BACKGROUND: The aim of our study was to measure concentrations of vascular endothelial growth factor (VEGF), platelet endothelial cell adhesion molecule-1 (PECAM-1) CD31 and vascular cell adhesion molecules (VCAM-1) in the follicular fluid of women treated with assisted reproduction technology to determine whether these proteins might be outcome markers. METHODS: Follicular fluid was collected from 75 patients ≤40 years undergoing oocyte retrieval procedures at our tertiary hospital during 1997 and 1998: 50 with tubal disease, 12 with endometriosis, and 13 whose partners had been diagnosed with severe oligoasthenoteratozoospermia. This retrospective analysis considered age and information about treatment and outcome for all these women who had undergone fewer than three assisted reproduction attempts. RESULTS: Nineteen women became pregnant (defined by human chorionic gonadotrophin concentrations and embryonic cardiac activity 1 month after follicular aspiration); 56 did not. Women did not differ significantly in their follicular fluid concentrations of VEGF, sCD31 and VCAM-1 according to cause of infertility, or assisted reproduction outcome, or age. Follicular fluid concentrations of VEGF were significantly correlated with the number of gonadotrophin ampoules administered ($P < 0.012$), and follicular fluid concentrations of sVCAM-1 with the fertilization rate ($P < 0.01$). Follicular fluid concentrations of VEGF and sVCAM-1 were also correlated ($P < 0.007$). CONCLUSIONS: Our results do not suggest that VEGF, CD31, or sVCAM-1 in follicular fluid predict assisted reproduction outcome, especially among patients ≤40 years old. The correlation of a high fertilization rate and sVCAM-1 in follicular fluid suggests that sVCAM-1 might be a marker of fertilization.

Key words: endometriosis/follicular fluid/CD31/VCAM-1/VEGF

Introduction

Interest has recently focused on possible interactions between cytokines, growth factors and mitogens in the ovary (Adashi, 1992; Moncayo and Moncayo, 1995; Elchalal and Schenker, 1997). Vascular endothelial growth factor (VEGF) is a heparin-binding glycoprotein that enhances vascular permeability, is mitogenic for endothelial cells and promotes angiogenesis in normal and tumoral tissues (Ferrara et al., 1991, 1992). In the ovary, VEGF is produced both by granulosa and thecal cells (Ravindranath et al., 1992; Kamat et al., 1995). Recent study of macaque granulosa cells suggests that VEGF expression increases in response to gonadotrophins such as LH, FSH and human chorionic gonadotrophin (HCG) (Christenson and Stouffer, 1997). VEGF has been also implicated in the pathogenesis of capillary leakage, which in turn is a major mechanism of ovarian hyperstimulation syndrome (OHSS), a severe complication of IVF procedures (Yan et al., 1993; Neulen et al., 1995; Abramov et al., 1997; Elchalal and Schenker, 1997).

In women with normal non-stimulated cycles and those undergoing IVF, the local VEGF production in follicular fluid (follicular fluid) is correlated with the degree of follicular luteinization (Lee et al., 1997; Anasti et al., 1998). Progesterone also appears to play a role in determining VEGF concentration in follicular fluid (Moncayo et al., 1998). A positive correlation has also been observed between follicular fluid VEGF concentrations and patient age, especially in patients ≥38 years undergoing IVF (Friedman et al., 1997; Manau et al., 2000). Recently, it has been reported that elevated VEGF concentrations in follicular fluid might predict poor conception rates after IVF (Friedman et al., 1998).

Platelet endothelial cell adhesion molecule-1 (PECAM-1 or CD31) and vascular cell adhesion molecule-1 (VCAM-1) are transmembrane glycoproteins belonging to the immunoglobulin
superfamily (Gearing et al., 1993; Almendro et al., 1996). These molecules are known to be essential mediators of white blood cell adhesion and extravasation during inflammatory and immune reactions (Marik and Lo, 1996; Cotran and Mayadas-Norton, 1998). VCAM-1 and CD31 have also been used to assess angiogenesis; the expression of each is characterized by the release of an active soluble form (Banks et al., 1993; Gearing et al., 1993; Goldberger et al., 1994). Recent investigation of the plasma and peritoneal fluid concentrations of soluble VCAM-1 (sVCAM-1) indicates that, like VEGF, VCAM-1 seems to play a role in the pathogenesis and progression of OHSS (Daniel et al., 1999). To our knowledge, VCAM-1 and CD31 have never been studied in follicular fluid.

The aim of our study was to assess the concentrations of VEGF, sVCAM-1 and sCD31 in the follicular fluid of 75 women seeking to become pregnant with assisted reproductive technology and to assess their utility as markers or predictors of IVF outcome.

Materials and methods

Follicular fluid was collected from 75 consecutive patients ≤40 years who were undergoing oocyte retrieval for IVF procedures at the Department of Obstetrics and Gynaecology in the Bichat Claude Bernard tertiary hospital during 1997 and 1998.

Population

The 75 patients were divided into three groups according to the cause of their infertility: for 50 women it was due only to laparoscopically confirmed tubal disease, without any male factors; 12 women had laparoscopy-proven severe endometriosis with no male factors and 13 women had partners diagnosed with severe oligoasthenoteratozoospermia that required intracytoplasmic sperm injection (ICSI). The records of all IVF and ICSI procedures were retrospectively reviewed to collect the following data: age, duration of stimulation, total ampoules of administered gonadotrophins, plasma oestradiol concentration on the day of HCG administration, number of retrieved oocytes, fertilization rate and pregnancy rate. None of them had more than three attempts at assisted reproduction and no patient was included twice considering sequential cycles. Fifty-six women did not become pregnant after assisted reproduction (group 1). Pregnancy (n = 19, group 2) was defined by significant HCG concentrations and the observation of embryonic cardiac activity during the transvaginal ultrasound examination performed 1 month after follicular aspiration.

IVF stimulation procedure

All patients underwent a standard stimulation protocol, described below. First, hormonal down-regulation was begun with a gonadotrophin-releasing hormone (GnRH) agonist (Triptorelin, Decapeptyl; Ipsen/Biotech, Paris, France). This treatment started on the third day of the menstrual cycle preceding IVF and was given i.m. at a daily dose of 0.1 mg for a minimum of 2 weeks. When the serum oestradiol concentration was <50 pg/ml, three ampoules of human menopausal gonadotrophin (Menotropin, Humegon 75 IU; Organon, Saint-Denis, France) for 26 women or two ampoules of recombinant FSH (Follitropin alpha, Gonalf 75 IU; Serono, Boulogne, France; or Follitropin beta, Puregon 100 IU; Organon, Saint-Denis, France) for 49 women were given i.m. each evening in addition to 0.05 mg of GnRH agonist.

Follicular development was monitored by ultrasound with a 5 MHz transvaginal probe. The dose of gonadotrophin stimulation was then adjusted according to plasma oestradiol concentrations and the size of the ovarian follicles evaluated by ultrasound examination. Finally, when the plasma oestradiol concentration was >1800 pg/ml and when at least two follicles with a diameter of 20 mm were observed by ultrasound examination, 10 000 IU of HCG was given i.m. to induce follicular rupture. Transvaginal follicular aspiration was performed 34–36 h after the HCG injection, under general anaesthesia using propofol (Diprivan; Zeneca, Cergy, France). After oocytes were identified and separated, the clear follicular fluid was collected and pooled for each woman. When blood contamination was observed, follicular fluid was discarded. This study considered follicular fluid from only one cycle for each patient. All samples were immediately centrifuged at 400 g for 10 min and the supernatant stored frozen at −40°C until they were assayed.

Determination of VEGF, sVCAM-1 and sCD31 concentrations in follicular fluid

These concentrations were assessed with the appropriate enzyme-linked immunosorbent assay test kits for human VEGF, human sVCAM-1, and human sCD31 (R&D Systems Minneapolis, USA). All samples were run in duplicate. For VEGF, VCAM-1 and CD31,

Table I. Characteristics of patients treated with assisted reproduction

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Groups</th>
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<tbody>
<tr>
<td></td>
<td>Tubal disease</td>
</tr>
<tr>
<td>No. of patients</td>
<td>50</td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.7 ± 3.59</td>
</tr>
<tr>
<td>Oestradiol peak (pg/ml)</td>
<td>2402 ± 843</td>
</tr>
<tr>
<td>No. of ampoules of gonadotrophins</td>
<td>33 ± 16</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>9.32 ± 5.38</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>56 ± 31.7</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>16</td>
</tr>
<tr>
<td>Follicular fluid</td>
<td></td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>2632 ± 1485</td>
</tr>
<tr>
<td>sCD31 (ng/ml)</td>
<td>11.2 ± 3.13</td>
</tr>
<tr>
<td>sVCAM-1 (ng/ml)</td>
<td>199.22 ± 93.16</td>
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</tbody>
</table>

Values are mean ± SD unless otherwise indicated.

The groups did not differ significantly for any parameter, as assessed using the Kruskal-Wallis test.

VEGF = vascular endothelial growth factor; sCD31 = soluble platelet endothelial cell adhesion molecule-1; sVCAM-1 = soluble vascular cell adhesion molecules.
null samples were diluted to 1/4, 1/20 and 1/5 respectively with the appropriate diluent provided by the manufacturer. For VEGF, the sensitivity of the test was 9 pg/ml. The intra-assay and inter-assay coefficients of variation (CV) were 3.5 and 7% respectively. For sVCAM-1, the sensitivity of the assay was 2 ng/ml, and the intra-assay and inter-assay CV were 5 and 9% respectively. Finally, for sCD31, the sensitivity of the assay was 0.05 ng/ml, and the intra-assay and inter-assay CV were 7% and 6% respectively.

**Statistical analysis**

Student’s t-test, the Kruskal-Wallis test and a Spearman correlation were all used for the statistical analysis. P < 0.05 was considered as significant. The sample size of our study provided a power of 97% for the t-test to detect the difference in VEGF concentrations previously observed (Friedman et al., 1998), with the variance estimates they reported.

**Results**

Table I summarizes the characteristics of the 75 patients. The three groups defined according to the origin of their infertility did not differ significantly for any of the characteristics assessed: mean age, total number of gonadotrophin ampoules administered, oestradiol concentration on day of HCG injection, number of retrieved oocytes, and fertilization rate. Similarly, they did not differ significantly with respect to follicular fluid concentrations of VEGF, sCD31 or sVCAM-1.

No patient in this study developed OHSS.

Moreover, the assisted reproduction outcome was not related to follicular fluid concentrations of VEGF, sCD31 or sVCAM-1. Group 1 (the 56 women who did not become pregnant) and group 2 (the 19 women who did become pregnant) did not differ significantly for any of these growth factor concentrations, as Table II shows.

Of the 19 women who became pregnant, five miscarried during the first trimester. The remaining pregnancies resulted in viable offspring. As with the previous comparisons, these two groups of women (14 who gave birth at term and five who had miscarriages) did not differ significantly in their follicular fluid concentrations of VEGF, sCD31 or sVCAM-1 (Table III).

In this study, 15 patients were ≥38 years old. As Table IV shows, we compared them with the group of women <38 years, and found no statistically significant differences in the follicular fluid concentrations of VEGF, sCD31, or sVCAM-1. The only significant difference between the two groups concerned the oestradiol peak on the day HCG was administered; it was higher among the younger women.

Finally, the angiogenic factors (VEGF, CD31, and VCAM-1) we examined were not significantly correlated with patient age, the number of oocytes retrieved, or the concentration of oestradiol peak. As in recent studies (Friedman et al., 1998; Manau et al., 2000), follicular fluid concentrations of VEGF were weakly but significantly correlated with the number of gonadotrophin ampoules administered (P < 0.012) (Figure 1A). Follicular fluid concentrations of sVCAM-1 were also correlated with the fertilization rate (P < 0.01) (Figure 1B) and the follicular fluid concentration of VEGF (P < 0.007) (Figure 1C).

**Discussion**

Several authors have demonstrated that VEGF is produced by granulosa cells and released into the follicular fluid after
Table IV. Relation between follicular fluid concentrations of VEGF, sCD31 and sVCAM-1 and patient age

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Groups</th>
<th>t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤37 years</td>
<td>≥38 years</td>
</tr>
<tr>
<td>No. of patients</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.8 ± 2.9</td>
<td>38.93 ± 0.8</td>
</tr>
<tr>
<td>Oestradiol peak (pg/ml)</td>
<td>2439 ± 800</td>
<td>1969 ± 519</td>
</tr>
<tr>
<td>No. of ampoules of gonadotrophins</td>
<td>32 ± 14</td>
<td>36 ± 20</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>9.47 ± 5.2</td>
<td>7.53 ± 5.5</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>53.6 ± 31</td>
<td>64.5 ± 29.6</td>
</tr>
<tr>
<td>Follicular fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>2741.81 ± 1823.65</td>
<td>2866.31 ± 1337.64</td>
</tr>
<tr>
<td>sCD31 (ng/ml)</td>
<td>11.34 ± 3.6</td>
<td>10.47 ± 3.1</td>
</tr>
<tr>
<td>sVCAM-1 (ng/ml)</td>
<td>201.45 ± 90.83</td>
<td>192 ± 84.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD unless otherwise indicated.

VEGF = vascular endothelial growth factor; sCD31 = soluble platelet endothelial cell adhesion molecule-1; sVCAM-1 = soluble vascular cell adhesion molecules.

The increased follicular fluid concentrations of VEGF in women ≥38 years old undergoing follicular aspiration, compared with younger women, was not statistically significant. Finally, in our study we obtained (-46.3; 1419.9) as 95% CI of the difference in the follicular fluid VEGF between groups 1 and 2 (Table II) which makes unlikely any such correlation with IVF outcome noted by Friedman et al. (1998). Furthermore, we found no statistically significant correlation between the follicular fluid concentrations of sCD31 or sVCAM-1 and patient age. Our results also indicated that follicular fluid concentrations of VEGF, sCD31 and sVCAM-1 were similar for the three different causes of infertility studied, i.e. tubal factors, endometriosis, or male factors. This result must be interpreted cautiously in view of the fairly small size of the latter two groups.

Thus, contrary to the results obtained by Friedman et al. (1998), our data suggest that VEGF concentrations in follicular fluid do not predict assisted reproduction outcome. However, some authors (Van Blerkom et al., 1997) have shown that the VEGF measurements of follicular fluid indicated a potential role for this factor in perifollicular angiogenesis and in the gonadotrophin stimulation (Neulen et al., 1995; Christenson and Stouffer, 1997). Recently, it was observed that follicular fluid concentrations of VEGF were positively correlated with patient age, in particular, for patients ≥38 years undergoing IVF (Friedman et al., 1997). These authors subsequently observed a correlation between the follicular fluid concentration of VEGF and IVF outcome (conception rate) (Friedman et al., 1998), with high concentrations associated with poor outcome. They therefore suggested that follicular fluid VEGF might be a marker of a decreased ovarian reserve and of a hostile follicular environment. Recently, the same positive correlation between high concentration of follicular fluid VEGF and the patient’s age was reported (Manau et al., 2000). Our study did not confirm their findings that VEGF concentrations are positively correlated with patient age and a low conception rate. Like them, we did observe a moderate increase in follicular fluid concentrations of VEGF in women ≥38 years old undergoing follicular aspiration, compared with younger women, but the difference was not statistically significant. Finally, in our study we obtained (-46.3; 1419.9) as 95% CI of the difference in the follicular fluid VEGF between groups 1 and 2 (Table II) which makes unlikely any such correlation with IVF outcome noted by Friedman et al. (1998). Furthermore, we found no statistically significant correlation between the follicular fluid concentrations of sCD31 or sVCAM-1 and patient age. Our results also indicated that follicular fluid concentrations of VEGF, sCD31 and sVCAM-1 were similar for the three different causes of infertility studied, i.e. tubal factors, endometriosis, or male factors. This result must be interpreted cautiously in view of the fairly small size of the latter two groups.

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Figure 1. Relationship between follicular fluid concentrations of vascular endothelial growth factor (VEGF) and number of gonadotrophin ampoules administered (A), follicular fluid concentrations of soluble vascular cell adhesion molecules (sVCAM) and fertilization rate (B), follicular fluid concentrations of VEGF and of sVCAM (C) individually for all patients. Spearman’s rank correlation (p) was determined.
regulation of intrafollicular oxygen concentrations. Therefore, VEGF could be a marker for healthy individual follicle but not a clinical prognostic marker for the course of assisted reproduction, which would be consistent with our results. Moreover, the other angiogenesis factors we tested here, sCD31 and sVCAM-1, did not provide any additional information. It should nonetheless be borne in mind that the number of pregnancies in our series was limited and our sample collection design pooled follicular fluid of each woman (as did Friedman et al., 1998) and therefore did not allow identification of processes occurring in single follicles, which is a limitation of our study. However, our data showed that there was no statistically significant difference in the follicular fluid VEGF concentrations in patients conceiving and those failing to conceive. This suggests that VEGF production by granulosa cells is not directly correlated with assisted reproduction outcome or pregnancy potential.

VEGF has also been postulated to be a mediator of OHSS (Elchalal and Shenker, 1997; Rizk et al., 1997) but not an important clinical marker for the course of this condition (Ludwig et al., 1998). In this context, a recent study reports that follicular fluid concentrations of VEGF are higher than serum concentrations (Artini et al., 1998). No patient in our study had clinical OHSS, and only four patients had an oestradiol concentration that peaked at >3500 pg/ml on the day of HCG administration. The moderate increase in the follicular fluid concentration of VEGF in some patients, especially those >38 years old, might therefore simply reflect the variable ovarian response to gonadotrophin stimulation, rather than serve as a prognostic factor for assisted reproduction outcome.

Of the various endothelial markers known, CD31, which corresponds to the intercellular adhesion molecule PECAM-1, has been widely used in immunohistochemical analysis of different tumours (DeYoung et al., 1993). A soluble form of CD31 (sCD31), detected in the serum, has been proposed as a marker of angiogenesis (Goldberger et al., 1994). CD31 is also expressed by the trophoblaster (Baldwin et al., 1994) and appears to be involved in the adhesion of trophoblast cells to arterial endothelium during implantation (Blankenship and Enders, 1997). Some authors have also reported that sCD31 might be a predictive marker that could distinguish pre-eclamptic from normotensive pregnant women (Konijnenberg et al., 1997; Krauss et al., 1997). To our knowledge, our study was the first to analyse follicular fluid concentrations of sCD31 in patients treated with assisted reproduction, but we found no correlation between the sCD31 concentration and either the assisted reproduction outcome or patient age.

After cell activation, various cytokines induce VCAM-1 expression; it is released in an active soluble form (sVCAM-1) during inflammatory and immune reactions (Gearing et al., 1993; Marik and Lo, 1996). Recent reports indicate that sVCAM-1 concentration in plasma and peritoneal fluid may be a predictive marker of OHSS (Daniel et al., 1999). It is known to be expressed by the oocyte and early embryo (Campbell et al., 1995). Our study confirmed for the first time the existence of sVCAM-1 in the follicular fluid. Like the other angiogenic factors tested in our study, follicular fluid sVCAM-1 does not appear to predict assisted reproduction outcome. Our data did, however, show a highly significant positive relation between follicular fluid sVCAM-1 concentrations and the fertilization rate. This concentration might thus be a marker of fertilization.

Besides the gonadotrophin-dependent production of VEGF by granulosa cells, other indications that cytokine interactions may be involved in the local regulation of VEGF production have appeared in recent studies (Cohen et al., 1996; Moncayo et al., 1998). We observed a significant positive correlation between follicular fluid concentrations of sVCAM-1 and of VEGF. Thus, follicular fluid local production of these factors appears to be linked. Finally, our study suggests that both these angiogenic factors (VEGF and VCAM-1) are probably involved in fertilization, but their local regulation is currently unknown.

Conclusion

In conclusion, we found that VEGF, sCD31 and sVCAM-1 concentrations in follicular fluid were not correlated with assisted reproduction outcome. We were also unable to confirm previous reports that follicular fluid concentrations of VEGF are elevated in older women (>38 years) undergoing assisted reproduction. Our results also indicated that follicular fluid concentrations of VEGF, sCD31 and sVCAM-1 were similar in women whose infertility stems from different causes (tubal factors, endometriosis, or male factors). Finally, our data suggest that elevated follicular fluid sVCAM-1 concentration may be associated with a high fertilization rate. Elevated follicular fluid concentrations of sVCAM-1 appears therefore to be a fundamental marker of the process of human fertilization.

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References


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