Insulin-like growth factor (IGF)-I and IGF binding protein-3 concentrations in fluid from human stimulated follicles

G.J.E.Oosterhuis1,2,4, I.Vermes1, C.B.Lambalk3, H.W.B.Michgelsen2 and J.Schoemaker3

Departments of 1Clinical Chemistry and 2Obstetrics and Gynaecology, Medisch Spectrum Twente Hospital Group, PO Box 50000, 7500 KA Enschede and 3Research Institute for Endocrinology, Metabolism and Reproduction, Academic Hospital Vrije Universiteit, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

4To whom correspondence should be addressed

Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) play an important role in regulating follicle growth and maturation. We have evaluated whether responsiveness to gonadotrophins during an in-vitro fertilization (IVF) treatment is related to follicular fluid IGF-I and IGFBP-3 concentrations. We also investigated if a difference is present in IGF-I and IGFBP-3 concentrations between patients treated with human menopausal gonadotrophin (HMG) and patients treated with highly purified follicle stimulating hormone (FSH). We have measured IGF-I and IGFBP-3 in follicular fluid from pre-ovulatory follicles in an IVF programme. All 70 patients were stimulated after being down-regulated with a gonadotrophin-releasing hormone (GnRH) analogue. IGF-I concentrations in follicular fluid were significantly inversely correlated with the number of ampoules FSH administered and number of days of FSH administration, and significantly correlated with the number of follicles aspirated. IGFBP-3 concentrations were not correlated with any other parameter measured nor were IGF-I and IGFBP-3 concentrations correlated. IGFBP-3 concentrations were significantly higher in patients receiving highly purified FSH compared with patients receiving HMG (P < 0.005). These results are new evidence that IGF-I concentration in follicular fluid is higher in women who respond better to follicular stimulation, i.e. women who grow many follicles, women who need a shorter duration of stimulation and women who need fewer ampoules FSH before oocyte retrieval.

Key words: apoptosis/follicular fluid/folliculogenesis/IGF-I/IGFBP-3

Introduction
There has been extensive research on the role of insulin-like growth factor (IGF) and its binding proteins (IGFBP) in the ovary. In recent years, it has become clear that the IGF autocrine/paracrine system is comprised of IGF-I and -II, its binding proteins IGFBP-1–6, and their receptors in the target cells (for reviews see Giudice, 1992; Giudice et al., 1993; Jones and Clemmons, 1995; Monget et al., 1996).

IGF-I is a peptide with mitogenic effects which can induce cellular differentiation, e.g. in granulosa cells (Apa et al., 1996), and IGF can also activate cell motility (Leventhal and Feldman, 1997). IGF-I has a strong blocking effect on follicle apoptosis which is opposed by binding of IGF-I on IGFBP-3 (Adashi and Rohan, 1992; Chun et al., 1994; Barreca et al., 1996; Hsueh et al., 1996). Only recently has the exact pathway used by the IGF-I receptor to protect cells from apoptosis been described (Kulik et al., 1997).

In serum, IGFBP serve as carriers for IGF-I and -II, and IGFBP may also protect IGF from degradation and serve as a transport medium and a reservoir for IGF (Giudice, 1992; Jones and Clemmons, 1995).

IGF-I is present in follicular fluid, and it has been made clear by several studies that IGF-I and IGFBP-3 play central roles in follicular development in humans, either alone (Erickson et al., 1989; El-Roeiy et al., 1993) or in synergy with follicle stimulating hormone (FSH) (Eden et al., 1988; Erickson et al., 1989; Geisthovel et al., 1989a; Giudice et al., 1990; Adachi et al., 1995; Foster et al., 1995; Barreca et al., 1996), human chorionic gonadotrophin (HCG) (Erickson et al., 1988; Apa et al., 1996) or oxytocin (Sirotkin et al., 1996). Gonadotrophins regulate some elements of the IGF system, such as IGF-I receptor gene expression (Zhou and Bondy, 1993).

These findings support the hypothesis that the capacity of the ovary to grow follicles and mature oocytes is at least partially regulated by the IGF system, although there is evidence that, in spite of IGF-I deficiency, a normal follicular development and subsequent pregnancy can be achieved (Dor et al., 1992). If an increased death of follicles caused by apoptosis is the main cause for imminent ovarian failure (Hsueh et al., 1994), then poor responders in an in-vitro fertilization (IVF) programme may have lower concentrations of IGF-I and higher concentrations of IGFBP-3 in their follicular fluid than normal responders.

We have evaluated whether women who are less responsive to gonadotrophins during an IVF treatment have lower IGF-I or higher IGFBP-3 concentrations in their follicular fluid than women who are normally responsive.

Materials and methods

Subjects
Human follicular fluid was obtained from 70 women participating in an IVF programme. The women were under 40 years of age and entered our IVF programme for various reasons. All patients received

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a gonadotrophin-releasing hormone (GnRH) analogue (Decapeptyl; Ferring B.V., Hoofddorp, The Netherlands) and either human menopausal gonadotrophin (HMG) (Humegon, Organon, Oss, The Netherlands) or highly purified urinary FSH (Metrodin HP; Serono Benelux, The Hague, The Netherlands). During the treatment, follicle size was measured by ultrasound until at least three follicles ≥16 mm diameter were recorded. Then oocyte retrieval was planned. HCG (10 000 IU i.m., Profasi; Serono) was injected 35 h before oocyte retrieval. Follicles were not flushed after puncture.

After isolation of the oocytes from the follicular fluid, the fluid from each patient was pooled and centrifuged at 250 g for 10 min. The supernatant was aspirated and stored at −70°C until IGF-I and IGFBP-3 determination.

**Ethics**

Procedures followed were in accordance with the policies of the institutional review board of the hospital group. All patients gave verbal informed consent for their follicular fluid to be used for this study.

**Assays**

IGF-I and IGFBP-3 were both measured by radioimmunoassay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The sensitivity of the IGF-I assay was 14 ng/ml, and the intra- and interassay coefficients of variation were 2.4–3.0% and 5.2–8.4%, respectively. The sensitivity of the IGFBP-3 assay was 62.5 ng/ml, and the intra- and interassay coefficients of variation were 3.4–8.0% and 5.3–6.3% respectively.

**Statistics**

Results are presented as the mean ± SD. If variables were not normally distributed, a log transformation was carried out before correlations were calculated. Linear regression analysis was used and Pearson’s correlation coefficient was calculated to detect any correlation between IGF-I or IGFBP-3 concentrations in follicular fluid as dependent variables, and age, the number of days the patient had to take FSH before oocyte retrieval, the total number of ampoules FSH administered, the number of follicles aspirated, the number of oocytes retrieved, and the number of oocytes fertilized as independent variables. Comparison of means was done with the unpaired t-test or the Wilcoxon rank sum test when appropriate. Significance was set at P = 0.05. Statistics were calculated using the Statistical Package for Social Sciences (SPSS) program.

**Results**

In the 70 patients who participated in the study, a mean number of 11.6 ± 4.9 follicles were aspirated, 9.5 ± 5.7 oocytes were retrieved, and 4.4 ± 4.3 oocytes were fertilized. The mean duration of FSH administration was 12.3 ± 2.8 days, and 42.1 ± 18.7 ampoules of FSH were administered.

The mean concentration of IGF-I in follicular fluid was 129 ± 67 ng/ml. Linear regression analysis showed that IGF-I concentrations were significantly inversely correlated both with the log transformed number of ampoules of FSH administered (Pearson’s r = −0.405, P = 0.001; Figure 1) and with the number of days of FSH administration (Pearson’s r = −0.249, P = 0.039; Figure 2). IGF-I concentrations were significantly correlated with the number of follicles aspirated (Pearson’s r = 0.317, P = 0.008; Figure 3). There was no significant correlation between IGF-I concentrations and any other variable measured.

Patients who needed ≥36 ampoules had significantly lower IGF-I concentrations in their follicular fluid (97.6 ± 47.8 versus 151 ± 70.3 ng/ml in the group with ≤36 ampoules, P = 0.001). Patients with ≤12 follicles had significantly lower IGF-I concentrations than patients with >12 follicles (116 ± 57.3 versus 155 ± 78.1 ng/ml, P = 0.05). There was an apparent but non-significant trend towards lower IGF-I concentrations in follicular fluid from patients stimulated for >12 days compared with patients stimulated ≤12 days (110 ± 56.6 versus 140 ± 70.6 ng/ml, P = 0.06) (see Table 1).

The mean concentration of IGFBP-3 was 1403 ± 812 ng/ml. There was no correlation between IGFBP-3 concentrations and any other parameter measured, including IGF-I concentrations.
Figure 3. Correlation between insulin-like growth factor-I (IGF-I) levels in follicular fluid and number of follicles aspirated ●, human menopausal gonadotrophin; ○, highly purified urinary follicle stimulating hormone (Pearson’s \( r = 0.317, P = 0.008 \)).

### Table I. Mean insulin-like growth factor (IGF-I) and IGF binding protein (IGFBP)-3 concentrations in patients grouped according to number of ampoules of follicle stimulating hormone (FSH) administered, number of follicles aspirated, and duration of stimulation

<table>
<thead>
<tr>
<th>IGF-I (ng/ml)</th>
<th>IGFBP-3 (μg/ml)</th>
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<tbody>
<tr>
<td>≤36 ampoules FSH</td>
<td>151 ± 70.3 (^a) 1.58 ± 0.87</td>
</tr>
<tr>
<td>&gt;36 ampoules FSH</td>
<td>97.6 ± 47.8 1.16 ± 0.67</td>
</tr>
<tr>
<td>≤12 follicles aspirated</td>
<td>116 ± 57.3 (^b) 1.32 ± 0.78</td>
</tr>
<tr>
<td>&gt;12 follicles aspirate</td>
<td>155 ± 78.1 1.59 ± 0.85</td>
</tr>
<tr>
<td>≤12 days stimulated</td>
<td>140 ± 70.6 1.41 ± 0.84</td>
</tr>
<tr>
<td>&gt;12 days stimulated</td>
<td>110 ± 56.6 1.38 ± 0.78</td>
</tr>
</tbody>
</table>

Values are means ± SD. \(^a\) \( P = 0.001 \), IGF-I levels ≤36 ampoules versus >36 ampoules; \(^b\) \( P = 0.05 \), IGF-I levels ≤12 follicles versus >12 follicles; \(^c\) \( P = 0.06 \), IGF-I levels ≤12 days versus >12 days; differences between IGFBP-3 concentrations were not statistically significant.

Twenty-three patients were given HMG and 47 received highly purified urinary FSH. As shown in Figures 1–3, there was no difference in number of ampoules, number of days of treatment, and number of follicles aspirated, between patients who received HMG and those who received highly purified FSH. The medication only influenced IGFBP-3 concentrations. Patients who were treated with HMG had a mean concentration of IGFBP-3 of 1000 ± 598 ng/ml, which was significantly lower than that of patients treated with highly purified urinary FSH: 1599 ± 835 ng/ml (\( P < 0.005 \), 95% CI -0.9881 to -0.2098) (Table II).

### Discussion

To our knowledge, our report is the first to detect a significant inverse correlation between IGF-I concentrations in follicular fluid and number of ampoules of FSH administered and number of days of FSH administration. Moreover, we found a significant positive correlation between IGF-I concentrations in follicular fluid and number of follicles grown. Patients with higher IGF-I concentrations in their follicular fluid needed fewer ampoules of FSH and required fewer days of FSH administration to reach an adequate number of follicles.

Our study is in line with evidence that IGF-I concentrations in follicular fluid are higher in patients who respond better to treatment. Higher follicular fluid IGF-I concentrations have been found in IVF patients with larger follicles and larger follicular fluid volumes (Rabinovici et al., 1990). These findings, as well as the facts that human granulosa cells can release IGF-I in vitro (Strickin et al., 1996), and that dominant follicles have three times higher IGF-I concentrations in follicular fluid compared to cohort follicles in normal cycling women (Eden et al., 1988), emphasize the importance of IGF-I in human folliculogenesis. Not all studies give supportive evidence, however. No difference in follicular fluid IGF-I concentrations has been found between androgen-dominant follicles, oestrogen-dominant follicles, or luteinizing follicles from IVF patients (Van Dessel et al., 1996), and one study even recorded lower mean IGF-I concentrations in follicular fluid from women with low basal serum FSH concentrations and a good response to the IVF treatment compared to women with high basal serum FSH concentrations and a worse response to the treatment (Seifer et al., 1995). In that study only a small number of patients participated, but still these conflicting results stress the uncertainty about the exact role of IGF-I in folliculogenesis.

The IGF system probably plays a key role in preventing apoptosis. This has been extensively studied in various cell types (Singleton et al., 1996; Kulik et al., 1997). Atresia of follicles is an apoptotic event and IGF play an important role in preventing cell death in follicles (Adashi and Rohan, 1992; Hsueh et al., 1996).

Immunohistochemical localization of IGF-I shows that the thecal–interstitial cells are the main site of IGF-I biosynthesis (Hernandez et al., 1992). No follicular site other than theca has been found for IGF-I production (Geisthovel et al., 1989b; Hernandez et al., 1992; Zhou and Bondy, 1993; Mason et al., 1996; Voutilainen et al., 1996) and no IGF-I mRNA has been found in human luteinized granulosa cells (Hernandez et al., 1992). There are significant correlations between follicular fluid and serum IGF-I concentrations (Geisthovel et al., 1989a; Rabinovici et al., 1990; Hamori et al., 1991) and serum and follicular fluid IGF-I concentrations are significantly lower in women of advanced reproductive age (Klein et al., 1996).
Therefore, the vascular system might be the origin of IGF-I in follicular fluid.

One explanation of higher IGF-I concentrations might be more availability because of lowered concentrations of IGFBP-3. In our study, we found that better responders did not differ in follicular fluid IGFBP-3 concentrations compared to the poor responders. Moreover, we found no correlation between follicular fluid IGFBP-3 and the outcome of the IVF treatment. Our data conflict with those of another study, which show that follicular fluid IGF-I concentrations are correlated with follicular fluid IGFBP-3 (Rabinovici et al., 1997). The present study, however, is consistent with the study of Hamori et al. (1991) and one other study giving evidence that FSH inhibits the binding activity of IGFBP-3 rather than affecting the secretion of IGFBP-3 (Adachi et al., 1995). Therefore, although IGF-I in follicular fluid is bound by IGFBP-3 (Barreca et al., 1996), and IGFBP-3 mRNA has been found in human luteinizing granulosa cells (Giudice et al., 1991), IGFBP-3 probably plays no active role in the regulation of folliculogenesis in humans. One study did report higher IGFBP-3 concentrations in oestrogen-dominant follicles than in androgen-dominant follicles in spontaneously menstruating women (Van Dessel et al., 1996), but this is contradicted by another study where it was found that IGFBP-3 concentrations in oestrogen-dominant follicles are lower than in androgen-dominant follicles and that low IGFBP-3 concentrations in follicular fluid are associated with dominance of the follicle (San Roman and Magoffin, 1993). It seems that IGFBP-3 concentrations in follicular fluid are not dependent on functional status of the follicle (Cataldo and Giudice, 1992). IGFBP-3 concentrations in serum from IVF patients, however, have been shown to be significantly higher in women who respond well to treatment compared to women who do not respond so well (Salobir et al., 1996). Serum IGFBP-3 might thus serve as a pool for IGF-I in follicular fluid.

Surprisingly, we found significantly higher IGFBP-3 concentrations in follicular fluid from patients who had been treated with highly purified urinary FSH compared with patients treated with HMG. There has been no previous report of a similar finding. Our finding is in contrast to one study, where in theca cell culture medium a trend towards increased IGFBP accumulation was seen in response to luteinizing hormone (LH) (Mason et al., 1996), a finding which could not be confirmed in another study (Voutilainen et al., 1996). The meaning of our finding is not clear. It might be that the LH in HMG is responsible for this difference. There was no difference in number of ampoules of FSH, duration of treatment, number of follicles or oocytes between the different medication groups, indicating no clear clinically relevant differences.

In summary, this study presents new evidence for the importance of the role of IGF-I in follicular development. The exact mechanism of IGF-I function and the relevance of IGFBP-3 in the metabolism of the human follicle remains unclear.

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References


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