Serum IGF-1 concentrations following pituitary desensitization do not predict the ovarian response to gonadotrophin stimulation prior to IVF

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BACKGROUND: Insulin-like growth factor-1 (IGF-1) is known to play a role in ovarian follicular development augmenting the action of FSH. Low intrafollicular concentrations have been detected in women who respond poorly to gonadotrophins. This study addresses the relationship between serum IGF-1 levels following pituitary desensitization and ovarian response to gonadotrophin stimulation. METHODS: This is a case–control study of 78 patients undergoing IVF–embryo transfer treatment. Thirty-nine strictly-defined poor responder patients requiring 50 or more ampoules (75 IU FSH) to reach oocyte retrieval were compared with 39 age-matched normal responders, requiring fewer than 50 ampoules. IGF-1 concentrations were determined by extraction radioimmunoassay on serum samples obtained after pituitary desensitization but prior to gonadotrophin stimulation. RESULTS: Despite highly significant differences in measures of ovarian response between groups, the mean serum IGF-1 concentration was not statistically significantly different between poor and normal responders (31.5 nmol/l [95% confidence interval (CI) 28.5–34.5] versus 34.5 nmol/l (95% CI 31.8–37.2)) respectively. No correlation between oocyte number or total gonadotrophin used and serum IGF-1 concentration was observed. CONCLUSION: Whilst IGF-1 influences ovarian follicular development this study suggests that serum IGF-1 does not predict ovarian response and does not differentiate between critically-defined poor and normal responders.

Key words: gonadotrophins/IGF-1/IVF/poor ovarian response

Introduction

Poor ovarian response, in assisted conception, presents a significant problem by reducing the prospect of successful treatment (Keay et al., 1997). Predicting it is difficult (Keay, 2002) as ovarian ageing may occur in advance and independently of chronological age (Akande et al., 2002). Basal serum FSH is the most widely used test of ovarian reserve with elevated levels being strongly associated with poor response (Akande et al., 2002). However, there remains a group of young women with apparently normal ovarian reserve who do not respond well.

The paracrine control of multiple follicle development is not fully understood but the detection of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) receptors on granulosa cell infers a direct ovarian action (Gates et al., 1987; Carlsson et al., 1992). GH stimulates hepatic IGF-1 synthesis and determines systemic concentrations. This is important as intrafollicular IGF-1, in women, is derived from the circulation rather than being produced locally (Pellegrini et al., 1995). In vitro, IGF-1 acts synergistically with FSH, (Adashi et al., 1985) enhancing granulosa cell proliferation and stimulating aromatase enzyme activity. Conversely, IGF-1 gene-deleted mice have impaired fertility with follicles arrested at the preantral stage (Kadakia et al., 2001) and reduced FSH receptor expression (Zhou et al., 1997) leading to reduced responsiveness to administered FSH.

In vivo data supports the assertion that GH/IGF-1 influences ovarian responsiveness. Total daily GH secretion predicts ovarian response in both natural (Potashnik et al., 1995) and controlled ovarian stimulation cycles (Stone and Marrs, 1992). Furthermore, poor responders demonstrate lower intrafollicular IGF-1 concentrations (Oosterhuis et al., 1998) suggesting the peptides’ availability may limit the follicles capacity to respond to FSH.

The few studies reporting the possible effect of pituitary desensitization on serum IGF-1 levels have given inconsistent results. A study of clomiphene-resistant women with polycystic ovary syndrome (PCOS) (a group known to have disturbed GH kinetics and lower GH reserve) showed no significant reduction in IGF-1 levels after down-regulation (Homburg et al., 1995). In contrast we observed a significant fall in IGF-1 in an unselected group of women undergoing controlled ovarian stimulation prior to IVF–embryo transfer.
with apparently normal pituitary function (Gadd et al., 1991). The decrease seen in serum IGF-1 levels following pituitary desensitization (Gadd et al., 1991) may therefore restrict the effectiveness of exogenous gonadotrophin in stimulating multiple follicle development in some women. Measuring overall GH secretion is complex and impractical outside a research setting. Furthermore, a single sample, because of pulsatile GH release, may be unrepresentative of an individual's total GH secretion over 24 h. The aim of this study was to determine if serum IGF-1, a stable reflection of overall GH secretion, following desensitization discriminated between two critically defined response groups and correlated with measures of ovarian response.

Materials and methods

Defining response groups

The inverse relationship between the total gonadotrophin used and IVF outcome is well recognized but studies report different thresholds at which outcome is affected (Stadtmauer et al., 1994). As a prelude to this basal IGF-1 study we determined IVF outcome in our own programme in relation to the total gonadotrophin used in 1200 IVF cycles. Factors known to adversely affect embryo implantation (female age >39 years, male factor infertility, hydrosalpinx or uterine abnormality) were excluded. Using receiver-operator characteristic (ROC) curves we demonstrated women requiring 50 or more ampoules (containing 75 IU FSH) had a significantly reduced clinical pregnancy and embryo implantation rate (10.6 versus 26.5% and 5.9 versus 14.0% respectively) compared with fewer than 50 ampoules (Keay et al., 1999). The poor and normal response groups were selected using this definition.

Patients and protocols

A consecutive series of 39 women from the University of Bristol IVF programme reaching oocyte retrieval and requiring a total of more than 50 ampoules of gonadotrophin (75 IU FSH), were age-matched (to within 2 years) with 39 women reaching oocyte retrieval requiring 50 or fewer ampoules. Women with PCOS or diabetes mellitus were excluded. The study was conducted within the guidelines of the local research ethics committee and women gave written informed consent for all clinical procedures. Two samples from the poor responder group were damaged leaving 37 for analysis. Power analysis, based on previous published data which demonstrated a 50% difference in intrafollicular IGF-1 levels between poor and normal responders, determined that 70 patients (35 in each arm) were required (for a type 1 error of 5% and a power of 95%) to detect a difference of 6 nmol/l between poor and normal responders.

Stimulation protocol

The stimulation protocol and laboratory methods have been described in detail previously (Keay et al., 1998). Briefly, pituitary desensitization was started in the mid-luteal phase of the cycle preceding IVF treatment, using intranasal buserelin acetate spray (Suprefact; Hoeschst, Hounslow, UK). A total of 600 mg daily in five divided doses, 100 mg at 4-h intervals during the day and 200 mg at bedtime. After 10 days of buserelin treatment, following the onset of menses, an early morning serum sample was assayed for estradiol (E2) to ensure adequate pituitary desensitization. Surplus serum was stored at -20°C for subsequent IGF-1 assay. Ovarian stimulation was begun by s.c. injection of urinary purified HMG (Metrodin HP; Serono, Welwyn Garden City, UK). In a first stimulation cycle with a normal basal FSH, 150 IU FSH daily was used initially, increased after 7 days if necessary. The stimulation dose was selected on an individual basis depending on previous ovarian response, age and basal FSH and increased to a maximum of 450 IU FSH. Ovarian follicular development was monitored by vaginal ultrasonography and serum E2 measurement. Oocyte retrieval was only undertaken if three or more follicles of 18 mm were recruited.

Fertilization was defined as normal by the development of two pronuclei and progressive cleavage up to the time of embryo transfer after 2–3 days. Implantation rates were defined by the proportion of individual embryos transferred resulting in a gestation sac including ectopic gestations (also including ectopic sacs without an evident fetal heartbeat). Clinical pregnancy was indicated by a gestation sac in which a fetal heartbeat could be seen on ultrasound examination 4 weeks after embryo transfer, the number of sacs, and pregnancy outcome.

Assays

IGF-1 assay

The methodology of the extraction radioimmunoassay has been previously detailed (Morrell et al., 1986) and was carried out after...
extracting the serum with acid-ethanol (Daughaday et al., 1980). During transport two samples from the poor response group were damaged leaving 37 samples to analyse. The intra- and interassay imprecision (coefficients of variation) of the IGF-1 assay were 3.5 and 7.5% respectively.

**Estradiol**

Serum E₂ was assayed using a radioimmunoassay (Delfia; Wallac, Milton Keynes, Bucks, UK). The intra- and interassay imprecision (coefficients of variation) of the E₂ assay were 7.1 and 9.7% respectively.

**FSH**

Serum FSH concentration was measured using a two-site immuno-fluorimetric method employing two monoclonal antibodies directed first against the β-subunit and then the α-subunit (Delfia; Wallac Oy, Turku, Finland). Interassay variation over the useful range was 4–6% and intra-assay variation <5%. The normal range found by us during the early follicular phase was 0.8–8.9 IU/l (95% limits) and these values and the assay performance were consistent with most laboratories participating in the UK Quality Assurance Scheme for Peptide Hormones.

**Analysis**

The Mann–Whitney U-test, Student’s t-test, χ²-test and Fisher’s exact test were used for comparison between groups where appropriate using the statistical package ARCUS Prostat (Medical Computers, Aughton, UK). The relationship between serum basal IGF-1 concentrations and parameters of ovarian response were assessed using the linear regression method. A P value of < 0.05 was considered statistically significant.

**Results**

Patient characteristics were similar in each response group with respect to infertility diagnosis, infertility duration and body mass index (BMI) but significantly more previously-poor responses were recorded in the poor responder group (Table I). Despite female age and basal serum FSH levels being similar, highly significant differences existed with respect to the number of oocytes retrieved, the total gonadotrophin used and peak serum E₂ achieved (Table II) between study groups. All poor responders required ovarian stimulation of at least 300 IU FSH daily. The difference in mean serum IGF-1 concentration between response groups was not statistically significant (Figure 1) (31.5 nmol/l [95% confidence interval (CI) 28.5–34.5] versus 34.5 nmol/l [95% CI 31.8–37.2] respectively, P = 0.10). No significant correlation was observed between the IGF-1 concentration following pituitary

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**Table II. Comparison of measures of ovarian response and clinical outcome in normal versus poor responders.** Values are means ± SD

<table>
<thead>
<tr>
<th></th>
<th>Normal responders (n = 39)</th>
<th>Poor responders (n = 37)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 8 serum E₂ (pmol/l)</td>
<td>1197 ± 1107</td>
<td>483 ± 576</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peak serum E₂ (pmol/l)</td>
<td>5230 ± 2382</td>
<td>3511 ± 1914</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>13.5 ± 2.3</td>
<td>16.1 ± 2.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oocyte number</td>
<td>9.3 ± 4.1</td>
<td>5.9 ± 2.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cycles fewer than five oocytes %</td>
<td>17.9 (7/39)</td>
<td>45.9 (17/37)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Number ampoules (75 IU FSH)</td>
<td>32.5 ± 9.1</td>
<td>65.4 ± 12.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ampoules/oocyte</td>
<td>4.6 ± 3.7</td>
<td>11.4 ± 7.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Embryos available</td>
<td>4.8 ± 2.6</td>
<td>3.3 ± 2.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>2.6 ± 0.9</td>
<td>2.4 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate %</td>
<td>56.4 ± 23.8</td>
<td>61.5 ± 24.6</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation rate %</td>
<td>10 (10/99)</td>
<td>5.5 (5/90)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy rate %</td>
<td>20.6 (8/39)</td>
<td>13.5 (5/37)</td>
<td>NS</td>
</tr>
<tr>
<td>Live birth rate %</td>
<td>20.6 (8/39)</td>
<td>8.1 (3/37)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mann-Whitney U-test, Student’s t-test and χ²-test used as appropriate. NS = not significant.

**Figure 1.** Comparison of basal serum IGF-1 concentrations between poor and normal responder groups. Difference between means P = 0.10.
desensitization and the number of oocytes retrieved (Figure 2) or the total gonadotrophin dose used (data not shown).

Discussion
We tested the hypothesis that low endogenous serum IGF-1 concentrations are associated with poor ovarian response, but rather we discovered serum IGF-1 levels following pituitary desensitization did not differentiate between the poor and normal responders despite significant differences in oocytes retrieved, peak serum E2 attained and total gonadotrophin dose required (see Figure 1). Furthermore, no correlation was observed between serum IGF-1 and the number of oocytes obtained or the total gonadotrophin dose used (see Figure 2). From this data we conclude that IGF-1 following pituitary desensitization is not sufficiently discriminatory to be of clinical use in predicting poor response. We did not specifically investigate whether measuring basal serum IGF-1 in spontaneous menstrual cycles would identify potential poor responders but from our observations it appears unlikely. However, formal testing would be required to fully substantiate this.

We studied IGF-1 but IGF-2, in addition to IGF-1, has recently been shown to promote non-human primate granulosa cell steroidogenesis and vascular endothelial growth factor (VEGF) production from mature preovulatory follicles (Martinez-Chequer et al., 2003) albeit at a lower rate than IGF-1. Intraovarian regulation of IGF-1, its binding proteins and IGF-2 are highly complex (Jones and Clemmons, 1995) and investigation of IGF-2 and the IGF-binding proteins in relation to ovarian responsiveness is warranted in future prospective studies.

Provocative stimulation testing has been used to identify relative GH deficiency (Menashe et al., 1990). Rising serum GH levels following clonidine administration were associated with a good response to stimulation whereas static GH levels indicated a high chance of poor response. A further study demonstrated GH co-treatment improved subsequent ovarian response in clonidine-negative patients (Blumenfield et al., 1991). Dynamic testing is involved and the advantage of a simple single serum test, as we investigated, is clear.

GH receptor knockout mouse studies demonstrated significantly reduced ovulation rates and a 3-fold reduction in response to exogenous gonadotrophin compared with wild type (Bachelor et al., 2002). Interestingly the depleted numbers of antral follicles observed were not reversed by the addition of recombinant IGF-1 suggesting an additional direct GH effect on ovarian follicles, independent of IGF-1.

Including synthetic glucocorticoids, potent inducers of GH (and consequently IGF-1) secretion (Miell et al., 1993) within a standard regime reduced the incidence of poor ovarian response compared with placebo (Keay et al., 2001). This approach may overtreat some women whose endogenous GH/IGF-1 secretion is already optimal but would minimize the risk of a poor response being due to inadequate stimulation of the somatotropic axis. The precise mechanism of action of glucocorticoids on ovarian response is not known and the effect of dexamethasone at cellular level may be mediated through a number of paracrine modulators (Keay, 2002). Although it has been suggested that low intraovarian IGF-1 levels may be a cause of reduced ovarian reserve (Karande and Gleicher, 1999) the capacity of the ovary to respond in incipient ovarian failure is limited by the reduced pool of primordial follicles. Higher GH/IGF-1 levels would not reverse this but may enhance the existing follicles’ sensitivity to FSH.

GH releasing hormone (GHRH) has not been demonstrated to be effective in improving the outcome for critically defined poor responders (Howles et al., 1999). Initial studies with GH co-treatment suggested more follicles developed and less gonadotrophin was required (Homburg and Ostergaard, 1995). A systematic review of GH co-treatment in assisted conception reported data from six small trials and suggested that GH was of no benefit in normal responders. Poor responders, however demonstrated a trend towards an improved outcome with GH co-treatment and concluded further study was warranted (Kotarba et al., 2000). A small case series employed dehydroepiandrosterone (DHEA) supplementation for 2 months prior to and during gonadotrophin stimulation in poor responder patients and observed an increase in follicular recruitment (Casson et al., 2000). This raises an interesting question about whether extended pre-treatment enhances follicle sensitivity to gonadotrophin.

In conclusion, although there is considerable experimental evidence to support the GH/IGF-1 axis having an important role in follicular development and ovarian response to gonadotrophins, this study suggests that measuring serum IGF-1, following pituitary desensitization, will not identify potential poor responders to gonadotrophin stimulation.

References


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