To redress this, our study is the third in a series of studies progressively exploring the concept of AGHD underlying infertility where there is a defined poor-prognosis in current ART management [8]. The idea is to explore those very same parameters which apply to the identification of GH-deficiency in children with low stature [9], to the adult female with infertility. Apart from the sequential measurement of stature and radiographic assessment of bone age, identifying the individual who should undergo provocative testing such as an insulin sensitivity challenge, relies on serum screening of the Insulin-like Growth Factor (IGF) profile which includes IGF-1, its main binding protein IGFBP-3, and the IGF ratio of one against the other; our preference being IGFBP-3/ IGF-1 where a ratio >5.0 corresponds with GHD. Measuring serum human GH (hGH) is considered unreliable as day-time levels are mostly very low because GH secretion from the pituitary is pulsatile and mostly nocturnal, disappearing rapidly from the serum [10]. Whereas our first study examined the IGF profile in women attending for ART treatment according to clinical parameters including age, body stature and body mass index [11], a second study compared the measurements against tests for ovarian reserve, as women with lower reserve are considered to have higher likelihood for a poor prognosis [12]. This third study examines the IGF profile against hGH levels on the same blood samples. It also studies the hGH levels separately and specifically against the same clinical parameters (age, BMI, stature) and tests of ovarian reserve, namely anti-Mullerian hormone (AMH) and antral follicle count (AFC).

2. Material and methods

This study examines those same women who completed an Assessment Cycle (AC) at the PIVET ART facility during the complete 9-year period from 2011 to 2019. As previously described this period embraces a highly stable program focusing on blastocyst culture (~90%), single embryo transfers (>95%) and a strong focus on cryopreservation (exclusively undertaken with the Cryotop vitrification method) [13]. During this period 3751 women entered into 10,728 treatments of various ART categories. Figure 1 shows the derivation of 1633 women from a total 2319 women who undertook an AC, and which included an IGF profile as well as an antral follicle count (AFC) estimation along with serum AMH testing. All these tests were performed around Day-5±1 of the menstrual cycle, in the morning, mostly between 0800 hours and 1000 hours following a minimal "tea and toast" breakfast. Stature and BMI assessments were undertaken and reported in the first study [11] which correlated with the IGF profile assays. These ACs were one-off evaluations of ART-naïve women, designed to determine the underlying infertility factors and were not repeated between ART treatments.

Figure 1. Flow diagram showing derivation of 1633 women who had an IGF profile (IGF-1 with IGFBP-3 and the IGF Ratio) in the early follicular phase of an Assessment Cycle undertaken prior to any definitive treatment. Of these ART-naïve women, 408 had hGH measurements on the same IGF serum sample and these levels were analysed with respect to age groups, BMI groups, stature, AMH and AFC. A further group of 945 ART-interval women also had IGF profiles determined and similar correlative analyses were undertaken.

The specific evaluations undertaken in this third study are described as follows:

2.1. IGF profile

The IGF profile included chemiluminescent immunoassays for both IGF-1 and IGFBP-3 with full details described in Study 1 [11]. The IGF Ratio is calculated from the division of IGFBP-3/ IGF-1 and the normal range is 1.6 to 5.0, with ratios >5.0 considered to be in the potential AGHD range.

2.2. AMH assays

The AMH assays involved two methods. The early estimations (across the years 2011 to 2016) were undertaken on the Beckman Coulter Immunotech Gen 2 Elisa platform (Danaher Corporation; California, USA). From 2017 the AMH assay has been performed on the Cobas Elecsys e411 platform (Roche Diagnostics; Basel, Switzerland). These assays were fully described in Study 2 [12] and have shown tight correlations on quality assurance programs (RANDOX RIQAS) monitored under accreditation with the National Australian Testing Authority (NATA). Furthermore, they have shown reasonably close correlation with AFC in grouped arrangements [14].

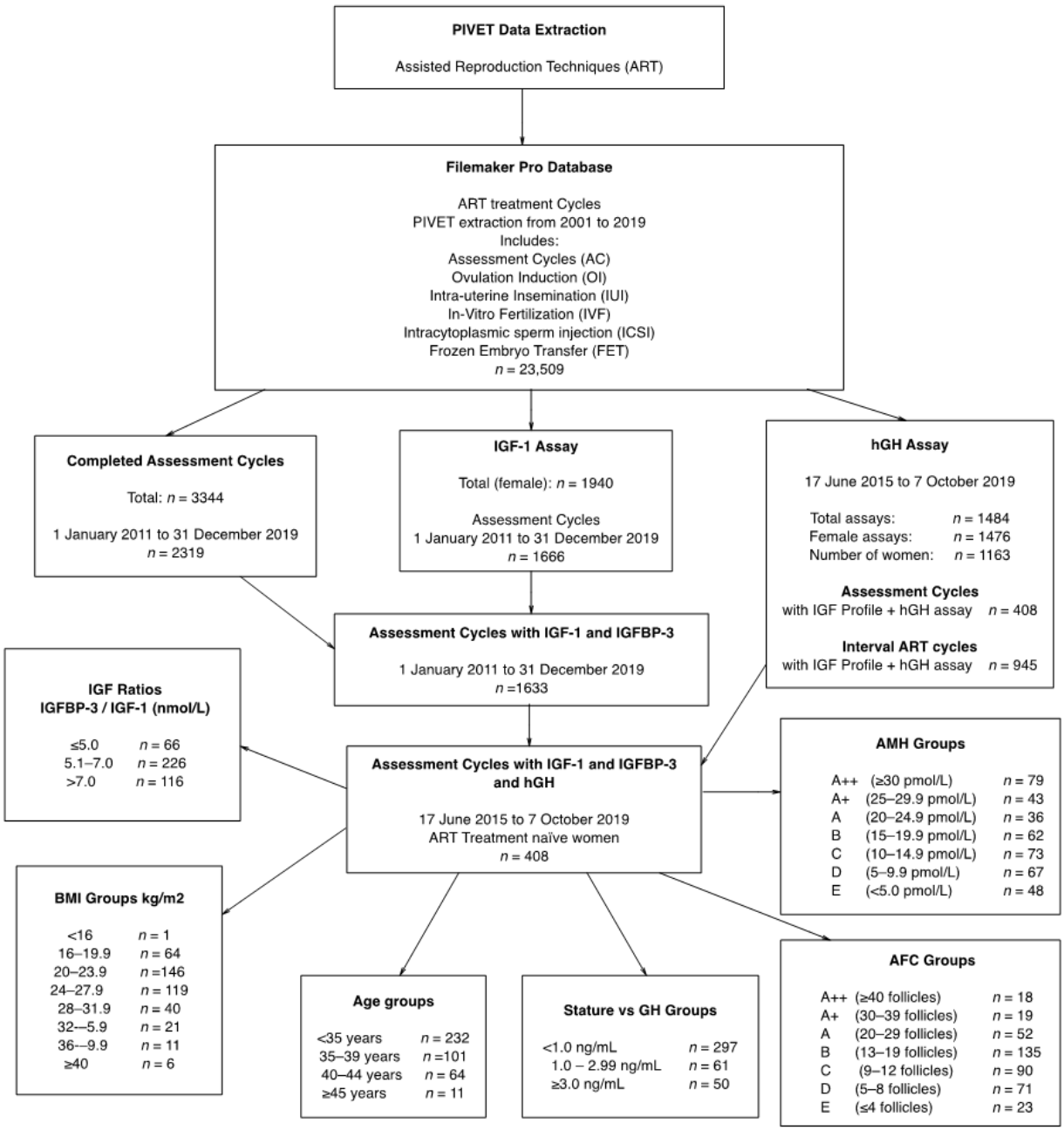

Figure 1 Flow diagram

2.3. AFC determination

Antral follicle counts were performed on Day-5±1 of the AC applying trans-vaginal ultrasound, using Voluson scanning machines supplied from General Electric Australia; initially using the Voluson 730 Expert for 5-years, followed by the Voluson P6 in the most recent 4-years. The ovary is viewed in 3 planes and all detectable (antral) follicles up to 10mm (2-10mm) are counted from both ovaries (a single ovary if the other is absent). The AFC determination is the key measurement to decide FSH dosing by the PIVET Algorithm [15,16] which also takes several other parameters into account, including the woman's age, her BMI and her AMH level. According to the PIVET Algorithm the AFC is categorised as Grade A if ≥ 20 antral follicles are counted. This grouping is sub-categorised as Grade A+ for 30-39 antral follicles and Grade A++ if ≥40 antral follicles are counted. For antral follicle counts 13-19, the classification is Group B, for 9-12 the classification is Group C, for 5-8 the classification is Grade D and the lowest category is Grade E comprising ≤4 antral follicles (Table 1).

Table 1. One of the PIVET FSH-dosing Algorithms which have been applied throughout this study period; this one being particularly suited to the Puregon pen which provides ~8.3 IU increments of FSH. The Algorithms have been reported [15,16] with validation indicating that 10±2 oocytes are generated for the majority of women and ovarian hyperstimulation syndrome is uncommon at <0.3% of cases

Table 1 PIVET FSH-dosing Algorithm

Puregon, Gonal-F & Elonva Desk Chart

AMH	>30 pm/L					25-29.9 pm/L					20-24.9 pm/L					15-19.9 pm/L					10-14.9 pm/L					5-9.9 pm/L					< 5.0 pm/L				
AFC*	A++ (≥ 40 follicles)					A+ (30-39 follicles)					A (20-29 follicles)					B (13-19 follicles)					C (9-12 follicles)					D (5-8 follicles)					E (≤4 follicles)				
BMI	16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35
Age (years) 20	41.7	41.7	41.7	50.0	58.3	58.3	58.3	58.3	66.7	66.7	66.7	75.0	75.0	75.0	83.3	91.7	91.7	91.7	100.0	108.3	116.7	116.7	116.7	125.0	133.3	141.7	141.7	141.7	150.0	150.0	166.7	166.7	166.7	175.0	175.0
21	41.7	41.7	41.7	50.0	58.3	58.4	58.3	58.3	66.7	75.0	75.0	75.0	83.3	83.3	91.6	91.7	100.0	100.0	108.3	116.6	125.0	125.0	125.0	133.3	141.6	150.0	150.0	150.0	158.3	158.3	175.0	175.0	175.0	183.3	183.3
22	41.7	41.7	41.7	50.0	58.3	58.4	58.3	58.3	66.7	75.0	75.0	75.0	83.3	83.3	91.6	100.0	100.0	100.0	108.3	116.6	125.0	125.0	125.0	133.3	141.6	150.0	150.0	150.0	158.3	158.3	175.0	175.0	175.0	183.3	183.3
23	50.0	50.0	50.0	58.3	66.6	66.7	66.7	66.7	75.0	83.3	83.3	83.3	83.3	91.7	100.0	108.3	108.3	108.3	116.7	125.0	133.3	133.3	133.3	141.7	150.0	158.3	158.3	158.3	166.7	166.7	183.3	183.3	183.3	191.7	200.0
24	50.0	50.0	50.0	58.3	66.6	66.7	66.7	66.7	75.0	83.3	83.3	83.3	91.7	91.7	100.0	108.3	108.3	116.7	116.7	125.0	133.3	141.7	141.7	150.0	150.0	158.3	158.3	158.3	166.7	166.7	183.3	183.3	191.7	200.0	200.0
25	50.0	50.0	50.0	58.3	66.6	66.7	66.7	66.7	75.0	83.3	91.7	91.7	91.7	100.0	108.3	116.7	116.7	116.7	125.0	133.3	141.7	141.7	141.7	150.0	158.3	166.7	166.7	166.7	175.0	175.0	191.7	191.7	200.0	20-21	200.0
26	58.3	58.3	58.3	66.7	75.0	75.0	75.0	75.0	83.3	91.6	91.7	91.7	100.0	108.3	108.3	125.0	125.0	125.0	133.3	141.6	150.0	150.0	150.0	158.3	166.6	175.0	175.0	175.0	183.3	183.3	191.7	200.0	200.0	208.3	208.3
27	58.3	58.3	58.3	66.7	75.0	75.0	75.0	75.0	83.3	91.6	100.0	100.0	100.0	108.3	116.6	125.0	125.0	125.0	133.3	141.6	150.0	150.0	150.0	158.3	166.6	175.0	175.0	175.0	183.3	183.3	200.0	208.3	208.3	216.7	216.7
28	58.3	58.3	58.3	66.7	75.0	83.3	83.3	83.3	91.7	100.0	108.3	108.3	108.3	116.7	125.0	133.3	133.3	133.3	141.7	150.0	158.3	158.3	158.3	166.7	175.0	183.3	183.3	183.3	191.7	200.0	216.7	225.0	225.0	233.3	233.3
29	66.7	66.7	66.7	75.0	83.3	83.3	83.3	83.3	91.7	100.0	108.3	108.3	108.3	116.7	125.0	141.7	141.7	141.7	150.0	158.3	158.3	158.3	158.3	166.7	175.0	183.3	183.3	183.3	200.0	208.3	233.3	233.3	233.3	241.7	241.7
30	66.7	66.7	66.7	75.0	83.3	91.7	91.7	91.7	100.0	108.3	116.7	116.7	116.7	125.0	133.3	141.7	141.7	141.7	150.0	158.3	166.7	166.7	166.7	175.0	183.3	191.7	200.0	208.3	208.3	216.7	241.7	241.7	241.7	250.0	275.0
31	75.0	75.0	75.0	83.3	91.6	91.7	91.7	91.7	100.0	108.3	125.0	125.0	125.0	133.3	141.6	141.7	141.7	141.7	150.0	158.3	175.0	175.0	175.0	183.3	191.6	200.0	200.0	200.0	208.3	216.7	225.0	250.0	250.0	275.0	300.0
32	75.0	75.0	75.0	83.3	91.6	100.0	100.0	100.0	108.3	116.6	125.0	125.0	125.0	133.3	141.6	150.0	150.0	150.0	158.3	166.6	175.0	175.0	175.0	183.3	200.0	208.3	208.3	208.3	216.7	225.0	250.0	275.0	300.0	325.0	350.0
33	83.3	83.3	83.3	91.7	100.0	108.3	108.3	108.3	116.7	125.0	133.3	133.3	133.3	141.7	150.0	158.3	158.3	158.3	166.7	175.0	183.3	183.3	183.3	200.0	208.3	225.0	225.0	225.0	233.3	250.0	275.0	300.0	325.0	350.0	375.0
34	83.3	83.3	83.3	91.7	100.0	108.3	108.3	108.3	116.7	125.0	141.7	141.7	141.7	150.0	158.3	158.3	158.3	158.3	166.7	175.0	183.3	183.3	200.0	208.3	216.7	233.3	233.3	233.3	250.0	275.0	300.0	350.0	400.0	425.0	450.0
35	83.3	83.3	83.3	91.7	100.0	116.7	116.7	116.7	125.0	133.3	141.7	141.7	141.7	150.0	158.3	166.7	166.7	166.7	175.0	183.3	191.7	200.0	208.3	216.7	225.0	241.7	241.7	250.0	275.0	300.0	350.0	400.0	425.0	450.0	450.0
36	100.0	100.0	100.0	108.3	116.6	100.0	100.0	100.0	108.3	116.6	150.0	150.0	150.0	158.3	166.6	183.3	183.3	191.7	200.0	208.3	216.7	216.7	225.0	225.0	250.0	275.0	300.0	325.0	350.0	375.0	400.0	425.0	450.0	450.0	450.0
37	100.0	100.0	100.0	108.3	116.6	100.0	100.0	100.0	108.3	116.6	158.3	158.3	158.3	166.7	175.0	183.3	191.7	200.0	208.3	216.6	225.0	225.0	233.3	250.0	275.0	300.0	325.0	350.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0
38	108.3	108.3	108.4	116.7	125.0	108.3	108.3	108.3	116.7	125.0	166.7	166.7	166.7	175.0	183.3	191.7	208.3	208.3	216.7	225.0	250.0	250.0	250.0	275.0	300.0	325.0	350.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0
39	108.3	108.3	108.4	116.7	125.0	108.3	108.3	108.3	116.7	125.0	183.3	183.3	183.3	191.7	200.0	208.3	216.7	225.0	233.3	241.6	266.7	275.0	275.0	300.0	325.0	350.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0	450.0
40	116.7	116.7	116.7	125.0	133.3	116.7	116.7	116.7	125.0	133.3	191.7	191.7	191.7	200.0	208.3	225.0	241.7	241.7	250.0	258.3	275.0	275.0	300.0	325.0	350.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
41	125.0	125.0	125.0	133.3	141.6	125.0	125.0	125.0	133.3	141.6	200.0	200.0	200.0	208.3	216.6	258.3	258.3	258.3	266.7	275.0	300.0	300.0	325.0	350.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
42	125.0	125.0	125.0	133.3	141.6	125.0	125.0	125.0	133.3	141.6	208.3	208.3	208.3	216.7	225.0	283.3	283.3	283.3	300.0	300.0	325.0	350.0	350.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
43	133.3	133.3	133.3	141.7	150.0	133.3	133.3	133.3	141.7	150.0	216.7	216.7	216.7	225.0	233.3	300.0	300.0	300.0	300.0	325.0	350.0	375.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
44	133.3	133.3	133.3	141.7	150.0	133.3	133.3	133.3	141.7	150.0	233.3	233.3	233.3	241.7	250.0	300.0	300.0	300.0	325.0	350.0	375.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
45	133.3	133.3	133.3	141.7	150.0	141.7	141.7	141.7	150.0	158.3	241.7	241.7	241.7	250.0	258.3	300.0	300.0	300.0	350.0	375.0	400.0	425.0	425.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0

Increased FSH and Smokers

Where FSH is less than 8 IU/L, with no history of smoking, use values as shown
— Smokers move two columns to the right

Where FSH is between 8 & 12 IU/L, with no history of smoking, move one column to the right
— Smokers move two columns to the right

Where FSH is greater than 12 IU/L, move two columns to the right
— Smokers and non-smokers read same column

*Antral Follicle Count based on number of antral follicles <1.0 cm

Oocyte Donors

Aiming for 10-12 oocytes, move four columns to the right

Consider GnRH Agonist trigger if >10 follicles e.g., Tryptorelin 100 mcg x2

8.3 IU increments suit Puregon Pen

25 IU increments also suits Gonal-F Pen

Elonva — 1 x 100µg for wt ≤60kg
1 x 150µg for wt >60kg

Version 12.1 3 April 2017

2.4. Growth Hormone (hGH) Estimations

At PIVET Medical Centre hGH estimations are undertaken on the Cobas Elecsys e411 platform (Roche Diagnostics GmbH, Mannheim, Germany). The chemiluminescent immunoassay applies the sandwich methodology and applies to hGH isoforms with molecular masses of 20kDa and 22kDa [17,18]. There are two incubation periods with total assay duration of 18 minutes.

2.4.1. The hGH assay testing process

The first incubation utilises 40µL of the serum sample applying a biotinylated monoclonal hGH-specific antibody and a polyclonal hGH-specific antibody radio-labeled with ruthenium to form a sandwich complex. Thereafter a second incubation involves the addition of streptavidin-coated microparticles. This complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/Procell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

2.4.2. Specimen collection and preparation

Patients are advised that biotin (Vitamin H, one of the B-Group of vitamins) will interfere with the assay hence to avoid B-group Vitamin ingestion in the preceding 24-hours. Also, the assay will be affected by Pegvisomant (treatment for acromegaly) hence this should be ceased for at least 24-hours prior. The assay is considered unsuitable for the determination of hGH in samples from pregnant women due to cross-reactivity with placental hGH. Serum is collected using standard sampling tubes containing separating gel with heparin and EDTA. The serum samples are considered stable for 8-hours at 15-25 °C, 24-hours at 2-8 °C and 28-days at -20 °C. It is advised to freeze samples only once if

required. At PIVET assays were performed each week hence 60% of specimens were frozen at -20°C. At 20-25°C samples are centrifuged after 5 minutes following collection or thawing, thereafter analysed within 2 hours.

2.4.3. Limits of measurement (Cobas e 411)

The measuring range of the assay is between 0.030 ng/mL (limit of detection) and 50.0 ng/mL (maximum of the master curve). Values below 0.030 ng/mL are reported as <0.030 ng/mL and values above the measuring range are reported as >50 ng/mL. The CV% ranges between 1.3% (Repeatability) at 0.099 ng/mL (SD) to 2.7% (Intermediate precision) at 0.211ng/mL (SD). The limit of quantitation (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤20%. The limit of Blank is 0.020 ng/mL; the limit of detection is 0.030 ng/mL; and the limit of quantitation is 0.050 ng/mL.

2.5. Reporting units of measurement

Here we are reporting serum hGH levels in conventional mass units, namely ng/mL which equates with µg/L. To convert ng/mL to mIU/L requires a 3-fold conversion (i.e. 3.0 ng/mL equates to 9.0 mIU/L; to convert in reverse requires mIU/L by 0.333-fold to read ng/mL). For the IGF profile we are reporting in SI units (Système Internationale; International System) as these are applied in our clinical practice. The conversion of SI units nmol/L to conventional mass units (ng/mL) is 7.65; hence 25nmol/L can be read as 191ng/mL. This conversion factor of 7.65 applies for both IGF-1 and IGFBP-3. For AMH the conversion from mass units (ng/mL) to SI units (pmol/L) is 7.14 hence AMH 5ng/mL can be read as 35.7pmol/L.

2.6. Statistical Analysis

Data extractions from the Filemaker database were placed in Microsoft Excel spreadsheets and sorted according to the relevant tests. Thereafter the sorted data was placed in the application Past 4.03 (developed by Øyvind Hammer) [19] for statistical data analysis. This application also generated the Tables comprising the statistical summaries, finally placed in Microsoft Word for clearer display. Having demonstrated that the data comprising the IGF profile (IGF-1, IGFBP-3 and IGF Ratio) are all distributed in a Normal fashion, the relationship among the means was examined by one-way ANOVA for overall comparison. Both Mann-Whitney and Tukey's pair-wise plots compared the individual means which ranged from three (in percentile studies of stature) to eight (in BMI comparisons) for various analyses. The Kruskal-Wallis test was applied to examine equality between sample medians and Mann-Whitney applied for pairwise comparisons between individual sub-groups. Bonferroni correction was applied for sub-group comparisons. Following corrections, probability values of $p<0.05$ were considered significant for any test. The Past 4.03 application also generated the Figures which were then upgraded in the xDiagram 5.4 application (developed by Vu Tien Thinh) enabling optimal display for this publication.

3. Results

The derivation of the 1633 women who had an IGF profile (IGF-1 with IGFBP-3 and the IGF Ratio) in the early follicular phase of an AC undertaken prior to any definitive treatment have been described in the methodology section. This is shown in Figure 1 which also indicates the distribution and categorization of those women also having hGH assays performed. It can be seen that 1163 women had a total of 1476 hGH tests, but only 408 tests were performed on 408 ART-naïve women undertaking an AC and which included complete IGF profiling. It is these 408 women whose data was analysed for this study and whose serum hGH results were compared with their age, their BMI, their stature and their tests of ovarian reserve, namely AFC and AMH groupings. The remaining tests were performed between ART treatment cycles, particularly when clinical outcomes were disappointing. There were 945 such ART-interval cases where both IGF profiling and hGH assays were performed. The data from those cases were also analysed but not fully reported here as the results did not differ except with respect to the age profiling when larger numbers were required to clarify borderline statistical significance.

3.1. hGH levels

The summary statistics of the distribution of hGH levels is shown in Table 2. Both the ART-naïve study group (n=408) and the ART-interval group (n=945) are shown alongside, demonstrating very similar profiles. Although the mean levels are similar, between 1.24 and 1.47 ng/mL, the median levels are low at 0.31 to 0.39 ng/mL, indicating the data is heavily grouped in the low region (<1ng/mL) and negatively skewed. Clearly, although the hGH levels are continuous, the distribution does not fit a Normal, Gaussian model.

Table 2 Summary statistics of the distribution of hGH levels for the 2 groups of women**.**

Distribution of hGH levels ng/mL		
Study Group	GH 945	GH 408
Number of women	945	408
Min	0.03	0.03
Max	23.32	20.63
Sum	1389.62	505.44
Mean	1.47	1.24
Std. error	0.09	0.12
Variance	7.15	6.18
Stand. dev	2.67	2.49
Median	0.39	0.31
25^{th} centile	0.15	0.12
75^{th} centile	1.55	1.18
Skewness	3.75	4.24
Kurtosis	18.09	22.56
Geom. mean	0.51	0.42
Coeff. var	181.82	200.74

The distribution of the serum hGH levels of the 408 ART-naïve women are shown as a histogram in Figure 2a, demonstrating a marked negative skew (to the left). The distribution is also compared with the ART-interval group of women, showing a bar-chart in Figure 2b and a box and whisker chart in Figure 2c, the latter best displaying the wide range and variance across the hGH distribution. There are no significant differences across the profiles of the two groups of women.

Figure 2. The hGH distribution of the study group (n=408 women) is shown as a histogram (Figure 2a), and the two groups as a bar-chart (Figure 2b) as well as a box and whisker chart (Figure 2c) to better display the profile. The data is drawn from Table 2.

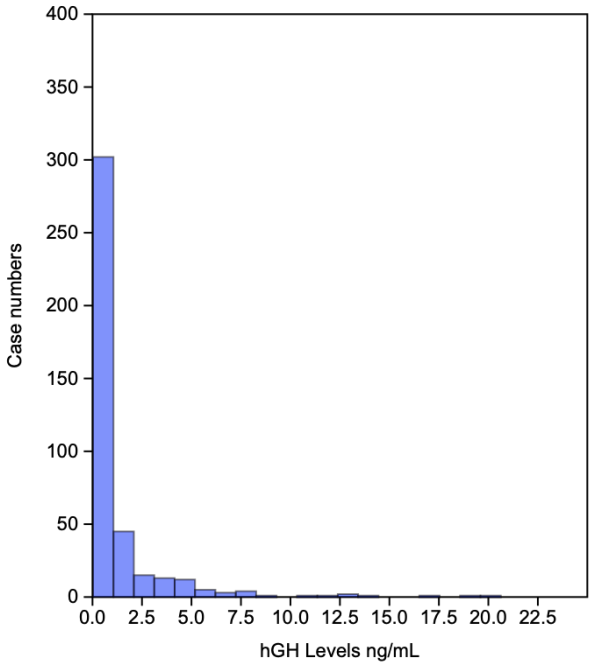

Figure 2a Histogram of hGH distribution

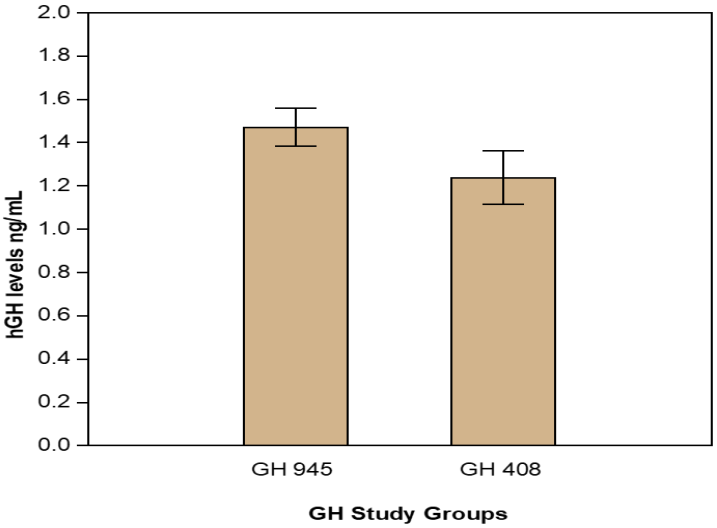

Figure 2b Bar-chart of hGH distribution

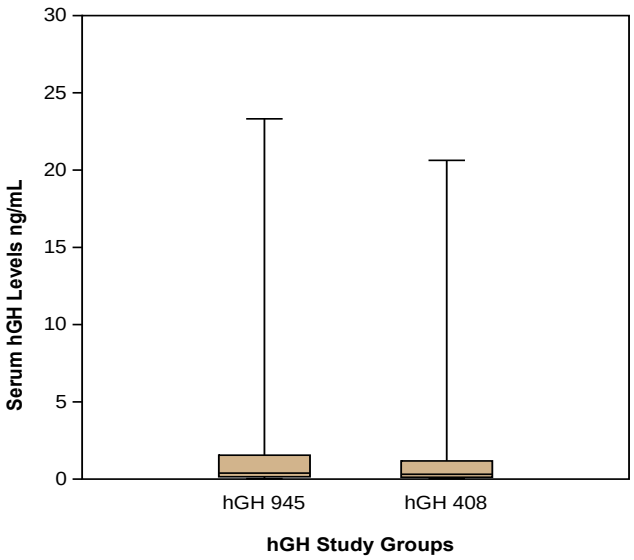

Figure 2c Box-plot of hGH distribution

These 408 tests of the ART-naïve women are set alongside the 945 tests performed outside the study group on ART-interval women for comparison, noting similar findings. The study group showed hGH mean level of 1.24 ng/mL which is well below the required hGH level of 3.0 ng/mL required to exclude the consideration of AGHD. The median level of 0.31 ng/mL indicates the majority of readings are skewed well below the mean and this is shown with a skewness rating of 2.44. Clearly this is not a Normal distribution as best displayed in the histogram of Figure 2a, hence non-parametric statistics are required for the data comparisons, mostly focusing on the median levels. The box-plot distribution in Figure 2c expresses this even more clearly as the variance is quite wide with 73% of readings being <1.0 ng/mL (297/408) and only 12% with readings being ≥3.0 ng/mL (50/408). These aspects are summarized in Table 2 with both ANOVA (for means) and Kruskal-Wallis (for medians) indicating no significant differences between the two study groups.

3.2. hGH vs IGF profile

The IGF profile comprises IGF-1, its main binding protein IGFBP-3 and the ratio of IGFBP-3/ IGF-1 termed IGF Ratio. The data is from the 408 ART-naïve women and is categorized according to three hGH groupings, namely very low being <1.0 ng/mL; low being in the range of 1.0 to 2.99 ng/mL; and normal range being ≥3.0 ng/mL. The distribution of IGF-1 levels are summarized in Table 3a and depicted in Figure 3a. It can be seen that the vast majority of IGF-1 levels are contained within the very low hGH group (297/408; 73%) but both the mean levels (27–28 nmol/L) and median levels

(26-27 nmol/L) are similar across the hGH categories (no significant differences). The distribution of IGFBP-3 is shown in Figure 3b and the data is summarized in Table 3b. As with IGF-1 the vast majority of cases fall within the very low hGH group but both the mean levels (160 to 169 nmol/L) and the median levels (157 to 164 nmol/L) are similar across the three hGH categories (no significant differences). Finally, the IGF Ratio, considered by some to be a better reflection of IGF-1 activity, is summarized in Table 3c which shows that only a minority of cases (16.2%) are within the normal range of 2.5 to 5.0 (≤5.0; 66/408). Half the cases (n=226; 55.4%) are in the elevated range of 5.1 to 7.0 and 24.4% (n=116) have very high ratios >7.0. From Figure 3c there is an apparent inverse relationship between hGH levels and the IGF Ratio with mean hGH levels declining with rising IGF Ratio (0.9 to 1.6 ng/mL) but the medians of hGH are all contained in a tighter range within the very low category (0.28 to 0.36 ng/mL) hence the differences are not significant. Neither are they particularly relevant with the vast majority of the IGF profile data residing within the very low hGH category of assays.

Table 3a Shows the summary statistics of IGF-1 levels across the 3 categories of hGH levels noting the vast majority are measured in the very low hGH group, <1.0 ng/mL

IGF-1 Levels nmol/L vs hGH Groups ng/mL - Summary Statistics			
GH Groups	<1.0 ng/mL	1.0 – 2.99 ng/mL	≥3.0 ng/mL
N	297	61	50
Min	10	12	16
Max	50	49	63
Sum	7902	1667	1416
Mean	26.6	27.3	28.3
Std. error	0.4	0.9	1.1
Variance	42.4	52.7	65.0
Stand. dev	6.5	7.3	8.1
Median	26	27	26
25th centile	22	23	23.75
75th centile	31	31.5	34
Skewness	0.33	0.61	1.59
Kurtosis	0.29	0.91	5.63
Geom. mean	25.79	26.38	27.33
Coeff. var	24.49	26.57	28.48

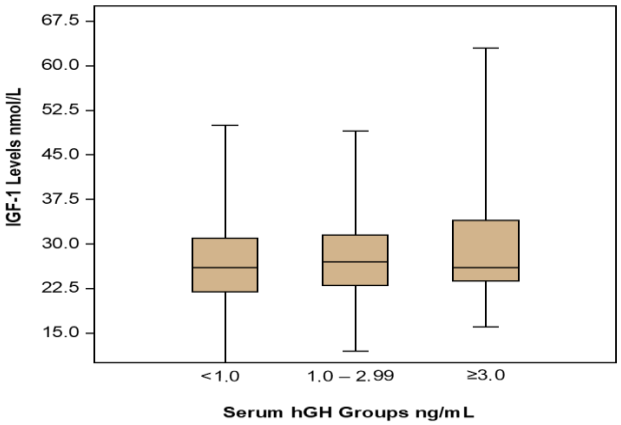

Figure 3a Applying data from Table 3a, this box-plot shows that IGF-1 levels do not differ across the 3 hGH categories.

Table 3b Shows the summary statistics of IGFBP-3 levels across the 3 categories of hGH levels noting the vast majority are measured in the very low hGH group, <1.0 ng/mL.

IGFBP-3 Levels nmol/L vs hGH Groups ng/mL - Summary Statistics			
GH Groups	<1.0 ng/mL	1.0 – 2.99 ng/mL	≥3.0 ng/mL
N	297	61	50
Min	59	66	108
Max	246	243	253
Sum	49619	9773	8443
Mean	167.07	160.21	168.86
Std. error	1.64	4.48	4.56
Variance	800.84	1225.24	1038.90
Stand. dev	28.30	35.00	32.23
Median	164	163	156.5
25th centile	150	137	147.25
75th centile	184.5	178	188.25
Skewness	-0.02	-0.05	0.85
Kurtosis	0.80	0.32	0.35
Geom. mean	164.52	156.13	166.04
Coeff. var	16.94	21.85	19.09

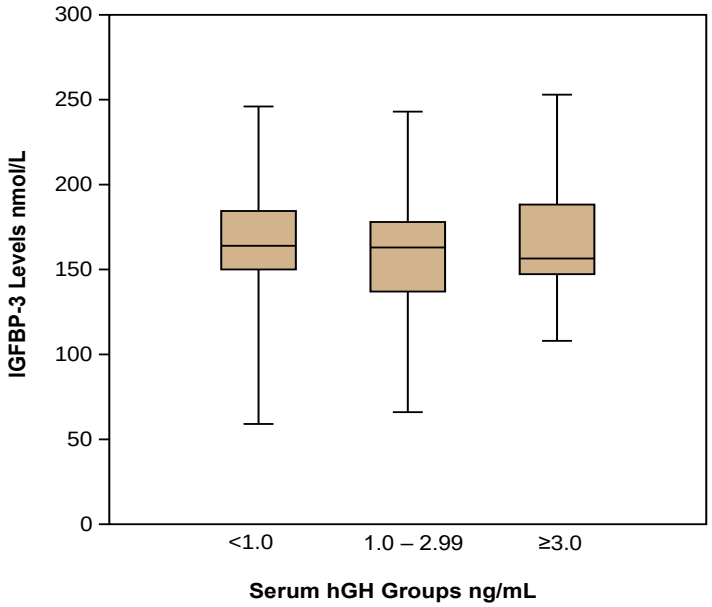

Figure 3b Applying data from Table 3b, this box-plot shows that IGFBP-3 levels do not differ across the 3 hGH categories.

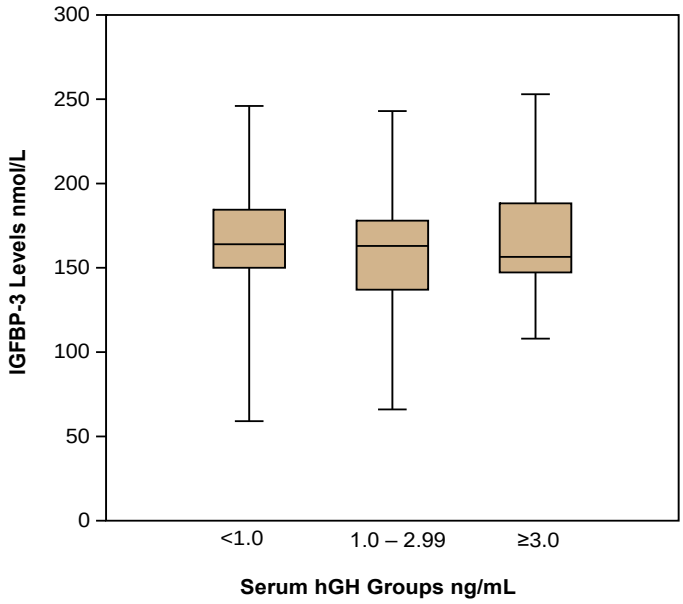

Table 3c Shows the summary statistics of the IGF Ratio of IGFBP-3/ IGF-1 across 3 categories (normal ≤5.0, elevated 5.1 to 7.0, highly elevated ≥7.0) noting that the vast majority of hGH levels are situated above the upper limit of the normal range.

IGF Ratio vs hGH levels ng/mL - Summary Statistics			
IGFBP-3/ IGF-1 Ratio	≤5.0	5.1 – 7.0	>7.0
N	66	226	116
Min	0.1	0.03	0.03
Max	12.75	20.63	17.03
Sum	102.72	297.375	105.34
Mean	1.56	1.32	0.91
Std. error	0.36	0.17	0.18
Variance	8.46	6.63	3.96
Stand. dev	2.91	2.57	1.99
Median	0.36	0.30	0.28
25^{th} centile	0.1	0.1	0.1
75^{th} centile	1.6	1.4	0.7
Skewness	2.95	4.37	5.36
Kurtosis	8.23	24.87	37.99
Geom. mean	0.51	0.44	0.33
Coeff. var	186.94	195.63	219.05

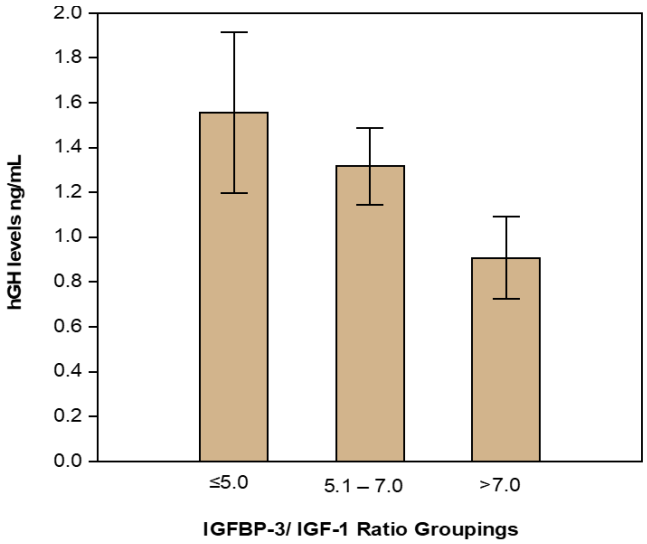

Figure 3c Applying data from Table 3c, this bar-chart shows that hGH levels apparently fall inversely to the category of IGF ratio with the lowest hGH levels associated with the highest IGF ratio. However, this apparent feature is not statistically significant in this study group as explained in the main text.

3.3. hGH vs Age

Table 4a shows the distribution of hGH levels across the four age groups ranging from <35 years to ≥45 years, with the vast majority of cases being women in the youngest grouping (232/408; 56.9%). Whilst this uneven distribution of women among the age groups gives an apparent higher mean level of hGH for the oldest women (Figure 4a), the observation is not statistically significant and is corrected by examining the box-plot of Figure 4b which provides a clearer perspective on the distortions in the older age groups which had fewer case numbers; as low as 11 cases in the ≥45 year grouping. The result is clearer in Figure 4c which provides a bar-chart on the larger study group of 945 women which now comprises 22 cases in the oldest group. Table 3b provides the summary statistics for the 945-study group and reverses the apparent observation on the oldest group. Furthermore, it should be noted that the hGH levels in all groups are concentrated in the low to very low category, hence the apparent differences among the groups carries little importance. In conclusion, there are no significant age-related differences in the hGH levels among the categorized age groups.

Table 4a Distribution of hGH levels among the 4 age categories of the 408 ART-naïve women, noting fewer numbers in the higher age group.

hGH Levels ng/mL vs Age Groups - Summary Statistics for Study Group 408				
Age groups	<35 years	35 – 39 years	40 – 44 years	≥45 years
N	232	101	64	11
Min	0.03	0.03	0.1	0.04
Max	20.63	17.03	5.28	7.54
Sum	286.3	144.0	54.7	20.5
Mean	1.2	1.4	0.9	1.9
Std. error	0.2	0.3	0.2	0.7
Variance	7.2	6.8	1.7	6.0
Stand. dev	2.7	2.6	1.3	2.5
Median	0.25	0.45	0.28	0.57
25^{th} centile	0.11	0.16	0.11	0.1
75^{th} centile	1.04	1.535	0.86	4.02
Skewness	4.37	3.67	2.32	1.54
Kurtosis	23.07	16.16	4.61	1.67
Geom. mean	0.38	0.52	0.38	0.65
Coeff. var	216.82	183.08	151.06	131.62

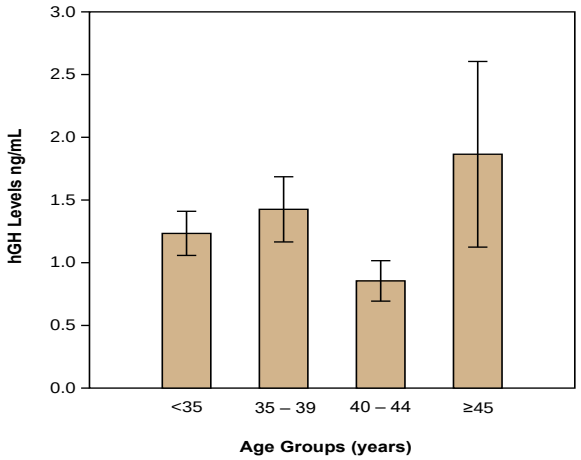

Figure 4a Bar-charts showing the hGH levels across the 4 age categories among the 408 ART-naïve women**.**

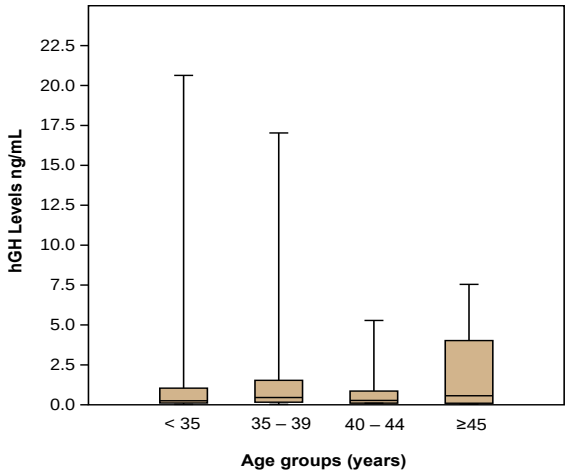

Figure 4b Box-plots showing the hGH levels across the 4 age categories among the 408 ART-naïve women.

Table 4b Distribution of hGH levels among the 4 age categories of the 945 ART-interval women, noting larger numbers in the higher age groups**.**

hGH levels ng/mL vs Age Groups - Summary Statistics for Study Group 945				
Age groups	<35 years	35 – 39 years	40 – 44 years	≥45 years
N	547	237	139	22
Min	0.03	0.03	0.03	0.1
Max	23.32	18.71	17.03	4.32
Sum	887.09	300.63	190.63	11.27
Mean	1.62	1.27	1.37	0.51
Std. error	0.12	0.16	0.22	0.20
Variance	8.07	5.83	6.57	0.84
Stand. dev	2.84	2.41	2.56	0.91
Median	0.46	0.34	0.36	0.2
25th centile	0.16	0.13	0.11	0.1325
75th centile	1.74	1.075	1.28	0.4325
Skewness	3.70	3.74	3.56	3.79
Kurtosis	17.80	17.46	15.11	15.63
Geom. mean	0.56	0.44	0.47	0.27
Coeff. var	175.15	190.28	186.88	178.60

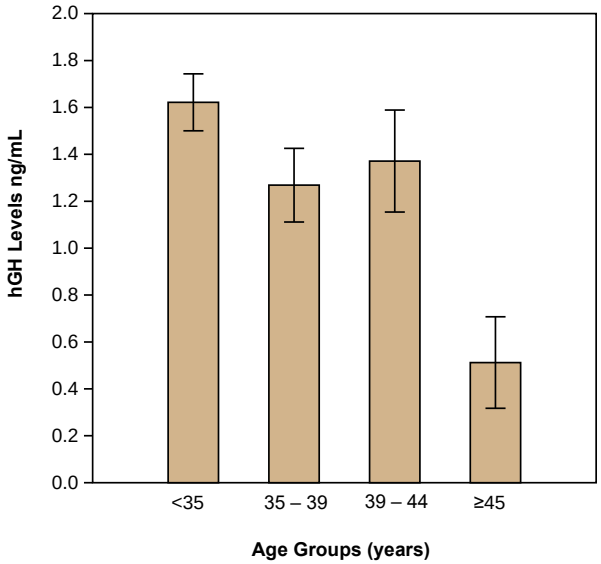

Figure 4b Bar-charts showing the hGH levels across the 4 age categories among the 945 ART-interval women. The larger numbers contained in the 40-44 year age group indicates that the group has the same hGH levels as the younger 2 groups. The oldest group ≥45 years appears to show lower hGH levels, but the number of women in this group (n=22) remains low and the variance is wide, hence the data is not significantly different.

3.4. hGH vs BMI

The influence of BMI on hGH levels is shown in the bar-chart of Figure 5a showing an apparent inverse relationship indicating that higher BMI groups display lower hGH. However, reference to the summary statistics show in Table 5 indicates that the median levels across the four BMI groups is well down amongst the very lowest hGH assay levels (0.21 to 0.37 ng/mL) such that the differences are entirely unimportant and not significant. This is clarified in the box-plot of Figure 5b where the variance of hGH for the younger groups is seen to be very wide up to 8.72 ng/mL in the BMI group with hGH ranging from 0.03 – 20.63 ng/mL. Therefore, there are no significant differences in hGH levels across the BMI groups.

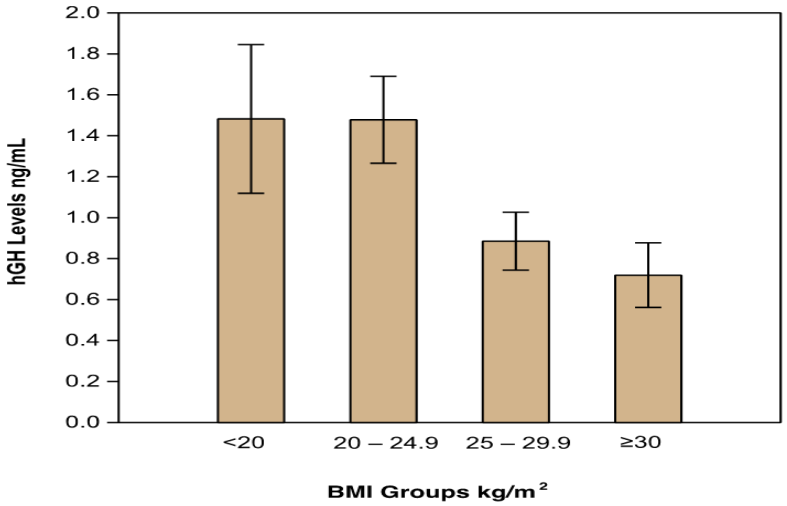

Figure 5a Bar-chart depicting hGH levels across 4 BMI categories. Although there is an apparent inverse relationship with lower hGH levels noted in the women with higher BMI status, the data in Table 5 and Figure 5b shows that this is not significant.

Table 5 Summary statistics showing the range of hGH levels among each of 4 BMI categories. Whilst the majority of women are in the normal BMI group of 20-24.9 kg/m^2, the hGH levels are not significantly different among the groups.

hGH levels ng/mL vs BMI Groups kg/m^2- Summary Statistics				
BMI Groups	<20	20 – 24.9	25 – 29.9	≥30
N	65	193	96	54
Min	0.07	0.03	0.03	0.03
Max	13.58	20.63	8.27	5.44
Sum	96.34	285.27	84.984	38.84
Mean	1.48	1.48	0.89	0.72
Std. error	0.36	0.21	0.14	0.16
Variance	8.57	8.72	1.92	1.34
Stand. dev	2.93	2.95	1.38	1.16
Median	0.37	0.33	0.25	0.21
25th centile	0.14	0.15	0.1	0.1
75th centile	1.03	1.47	1.2	0.865
Skewness	2.94	4.01	2.82	2.71
Kurtosis	8.25	19.26	9.53	7.50
Geom. mean	0.46	0.48	0.36	0.30
Coeff. var	197.54	199.77	156.33	160.72

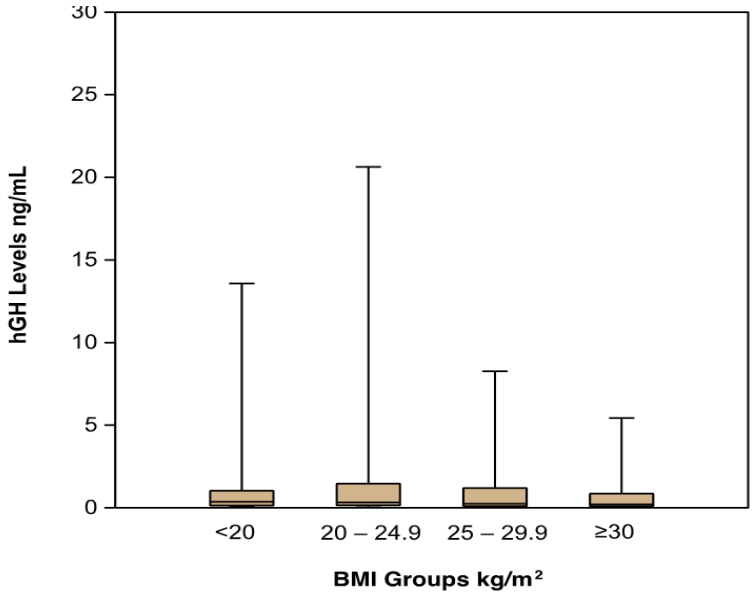

Figure 5b Box-plot depicting hGH levels across 4 BMI categories. Although there was an apparent inverse relationship with lower hGH levels noted in the women with higher BMI status in Figure 5a, this figure, also derived from the data in Table 5, better depicts the true, insignificant differences among the groups.

3.5. hGH vs Stature

As stature is the most relevant clinical parameter on which the diagnosis of GHD is suspected, we examined this parameter separately from the BMI consideration, albeit stature is a component in the BMI formulation. This is displayed in the statistical summary of Table 6 along with the derived Figure 6 where it can readily be seen that Stature is exactly the same across the very low, low and normal hGH groups. Table 6 shows that both the means of stature (at 1.64–1.67 meters) are very similar to the medians of stature (at 1.6–1.7 meters). Furthermore, the ranges (1.5–1.8

meters) were exactly the same in each hGH group. Clearly there are no significant differences in hGH levels related to the woman's stature.

Table 6 Summary statistics showing the full range of heights (stature) measured in each of the hGH groupings; namely very low hGH at <1.0ng/mL, low at 1.0 to 2.99 ng/mL and normal at ≥3.0 ng/mL.

hGH Levels ng/ml vs Stature (meters) - Summary Statistics			
GH Groups	<1.0 ng/ml	1.0 – 2.99 ng/mL	≥3.0 ng/mL
N	297	61	50
Min	1.5	1.5	1.5
Max	1.8	1.8	1.8
Sum	491.2	100.3	83.7
Mean	1.65	1.64	1.67
Std. error	0.00	0.01	0.01
Variance	0.01	0.01	0.00
Stand. dev	0.08	0.07	0.07
Median	1.7	1.6	1.7
25th centile	1.6	1.6	1.6
75th centile	1.7	1.7	1.7
Skewness	-0.07	0.21	-0.36
Kurtosis	-0.41	-0.11	0.31
Geom. mean	1.65	1.64	1.67
Coeff. var	4.76	4.38	4.15

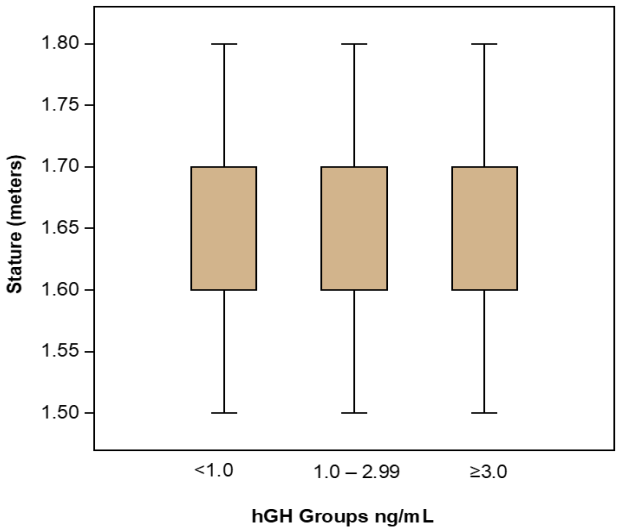

Figure 6 Box-plot display of stature in each of the 3 hGH categories summarized in Table 6. There are no differences in the stature profiles of the women comprising the normal, low or very low hGH groupings.

3.6. hGH vs AMH

One of the prognostic measures for clinical outcomes in ART is the AMH level as a measure of ovarian reserve, particularly when the level is very low at <8 pmol/L, which matches the PIVET Algorithm groups D and E (Table 1). The hGH levels across the 7 AMH groups is shown as summary statistics in Table 6a and depicted as a bar-chart in Figure 6a as well as a box-plot in Figure 6b. Whilst the bar-chart implies some variations in hGH levels among the AMH groups, the box-plot shows that the clustering effect in the very low hGH group makes the apparent findings irrelevant. The data shown in Table 6 indicates that the hGH levels in each AMH grouping range very widely, the greatest being from 0.03–20.63ng/mL and the lowest ranges being in disparate AMH groups A and E (from very low to around 5 ng/mL). The statistical assessment indicates no significant differences of hGH levels among the AFC groups.

Table 6 Distribution of hGH levels of ART-naïve women across 7 AMH groupings from the PIVET FSH-dosing algorithm.

hGH levels ng/mL vs AMH groups							
AMH Groups pmol/L	A++ ≥30	A+ 25 – 29.9	A 20 – 24.9	B 13 – 19	C 9 – 12	D 5 – 8	E <5.0
N	79	43	36	62	73	67	48
Min	0.03	0.03	0.1	0.1	0.03	0.1	0.04
Max	18.71	11.76	5.29	13.58	20.63	12.54	4.63
Sum	108.27	65.39	32.19	54.38	98.53	106.53	40.14
Mean	1.37	1.52	0.89	0.88	1.35	1.59	0.84
Std. error	0.31	0.39	0.22	0.24	0.40	0.31	0.17
Variance	7.40	6.47	1.77	3.67	11.85	6.55	1.35
Stand. dev	2.72	2.54	1.33	1.91	3.44	2.56	1.16
Median	0.27	0.36	0.23	0.25	0.31	0.44	0.33
25th centile	0.1	0.1	0.1025	0.1	0.125	0.17	0.11
75th centile	1.42	1.58	1.3075	0.865	0.745	1.78	1.17
Skewness	4.08	2.46	2.16	5.23	4.39	2.52	2.14
Kurtosis	21.44	6.28	4.18	32.54	20.09	6.64	4.18
Geom. mean	0.42	0.47	0.37	0.34	0.39	0.57	0.37
Coeff. var	198.5	167.2	148.7	218.3	255.1	160.9	138.9

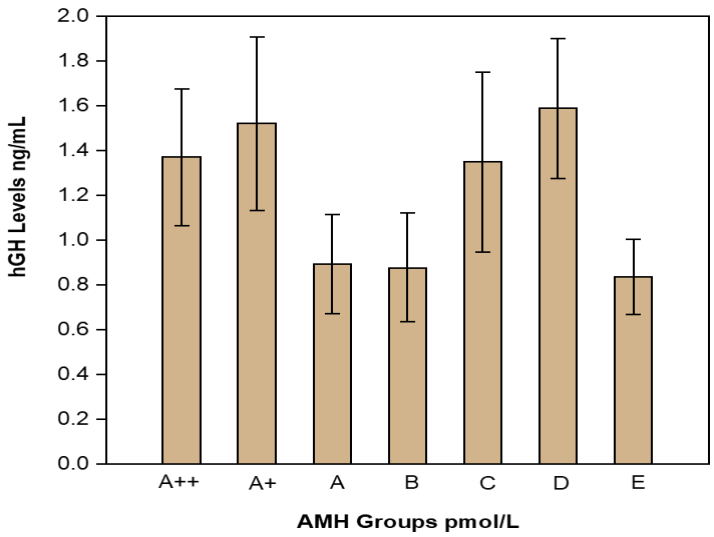

Figure 6a Bar-chart depicting hGH levels across the 7 AMH groups of the PIVET FSH-dosing algorithm. The apparent differences are not significant as the majority of hGH levels are within the low <3.0 ng/mL and very low <1.0 ng/mL ranges with very wide variance.

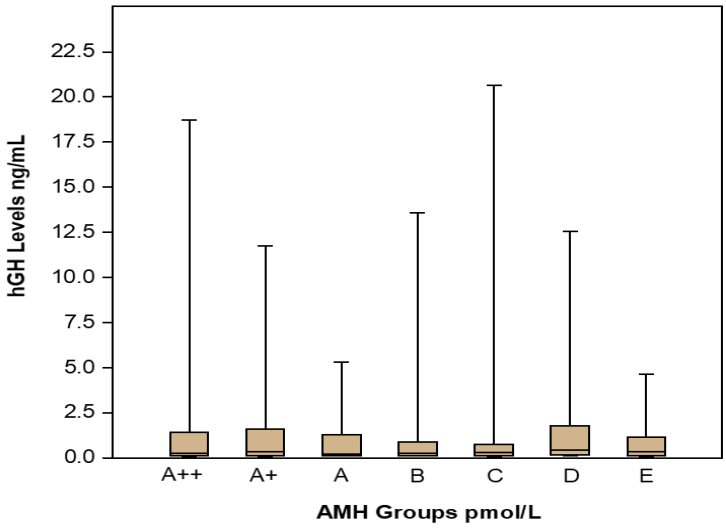

Figure 6b Box-plot depicting hGH levels across the 7 AMH groups of the PIVET FSH-dosing algorithm. The apparent differences noted in Figure 6a, are more clearly depicted and seen to be not significant as the majority of hGH levels are within the low <3.0 ng/mL and very low <1.0 ng/mL ranges and with very wide variance**.**

3.7. hGH vs AFC

As another parameter with prognostic value in ART, the AFC test of ovarian reserve was also analysed with respect to hGH levels. Although the number of cases in each AFC group shown in Table 7 is quite different from the AMH groups presented in Table 6, the profiles are quite similar. Figure 7a shows the profile as bar-charts which imply some variability of hGH among the AFC groups. However, the box-plot depiction in Figure 7b reveals that the majority of hGH levels are recorded in the very low category, and variations within this group are actually not particularly relevant. Furthermore, Table 7 shows the variance in several disparate groups such as A++, B and C is quite large (up to 11.84 ng/mL in group B) and hGH levels can range widely (from very low in all groups to a high of 20.63 ng/mL in group B). The statistical evaluation shows no significant differences of hGH levels among the AFC groups.

Table 7 Distribution of hGH levels of ART-naïve women across 7 AMH groupings from the PIVET FSH-dosing algorithm.

hGH levels ng/mL vs AFC groups							
AFC Groups antral follicles	A++ ≥40	A+ 30 – 39	A 20 – 29	B 13 – 19	C 9 – 12	D 5 – 8	E ≤4
N	18	19	52	135	90	71	23
Min	0.1	0.05	0.03	0.05	0.03	0.03	0.07
Max	8.27	7.17	4.77	20.63	12.75	6.19	4.63
Sum	24.46	24.61	43.39	214.294	112.511	60.58	25.59
Mean	1.36	1.30	0.83	1.59	1.25	0.85	1.11
Std. error	0.58	0.45	0.17	0.30	0.25	0.16	0.28
Variance	6.06	3.80	1.48	11.84	5.42	1.80	1.80
Stand. dev	2.46	1.95	1.22	3.44	2.33	1.34	1.34
Median	0.195	0.44	0.27	0.26	0.2905	0.34	0.56
25th centile	0.1075	0.11	0.1175	0.13	0.11	0.11	0.19
75th centile	1.145	1.26	0.8225	1.46	1.545	0.88	1.85
Skewness	2.07	2.05	2.01	3.68	3.40	2.63	1.62
Kurtosis	3.23	3.84	2.99	14.64	12.78	6.32	1.96
Geom. mean	0.37	0.48	0.36	0.44	0.43	0.37	0.53
Coeff. var	181.09	150.48	145.73	216.74	186.16	157.36	120.74

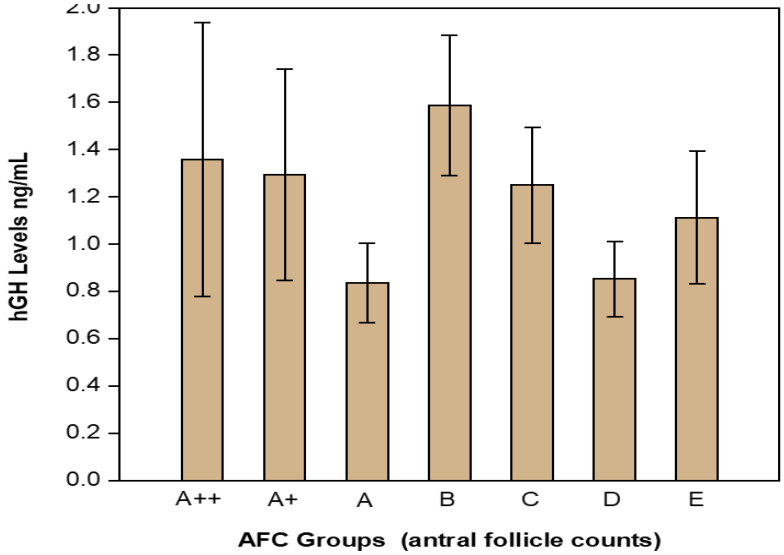

Figure 6a Bar-chart depicting hGH levels across the 7 AFC groups of the PIVET FSH-dosing algorithm. The apparent differences are not significant as the majority of hGH levels are within the low <3.0 ng/mL and very low <1.0 ng/mL ranges and with very wide variance.

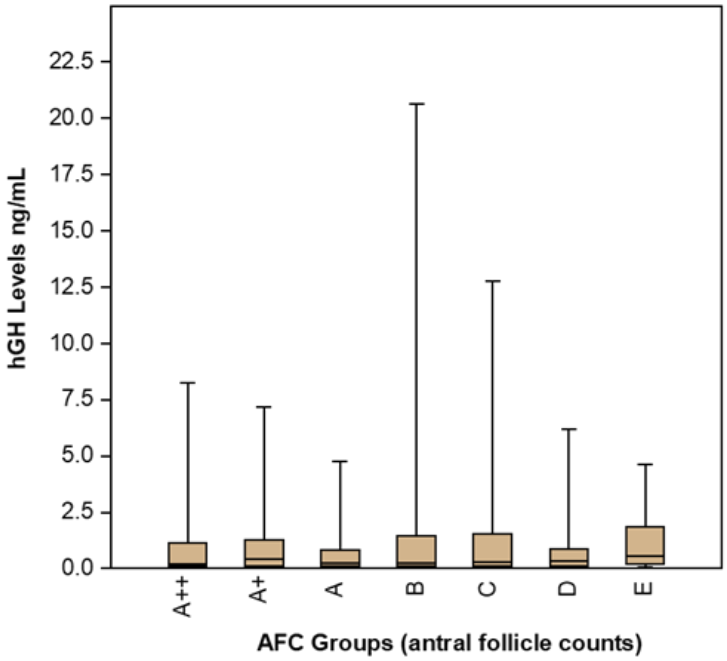

Figure 7b Box-plot depicting hGH levels across the 7 AFC groups of the PIVET FSH-dosing algorithm. The apparent differences noted in Figure 7a, are more clearly depicted and seen to be not significant as the majority of hGH levels are within the low <3.0 ng/mL and very low <1.0 ng/mL ranges and with very wide variance.

4. Discussion

Although the evolution of IVF over the past 42 years has seen a spectacular world-wide explosion in the number of children born from these and the associated reproductive technologies, there are current limitations which have not been met. These have been defined recently by the Annual Capri Workshop Group [3] which indicates that the age-related decline in oocyte quality is the main limitation with no clear advance pending in this aspect. There are other limitations also, with recent data showing that ART success rates, measured as live births achieved from the perspective of treatments initiated, are actually declining over the past two to three years, with wide variations among countries around the world [20]. It is hoped that adjuvants may improve the outcomes for some cases labelled as poor prognosis

couples, but most studies have been empirical, rather than structured on clear scientific evaluation. Only a couple of other studies have focussed on IGF-1 relevance in this area [21,22], so far without clear definition.

This study is the third in a series directed towards the development of a clear identification of the woman undergoing ART management who may be diagnosed as AGHD and is likely to benefit from the use of GH as an adjuvant. This requires an understanding of the factors impacting on the IGF/GH axis [23,24] and the limitations of measurement of the various parameters applied in the identification of women categorised as having a poor prognosis. The latter can be due to a range of fertility factors related to ovarian function, endometrial development, embryo quality as well as unclear reasons include lifestyle factors of both partners; but female age is the dominant consideration. These factors were detailed in our second study [12], along with the consideration of using any of the more than 50 adjuvants reported, but which so far have not reached high-level evidence-based confirmation [5,7]. The only one with moderate support is that of GH [4,25], but identification of the individual likely to benefit requires an understanding of the IGF-1/GH axis and the complex interaction of the relevant impacting factors.

This third study is therefore designed to answer the question of the relevance of directly assaying the hGH level in a morning serum sample carried out in the early follicular phase at the usual working time for other blood tests undertaken for fertility assessment and management. The study was performed with the current knowledge that hGH release from the human pituitary varies between males and females [23,26]. In males, adult patterning is established by 18–25 years with dominant peaks >3.0 ng/mL occurring during sleep, mostly a single pulse between midnight and 4:00 am, and with a rapid return to basal levels within 30 minutes of a pulse. Thereafter with increasing age, there is a progressive decline in GH pulses with total hGH release halving every 7-years. In the male, measurements of hGH between 8:00 am and midday are invariably less than 1 ng/mL mostly under 0.1 ng/mL. However, in the female the pattern diverges from the male with increased peak amplitude, frequency of pulses and total hGH release. The age-related decline seen in adult males does not commence in females until after the menopause. However, there are variations during the menstrual cycle with lowest GH pulse amplitude and frequency in the early follicular phase; highest GH pulse amplitude and frequency in the late follicular phase, directly related to rising estradiol (E2) and peak E2 levels; and a reduction in pulse frequency throughout the luteal phase. It appears that hGH release is directly responding to underlying E2 levels, irrespective of the presence of progesterone. Furthermore, the female displays specific GH effects during pregnancy when maternal pituitary hGH is suppressed by placental hGH feedback. Immediately following birth, both pituitary-derived hGH and maternal liver-derived IGF-1 rise dramatically, being involved in maintenance of the mammary glands, preventing involution of the breasts and the promotion of milk fat content.

The specific findings of the analyses performed in this third study can be summarised as follows:

1. The vast majority, 73% of hGH levels on these early follicular phase morning serum samples are very low at <1.0 ng/mL and 22% are extremely low <0.1 ng/mL, close to the detection level of the assay (0.03 ng/mL).
2. Only 12% of hGH levels are recorded in the normal range ≥3.0 ng/mL.
3. Although IGF-1 levels are regarded as a screening test for AGHD, our studies showed no correlation between hGH levels and the entire range of IGF-1 levels. Similarly, there was no correlation between hGH and IGFBP-3 levels. Sometimes regarded as a more sensitive test for detecting AGHD, there was no significant association with IGF Ratios, albeit there was an apparent inverse trend. In both childhood and adult GHD a high IGF Ratio is seen >5.0.
4. Across the entire age range, the hGH levels were not statistically different. As there were few cases ≥45 years in the ART-naïve study group (11/408), the study was expanded to include the ART-interval group (22/945). This clearly showed no significant differences in hGH levels across the entire age ranges, albeit the oldest group had the lowest hGH readings.
5. An examination of both BMI status and the single parameter of Stature showed no differences in hGH levels across the full ranges.
6. Applying the 7 groupings used in the PIVET dosing algorithms for FSH, the was no variation in hGH levels for either AMH or AFC.

This study confirms that the measurement of hGH in morning samples of serum collected in the early follicular phase (Day-5±1) detects only 12% of women with normal GH status. To determine the true status in the remaining 88% of cases would require either undertaking an integrated GH-secretion test over 24 hours or applying a provocative stimulation test.

It is known that GH secretion is not only subject to nocturnal pulses during slow-wave sleep, but they can be induced by hunger and even affected by food composition. Stresses included certain types of exercise can induce GH pulses which

may be suppressed in those persons with disturbed sleep and are usually attenuated in obese persons. We have already indicated that E2 stimulates the pulses, but so can testosterone, in both men and women. Low testosterone in men correlates with AGHD. In both genders, any pituitary disorder (low TSH, low MSH, low ACTH and low pro-opiomelanocortin; POMC) all associate with AGHD. GH is under the stimulatory control of GHRH and is inhibited by Somatostatin, the GH-inhibitory hormone; GHIH. Both of these regulators are intimately influenced by hypothalamic neuromodulators (adrenaline, 5-HT and Acetylcholine) as well as an array of external neuropeptides including ghrelin (from the stomach) and galanin from the brain and pancreas. In fact, the natural age-related decline in hGH activity is a net effect directly caused by declining GHRH secretion and a concomitant rise in somatostatin.

The secretion of hGH from the pituitary is known to occur in two different molecular forms with a molecular mass of 20kDa (more than 90% of circulating hGH) and a 22kDa isoform is co-secreted [17,18]. The 22kDa form carries 191 amino acids while the 20kDa variant carries 15 fewer amino acid residues, namely 186, losing numbers 32 – 46 from the larger molecule. However, the biological activity of both forms is thought to be comparable. The Elecsys electrochemiluminescence immunoassay applied in this study detects both isoforms as a single hGH entity and likely includes all the GH fragments recently reported to be part of the hGH complex [17], namely GH 1–43, GH 30–54, GH 44–191, GH 108–129, GH 147–191 and GH 177-191. It appears that each of these GH fragments has specific biological activities which may be pro- or contra- to the general bioactivity of the main hGH complex. In blood, hGH is bound to growth hormone binding protein (GHBP) which acts to dampen the oscillations arising from the pulsatile secretions of hGH which can vary from 5–45 ng/mL, lasting from 10 to 30 minutes, there-after returning to basal levels by 30 minutes.

The gold standard for diagnosing AGHD requires an Insulin Tolerance Test (ITT) demonstrating a peak hGH response of ≥3 ng/mL (NICE Guidelines and Endocrine Society). The ITT requires close supervision by an Endocrinologist within setting enabling the management of profound hypoglycemic reactions. However other, less dangerous tests may suffice including a clonidine challenge (binds to α_2 adrenergic receptors) or glucagon stimulation (induces insulin release) or a GHRH-arginine test (by reducing somatostatin)

5. Conclusion

This third study adds to our first 2 studies assessing the IGF Profiles in women presenting for ART, which we showed can be markedly influenced by the woman's age, but not other clinical parameters nor of the tests for ovarian reserve, namely AMH and AFC. It is expected that the IGF profile might help to screen women with suspected AGHD who could benefit by provocative testing such as the ITT. If so, the failure to generate levels of hGH ≥3.0 ng/mL would define a group of women who should benefit from adjuvant GH therapy. Whilst we would recommend undertaking IGF profile screening on all women presenting for ART, we would recognise that adding hGH screening would have limited benefit. Certainly the 12% of women with normal hGH levels should not be considered for GH adjuvant therapy, but we would caution that the remaining 88%, even the 73% with very low hGH levels, may not benefit. That group of cases could "trial" standard ART treatment and, if thereafter classified as poor prognosis, should undergo a provocative test for underlying AGHD. In Australia, if this is reported by an Endocrinologist, the Medicare system will cover the costs of the GH adjuvant, even enabling long-term therapeutic use.

Compliance with ethical standards

Acknowledgments

We are grateful for the close working relationship between PIVET Medical Centre and CLINIPATH Pathology which carried out the assays involved in the IGF profile and is also accredited by NATA.

Disclosure of conflict of interest

The entire project has been funded internally at PIVET without any external or commercial contributions. The authors declare no conflict of interest.

Statement of ethical approval

Reporting of the data was approved under Curtin University Human Ethics Committee approval no. RD_25–10 general approval for retrospective data analysis in 2010, updated in 2015, and again further updated recently, in August 2020.

Statement of informed consent

PIVET is accredited with both the self-regulatory National Australian Reproductive Technology Committee (RTAC) as well as the Reproductive Technology Council (RTC) of Western Australia. Consent forms received approval under both regulatory bodies. The assay laboratory is accredited on an annual basis by the National Australian Testing Authority (NATA).

Author Contributions

The study was conceived by PIVET Medical Director JLY who established the data-base at PIVET Medical Centre with the assistance of IT Consultant and data manager PMH. The first data extractions were undertaken by SZ who was on a sabbatical study period from her facility in Malaysia to which she has now returned. The Data has been further analysed by PMH supported by MDKN who has a managerial role for the Laboratory Assays at PIVET. All authors have assisted with the data analyses as well as the preparation of the Tables and Figures. The manuscript was written by JLY and each of the authors have read and agreed to its content.

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