Insulin-like growth factor binding protein-3 (IGFBP-3) serum concentrations and ovarian responsiveness in in-vitro fertilization

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The growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis seems to play an important role in ovarian responsiveness. Recently IGF binding protein-3 (IGFBP-3) serum concentrations have been reported to be a good marker of GH/IGF-I axis activity. In view of this finding, we measured IGFBP-3 serum concentrations in 29 women undergoing in-vitro fertilization. We found a significant correlation among IGFBP-3 serum concentrations and markers of ovarian stimulation including efficacy index, serum oestradiol concentrations and the number of follicles on the day of human chorionic gonadotrophin (HCG) administration. The results of our study add additional evidence to the importance of the GH/IGF-I system in regulating ovarian responsiveness to gonadotrophin stimulation.

Key words: IGFBP-3/IVF/ovarian responsiveness

Introduction

The outcome of in-vitro fertilization (IVF) and ovulation induction is strongly dependent on ovarian responsiveness to exogenous stimulation. The growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis seems to play an important role in ovarian responsiveness. The other major variables are age, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Ebrahim et al., 1993; Shoham et al., 1993; Seibel et al., 1995). The evidence for the importance of the GH/IGF-I axis for ovarian responsiveness to gonadotrophin stimulation comes from clinical studies where GH or growth hormone-releasing hormone (GHRH) treatments have been successfully used to improve the ovarian response to gonadotrophins, and to facilitate follicular growth (Homborg et al., 1988; Hughes et al., 1991; Ibrahim et al., 1991). In addition, decreased GH secretory capacity in women with ovulatory dysfunction and an inverse correlation between the amount of GH released after clonidine challenge and the amount of human menopausal gonadotrophin (HMG) used for ovulation induction have been described (Menashe et al., 1990; Ovesen et al., 1992). Furthermore, IGF-1 influences many aspects of ovarian function (Giudice, 1992).

IGF-I serum concentrations are claimed to be a better marker of long-term GH/IGF-I axis activity than GH serum concentrations (Barkan et al., 1988). Recently IGF binding protein-3 (IGFBP-3) serum concentrations have been reported to be an even better marker of GH/IGF-I axis activity in hypersecretory states (Grinspoon et al., 1995) and hyposecretory states (Hasegawa et al., 1993), as well as in a healthy population (Blum et al., 1993).

In view of these findings, we carried out a study to determine the relative contribution of IGFBP-3 serum levels in predicting ovarian responsiveness to exogenous gonadotrophin stimulation. We compared it with the contributions of age and basal LH, FSH and GH concentrations.

Patients and methods

Patients

The study involved 29 consecutive women with infertility who underwent treatment within the IVF programme. Infertility was caused by tubal disease in 25 women, male factor in two patients and the combination of tubal factor and male subfertility in two patients. In all, 24 patients were eumenorrheic, five had oligomenorrhea (defined as the cessation of menstrual flow for >35 and <90 days). The criteria for inclusion in this study were: (i) age <40 years (mean 31.4 years, range 25-40), (ii) body mass index (BMI) <30 kg/m² (mean 22.7 kg/m², range 28.9-29.1), (iii) exclusion of diabetes or any other serious medical disorder. The study was approved by the ethical committee and all patients gave informed consent to participation in the study.

Protocol

A careful history was obtained and gynaecological examination was performed in all patients before enrolment into the IVF programme. The stimulation protocol included the use of HMG (Pergonal; Serono S.A., Aubonne, Switzerland), which started on menstrual cycle day 2. Ovarian response was monitored from the fifth day of HMG administration by ovarian ultrasonography (Ultrasonic Scanner 1849 with a 7-MHz vaginal sector transducer: Bruel and Kjaer, Gentofo, Denmark) and measurement of serum oestradiol concentration. The dose of HMG was adjusted accordingly. Ovulation was triggered by l.m. administration of 5000 IU of human chorionic gonadotrophin (HCG; Primogonyl; Schering AG, Berlin, Germany), when the largest follicle reached >17 mm and the second one >15 mm in diameter. Oocyte retrieval was performed 34-36 h later using transvaginal, sonographically guided puncture.

Blood samples were obtained for the determination of LH, FSH, GH and plasma IGFBP-3 concentrations in the early follicular phase of the previous menstrual cycle. Sera were prepared and all samples stored at -20°C prior to assay.

As markers of ovulation stimulation we used the following parameters on the day of HCG administration: (i) serum oestradiol concentration, (ii) the ratio of the highest oestradiol serum concentra-
tion to the total number of ampoules of gonadotrophins used - efficacy index, (ii) numbers of follicles \( \geq 10 \) mm on the day of HCG administration, (iv) numbers of follicles \( \geq 17 \) mm in diameter on the day of HCG administration and (v) the number of oocytes retrieved.

**Assay methods**

Commercial immunoradiometric (IRMA) kits were used for assaying LH, FSH, GH (Delfia, Wallac - Turku, Finland) and IGF-I (Diagnostic System Laboratories, Webster, Texas, USA). A radioimmunoassay (RIA) kit was used for assaying oestradiol (Sorin Biomedica, Saluggia, Italy).

For determinations of IGFBP-3 serum concentrations, a highly specific double antibody RIA (Diagnostic System Laboratories, Webster, Texas, USA) was used. This assay has a sensitivity of 0.9 ng/ml and cross-reactivity with other IGFBPs is less than 0.3. In conditions characterized by increased protease activity (pregnancy, chronic renal failure, severe illness), immunoreactive degradation fragments of IGFBP-3 may cause falsely elevated serum IGFBP-3 levels and preliminary size-exclusion chromatography may be required. As there were no such conditions in our patients no preliminary chromatography was performed.

The intra- and interassay coefficients of variation for all the methods used were \(<6\) and \(<9\%\) respectively.

**Results**

According to the results of multiple regression analysis, the composite interaction of age, LH, FSH, GH, IGF-I and IGFBP-3 was significantly predictive for efficacy index \((r = 0.68; P = 0.026)\), oestradiol \((r = 0.67; P = 0.037)\), and the number of follicles \(\geq 10\) mm in diameter on the day of HCG administration \((r = 0.70; P = 0.018)\). Among single variables IGFBP-3 concentrations appeared as an independent predictor for all the markers of ovulation stimulation, while LH and FSH serum concentrations were predictive only for the number of follicles \(\geq 10\) mm at the time of HCG administration (Table I).

**Discussion**

Our results indicate that IGFBP-3 serum concentration is more predictive of ovarian responsiveness to exogenous gonadotrophin stimulation than age and other baseline hormone values. The significantly positive correlation between IGFBP-3 and ovarian responsiveness as assessed by the markers used could be explained in several ways.

First, IGFBP-3 serum concentration may be only a finely tuned marker of spontaneous GH secretion. IGFBP-3 is GH-dependent, but has a prolonged half-life compared to GH and does not exhibit pulsatility or a circadian pattern. Therefore it is a good integrated marker of somatotroph function (Blum and Ranke, 1990). The mechanism(s) whereby GH might influence ovarian function is not known, although the effect of GH on the ovary is believed to be mediated by IGF-I (Giudice, 1992), the evidence for functional GH receptors on human granulosa cells (Carlsson et al., 1992) makes a direct effect of GH on the ovary plausible.

Second, IGFBP-3 may potentiate the effect of IGF-I on the ovary. Though the follicular fluid (FF) concentration of IGFBP-3 was not measured in our study, a reported significant correlation between serum and FF concentrations of IGFBP-3 (Bergh et al., 1994) permits the assumption of similar FF concentrations in our patients. IGFBP-3 could thus potentiate the effect of IGF-I, whose serum values by themselves were not statistically significantly correlated to the markers of ovarian stimulation in our study. Possible mechanisms for potentiation of IGF-I bioactivity involve post-translational modification of the IGFBPs by phosphorylation (Jones et al., 1991) or proteolysis (Conover, 1991) to yield forms with decreased IGF-binding affinity.

Finally, IGFBP-3 concentrations may be under the influence of a third, so far unidentified factor, that affects ovarian responsiveness to exogenous stimulation. Sex steroids including oestradiol, free testosterone, androstenedione and dehydroepiandrosterone sulphate do not seem to be likely candidates. We calculated their contributions to IGFBP-3 serum concentrations by means of multiple regression analysis, but no interactions were statistically significant (data not shown).

Basal GH and IGF-I serum concentrations did not correlate with markers of ovarian stimulation. In contrast to many, though contradictory, reports of GH and IGF-I serum values during the IVF stimulation process (Huang et al., 1993, Bergh et al., 1994; Potashnik et al., 1995), there are few data on pre-treatment GH and IGF-I concentrations and the outcome of ovulation stimulation process (Tiitinen et al., 1993; Pellicer et al., 1994). Our data are consistent with reports which found no correlation between pre-treatment GH and IGF-I concentrations and the outcome of ovulation stimulation. For GH basal serum concentration the finding is not surprising, as its pulsatile secretion and short half-life make it an inappropriate representative of GH secretory status. The lack of correlation between serum IGF-I values and the markers of ovarian responsiveness as assessed by the markers used could be explained in several ways.

| Table I. Standardized regression coefficients (beta) among age, hormonal variables and markers of ovulation stimulation |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Efficacy index | Oestradiol (pmol/l) on the day of HCG | No. of follicles \( \geq 10\) mm on the day of HCG | No. of follicles \( \geq 17\) mm on the day of HCG |
| IGFBP-3                        | 0.643**        | 0.629**         | 0.384*          | 0.478*          |
| IGF-I                          | 0.050          | -0.022          | 0.008           | -0.208          |
| GH                             | -0.321         | -0.290          | -0.305          | 0.142           |
| FSH                            | -0.113         | -0.064          | -0.395*         | -0.047          |
| LH                             | 0.211          | 0.259           | 0.416*          | 0.198           |
| Age                            | 0.267          | 0.234           | -0.247          | 0.045           |

*The ratio of the highest oestradiol serum concentration at the time of HCG administration to the total number of ampoules of gonadotrophins used.

\( P < 0.05 \)

\( ** P < 0.01 \)

The intra- and interassay coefficients of variation for all the methods used were \(<6\) and \(<9\%\) respectively.
stimulation is more surprising because its ovarian actions are well described (Adashi et al., 1985). Also, follicular IGF-I is probably derived by diffusion from the peripheral circulation, and local production appears unlikely (Pellegrini et al., 1995).

Among other variables in the multiple regression model FSH and LH basal serum values were statistically significantly correlated with the number of follicles ≥10 mm in diameter on the day of HCG administration, but not with other markers of ovarian stimulation. This confirms previous reports of basal FSH (Ebrahim et al., 1993) and basal LH concentrations (Shoham et al., 1993; Seibel et al., 1995) as predictors of IVF performance.

Age did not appear as an independent predictor of ovarian stimulation in our study, though its value in predicting performance in assisted reproduction techniques is well established (Jacobs et al., 1990; Piette et al., 1990). The reasons for this discrepancy may be two-fold. First, the majority of our patients were in a relatively narrow age range. This may account for the failure to observe a significant correlation between age and markers of ovarian stimulation. Second, age dependency of IVF performance may be partly caused by an age-related increase in FSH serum concentrations (Lenton et al., 1988).

In our study we used multiple regression analysis where FSH concentrations as well as other independent variables were taken into account when examining the relationship between age and markers of ovarian stimulation. It may be that age as an independent variable lost statistical significance when the effect of serum FSH concentration was accounted for. Indeed, if we determined univariate relation between age and the number of follicles ≥10 mm in diameter on the day of HCG administration, the correlation coefficient reached borderline statistical significance (P < 0.1).

In summary, our study showed that IGFBP-3 serum concentration is more predictive of ovarian responsiveness to exogenous gonadotrophin stimulation than age and other baseline hormone values. Whatever the underlying mechanisms, this finding adds additional evidence to the importance of GH/IGF-1 system in regulating ovarian responsiveness to gonadotrophin stimulation.

References


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