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Serum vascular endothelial growth factor concentrations and ovarian stromal blood flow are increased in women with polycystic ovaries

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The aim of this study was to determine basal serum vascular endothelial growth factor (VEGF) concentrations and Doppler blood flow changes within the ovarian stroma of women with polycystic ovaries (PCO) and women with normal ovaries. Pulsed and colour Doppler blood flows within the ovarian stroma were recorded, and serum VEGF concentrations measured, in the early follicular phase (days 2–3 of a menstrual cycle) in 60 women undergoing ovarian stimulation for in-vitro fertilization. 36 women had normal ovaries, 14 women had PCO as seen on pelvic ultrasound examination and 10 had polycystic ovarian syndrome (PCOS). Mean ± SD serum VEGF concentrations were significantly higher ($P < 0.001$) in women with PCO and PCOS (3.4 ± 0.7 and 3.2 ± 0.66 ng/ml respectively) compared with women with normal ovaries (2.3 ± 0.5 ng/ml). Mean peak systolic blood flow velocity (PSV) and time-averaged maximum flow velocity (TAMXV) were significantly higher ($P < 0.001$) in women with PCO and PCOS compared with women with normal ovaries. The mean PSV were 15 ± 4 and 16 ± 4 cm/s in women with PCO and PCOS respectively, compared with 9 ± 2 cm/s in women with normal ovaries. The TAMXV were 9 ± 3 and 11 ± 3 cm/s in women with PCO and PCOS respectively compared with women with normal ovaries (5.8 ± 1.5 cm/s). Serum VEGF concentrations were positively correlated with PSV ($r = 0.44$, $P = 0.001$) and TAMXV ($r = 0.45$, $P < 0.000$) in all three groups of women. Higher serum concentrations of VEGF in women with PCO and PCOS may relate to the increased vascularity that underlies the increased blood flow demonstrated by Doppler blood flow velocity measurements in these women. The results may explain the higher risk of ovarian hyperstimulation syndrome in programmes of ovarian stimulation in patients with PCO compared with those with normal ovaries.

Key words: Doppler blood flow velocity/polycystic ovarian syndrome/polycystic ovary/vascular endothelial growth factor

Introduction

Several growth factors have been implicated in angiogenesis within the ovary, including basic fibroblast growth factor (bFGF; Gospodarowicz, 1974), transforming growth factor β (TGF-β; Pepper *et al.*, 1993) and platelet-derived growth factor (PDGF; Klagsbrun and D'Armore, 1991; Folkman and Shing, 1992). Luteinizing hormone (LH)-induced neoangiogenesis has also been described (Findlay, 1986).

Vascular endothelial growth factor (VEGF), an endothelial cell mitogen with potent angiogenic properties, is emerging as an important mediator of neoangiogenesis (Senger *et al.*, 1983; Ferrara and Henzel, 1989; Gospodarowicz *et al.*, 1989; Leung *et al.*, 1989; Phillips *et al.*, 1990; Connolly, 1991). VEGF mRNA has a wide distribution in normal and malignant tissue (Senger *et al.*, 1993) and is highly expressed in areas of active vascular proliferation. Unlike other growth factors, the mitogenic activity of VEGF is restricted to vascular endothelial cells. Increased expression of VEGF has been described recently in the hyperthecotic stroma of polycystic ovaries (PCO; Kamat *et al.*, 1995). Increased ovarian stromal blood flow in women with PCO has been demonstrated previously by colour Doppler blood flow imaging (Battaglia *et al.*, 1995; Zaidi *et al.*, 1995b; Aleem and Predanic, 1996). VEGF may also be a factor responsible for maintaining perifollicular blood flow and regulation of intrafollicular oxygen levels (Van Blerkom *et al.*, 1997).

The aim of this study was to determine serum VEGF concentrations and Doppler blood flow changes within the ovarian stroma of women with PCO and women with normal ovaries. We aimed to establish whether there is a relationship between serum VEGF concentrations and Doppler blood flow velocities within the ovarian stroma of women with normal and polycystic ovaries.

Materials and methods

Subjects

We recruited 60 consecutive women (46 nulliparous and 14 multiparous) who attended the London Women's Clinic for in-vitro fertilization (IVF) treatment. These women had no concomitant pelvic pathology, such as endometriosis, uterine fibroids or ovarian cysts. The 60 women were divided into three groups according to the following criteria. The 'normal ovary group' ($n = 36$) had regular ovulatory menstrual cycles and normal ovaries, as demonstrated on baseline ultrasound examination. The 'PCO group' ($n = 14$) had regular ovulatory menstrual cycles and PCO on pretreatment ultrasonographic assessment of the ovaries on days 2–3 of the menstrual cycle on at least one or on several occasions if the patient had been treated previously in the clinic (Adams *et al.*, 1985; Conway *et al.*,

Table I. Demographic data of the three groups of women

Variables	Normal ovaries (n = 36)	Polycystic ovaries (n = 14)	Polycystic ovarian syndrome (n = 10)
Mean age (years; range)	36.2 (28–43)	35.2 (25–40)	33.1 (28–41)
Mean ± SD body mass index (kg/m^2)	22.9 ± 2.8	24.0 ± 4.4	26.9 ± 2.2[a]
No. of parous women (%)	5 (13.8)	5 (35.7)	4 (40.0)
Duration of infertility (years)	6.8 ± 3.1	7.0 ± 3.8	6.7 ± 2.7
Causes of infertility			
Male factor (MF)	10 (27.7%)	5 (35.7%)	3 (30%)
Tubal factor (TF)	6 (16.6%)	3 (21.4%)	2 (20%)
Mixed (MF + TF)	5 (13.8%)	0	0
Endometriosis	7 (19.4%)	3 (21.4%)	2 (20%)
Unexplained	8 (22.2%)	3 (21.4%)	3 (30%)

[a]Statistically significant difference (P = 0.0001) between women with normal ovaries and polycystic ovaries compared with women with polycystic ovarian syndrome.

Table II. Serum vascular endothelial growth factor (VEGF) and hormone concentrations in the three groups of women

Hormones (mean ± SD)	Normal ovaries (n = 36)	Polycystic ovaries (n = 14)	Polycystic ovarian syndrome (n = 10)	Statistical significance
VEGF (ng/ml)	2.30 ± 0.55	3.4 ± 0.7[a]	3.20 ± 0.66[b]	<0.0001
Follicle stimulating hormone (IU/l)	6.8 ± 4.4	6.1 ± 1.4	5.9 ± 2.2	NS
Luteinizing hormone (IU/l)	4.5 ± 2.2	5.4 ± 2.4	9.2 ± 2.8[b]	<0.0001
Oestradiol (pmol/l)	158.3 ± 96.0	105 ± 45	134.6 ± 61.0	NS
Testosterone (nmol/l)	0.83 ± 0.50	0.88 ± 0.30	1.5 ± 1.2[b]	<0.05

NS = not significant.
[a]Statistically significant differences between women with normal ovaries and women with polycystic ovaries and polycystic ovary syndrome.
[b]Statistically significant differences between women with normal ovaries and women with polycystic ovarian syndrome.

1989; Fox *et al.*, 1991; Balen *et al.*, 1995) but did not have clinical or biochemical evidence of polycystic ovarian syndrome (PCOS). The 'PCOS group' (n = 10) had PCO on ultrasound examination and a history of anovulatory menstrual cycles and/or oligomenorrhoea, with or without hirsutism, acne and obesity and/or elevated serum LH (>10 IU/l) and/or elevated serum androgen concentrations. The descriptive data of the three groups of women are presented in Tables I and II.

Pelvic ultrasonography and Doppler blood flow velocity measurements

Ultrasound examinations were performed using a 5 MHz transvaginal transducer with colour and pulsed Doppler facilities (128XP/10 OB; Acuson, Mountain View, CA, USA). All examinations were performed at the beginning of a menstrual cycle (day 2 or 3) prior to starting IVF treatment.

The spatial peak temporal average intensity of ultrasound for B-mode and Doppler examinations was <50 mW/cm^2. Colour flow mapping and pulsed Doppler measurements were performed on ovarian stromal blood vessels once normal pelvic findings were confirmed. Areas of maximum colour intensity, representing the greatest Doppler frequency shifts, were selected for pulsed Doppler examination. Blood flow velocity waveforms were thus detected and recorded. The peak systolic blood flow velocity (PSV), time-averaged maximal velocity (TAMXV), pulsatility index (PI) and resistance index (RI) were assessed within both ovaries. PI values for each vessel were calculated from smooth curves fitted to the waveforms over three cardiac cycles according to the formula PI = (S – D)/TAMXV, where S is the peak systolic velocity, D is the minimum Doppler shifted frequency over a cardiac cycle and TAMXV is the time-averaged maximum velocity over a cardiac cycle (Gosling *et al.*, 1971). The PI and RI were used as measures of blood flow impedance distal to the point of sampling.

All observations were made by R.A., P.S. and L.E. The interobserver coefficient of variation (CV) for TAMXV was 24% and for PI was 14%, as described previously (Sladkevicius *et al.*, 1993). For TAMXV and PI, the intra-observer CV was <10%. All examinations were performed before midday to reduce the effects of diurnal variations in blood flow (Zaidi *et al.*, 1995a). Blood flow images were recorded on video and stored for later analysis.

VEGF assay

Blood samples for serum VEGF and hormone measurements were obtained between 08:00 and 12:00 h immediately after the ultrasound examination. Serum was stored at –70°C. VEGF concentrations were measured using an enzyme immunoassay (Cytokit Red™ EIA kits; Peninsula Labs Inc., College Park, MD, USA). The assay sensitivity was 0.195 ng/ml. The detectable range was 0.195–200 ng/ml, and cross-reactivity was <0.5% against cytokine standards (intra-assay CV 7.8%, inter-assay CV 12.2%).

Hormone assays

Follicle stimulating hormone (FSH) and LH were measured by a microparticle enzyme immunoassay (Abbot AxSYM reagent pack; Abbot, IL, USA). Oestradiol and testosterone concentrations were assessed using radioimmunoassays (Sorin clinical assays and coated

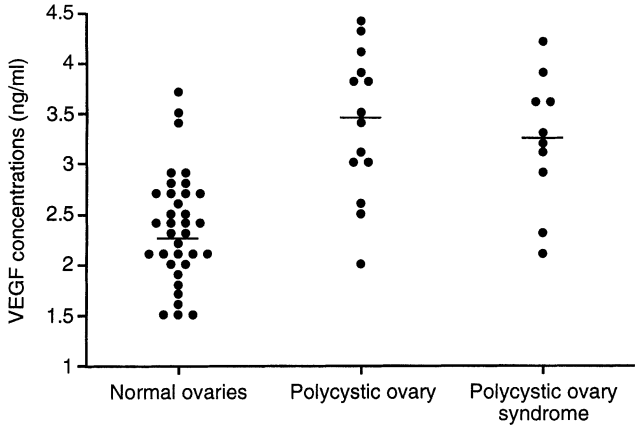

Figure 1. Serum vascular endothelial growth factor (VEGF) concentrations were higher in women with polycystic ovaries (3.4 ± 0.7 ng/ml) and polycystic ovarian syndrome (3.20 ± 0.66 ng/ml) than in women with normal ovaries (2.3 ± 0.5 ng/ml) ($P < 0.0001$).

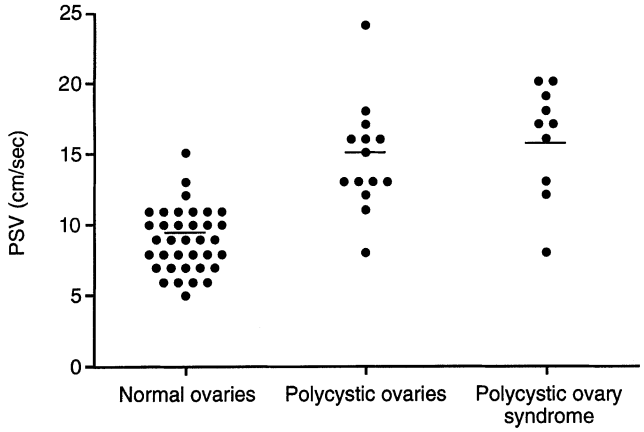

Figure 2. Peak systolic velocity (PSV) was higher in women with polycystic ovaries (15 ± 4 cm/s) and polycystic ovarian syndrome (16 ± 4 cm/s) than in women with normal ovaries (9 ± 2 cm/s) ($P < 0.001$).

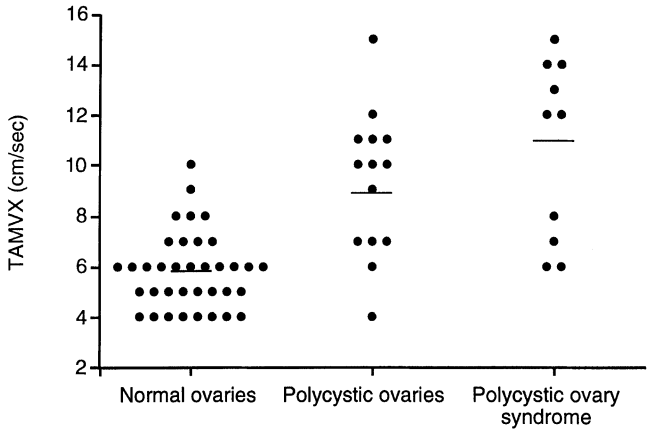

Figure 3. Time-averaged maximum velocity (TAMXV) was higher in women with polycystic ovaries (9 ± 3 cm/s) and polycystic ovarian syndrome (11 ± 3 cm/s) than in women with normal ovaries (5.8 ± 1.5 cm/s) ($P < 0.001$).

tubes; DPC, Los Angeles, CA, USA). The intra- and interassay CV were 4.0 and 7.5% for FSH, 4.0 and 7.5% for LH, 6.0 and 7.5% for oestradiol and 6.2 and 8.0% for testosterone respectively.

Statistical analysis

Data are represented as means ± SD. Comparisons between the three groups were performed using an analysis of variance. Student's *t*-test was used to compare continuous variables. P values < 0.05 were considered to be statistically significant. Correlations between variables were sought using Pearson's correlation coefficient.

Results

Demographic data from the three study groups were similar. However women with PCOS had a significantly higher body mass index ($P < 0.001$) than women with normal ovaries and PCO (Table I).

Serum VEGF and hormone concentrations of the three groups of women are shown in Table II. Mean serum VEGF concentrations in women with PCO and PCOS were significantly higher ($P < 0.0001$) than in women with normal ovaries (Figure 1). There were no statistically significant differences in serum VEGF concentrations between women with PCO and those with PCOS. Significantly higher LH and testosterone concentrations were found in women with PCOS than in the other two groups (Table II).

Recordings of blood flow velocity waveforms from the ovarian stroma were possible in 98.4% of women with PCO and in 88.4% in women with normal ovaries. Analyses of Doppler blood flow velocities of ovarian stromal blood vessels showed no significant differences in the PSV, TAMXV, PI or RI values between the right and left ovaries. Mean values of the two blood flow velocity measurements were therefore calculated and used for subsequent analyses. A subjective assessment of the ovaries demonstrated a larger number of blood vessels and a greater intensity of colour blood flow within the ovarian stroma of women with PCO and PCOS compared with women with normal ovaries.

PSV and TAMXV values were significantly greater in women with PCO and PCOS than in women with normal ovaries (Figures 2 and 3). There were no significant differences in the blood flow velocities (PSV and TAMXV) of women with PCO compared with women with PCOS. Mean RI and PI values were not different between the three groups of patients.

A positive correlation was demonstrated between serum VEGF concentrations and PSV ($r = +0.41$, $P = 0.001$) and TAMXV ($r = +0.45$, $P < 0.001$) in women in all three groups (Figure 4). No correlations were observed between VEGF concentrations and serum FSH, LH, oestradiol and testosterone concentrations. No relationship was found between serum hormone concentrations and peak blood flow velocities measured on day 2 or 3 of the menstrual cycle.

Discussion

The important findings of this study are that women with PCO have elevated serum VEGF concentrations and higher ovarian stromal blood flow velocities than women with normal ovaries. We also found a positive correlation between serum VEGF concentrations and blood flow velocities (PSV and TAMXV).

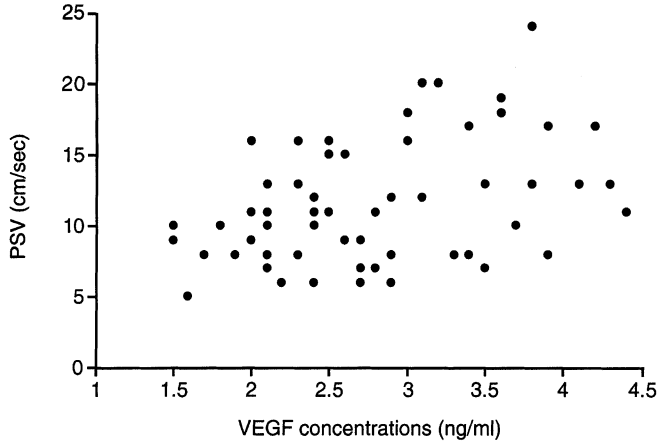

Figure 4. A positive correlation was observed between serum vascular endothelial growth factor (VEGF) concentrations and peak systolic velocity (PSV; $r = +0.44$, $P < 0.001$).

The results of this study confirm increased ovarian stromal blood flow velocities, as measured by colour and pulsed Doppler blood flow, in women with PCO and PCOS compared with women with normal ovaries. These findings were consistent with those published previously (Battaglia *et al.*, 1995; Zaidi *et al.*, 1995b; Aleem and Predanic, 1996), although we did not observe the low resistance indices in ovarian stromal blood vessels reported by Battaglia *et al.* (1995) and Aleem and Predanic (1996). Our present observations are in fact similar to those reported previously by Zaidi *et al.* (1995b). A recent study has also confirmed VEGF to be responsible for the maintenance of perifollicular blood flow (Van Blerkom *et al.*, 1997).

VEGF is a diffusable endothelial cell mitogen with potent angiogenic properties (Senger *et al.*, 1983; Ferrara and Henzel, 1989; Gospodarowicz *et al.*, 1989; Leung *et al.*, 1989; Connolly, 1991; Dvorak *et al.*, 1995). It mediates neovascularization in various biological processes, e.g. corpus luteum formation, embryogenesis, tumorigenesis and wound healing (Ferrara and Davis-Smith, 1997). VEGF mRNA is expressed in cells surrounding expanding vasculature. It is also constitutionally expressed in healthy human beings but, less so, in adults compared with fetuses, where it may play an important role in neoangiogenesis and organogenesis. VEGF is expressed more in pathological conditions, including tumours. Within the ovary it has been demonstrated in theca cells (Gordon *et al.*, 1996), granulosa and lutein cells (Phillips *et al.*, 1990; Shweiki *et al.*, 1993; Koos, 1995; Neulen *et al.*, 1995) and the interstitial tissue (Kamat *et al.*, 1995). VEGF not only mediates angiogenesis but also induces connective tissue stromal growth by increasing microvascular permeability, which leads to extravasation of plasma proteins. The extravascular matrix thus formed favours in-growth of new blood vessels and fibroblasts, which in turn organizes the avascular provisional fibrin matrix into a mature, vascularized connective tissue stroma (Kamat *et al.*, 1995).

These observations and our findings of elevated serum VEGF concentrations in women with PCO may explain in part the dense hyperechogenic and highly vascularized stroma of PCO, as demonstrated by Doppler blood flow studies in this and other studies (Battaglia *et al.*, 1995; Zaidi *et al.*, 1995b; Aleem and Predanic, 1996). The increased vascularity may result from overexpression of ovarian VEGF in women with PCO. This hypothesis is supported by the recent demonstration of a strong immunohistochemical staining of VEGF in the ovarian stroma of three patients with PCOS (Kamat *et al.*, 1995).

It is well established that growth factors are involved in intra-ovarian regulatory mechanisms. Insulin-like growth factor-I has been shown to induce LH receptors, and LH-mediated angiogenesis has been described previously (Findlay, 1986). Perhaps increased intra-ovarian concentrations of VEGF are related to increased secretion and pulsatility of LH, an important pathophysiological feature of PCOS. There are, however, large fluctuations of LH concentration in women with PCOS over time (Adashi *et al.*, 1995). Moreover, raised LH concentrations are seen in only 40% of women with PCOS (Conway *et al.*, 1989; Balen *et al.*, 1995). In addition, our study has shown that elevated concentrations of VEGF and increased stromal blood flow are found in women with PCO as well as those with PCOS, consistent with it being a constitutive feature of PCO. Unlike other growth factors responsible for angiogenesis, e.g. bFGF, which is largely intracellular and non-diffusable, VEGF is a soluble, diffusable growth factor. There is also evidence to suggest that angiogenic factors, like bFGF, TGF-β, platelet-derived growth factor and nitric oxide, act as agonists to the action of VEGF (Connolly, 1991).

Oestradiol plays an important role as a moderator of uterine and ovarian vascularity (Steer *et al.*, 1990; de Ziegler *et al.*, 1991). However, visualization of distinct stromal blood vessels in PCO in the early follicular phase of the menstrual cycle is the first striking difference from normal ovaries in the vascular pattern. We consider that this difference is unlikely to be caused by oestradiol, particularly because its concentrations are not raised in women with PCO.

It is well known that the risk of ovarian hyperstimulation syndrome (OHSS) in ovulation induction and in-vitro fertilization programmes is higher in women with PCO than women with normal ovaries (Rizk, 1991; MacDougall *et al.*, 1993). Again this finding might be the result of overexpression of VEGF in women with PCO, who characteristically recruit excess numbers of follicles with even small doses of gonadotrophin stimulation. Elevated concentrations of VEGF in various body fluids, e.g. ascitic fluid, follicular fluid, serum and urine, have been established recently in women undergoing ovarian stimulation who develop OHSS (McClure *et al.*, 1994; Robertson *et al.*, 1995; Krasnow *et al.*, 1996; Abramov *et al.*, 1997; Elchalal and Schenker, 1997; Rizk *et al.*, 1997). Our current findings provide a mechanism that helps to explain the link between VEGF, OHSS and PCO.

In conclusion, in women with PCO the increased vascularity that underlies the increased blood flow demonstrated by Doppler blood flow velocity measurements may be related to the higher serum concentrations of VEGF. In addition, VEGF also increases vascular permeability, which may contribute to the formation of the increased stroma in PCO and the increased capillary leakage associated with OHSS. The results may

explain the higher risk of OHSS in programmes of ovarian stimulation in patients with PCO compared with women with normal ovaries.

Though an elevated serum concentration of LH is a pathophysiological hallmark of PCOS, values of LH concentrations may fluctuate widely over time and are normal in women with PCO; indeed in our study they did not correlate with serum VEGF concentrations. Therefore we suggest that increased expression of ovarian VEGF, reflected in elevated serum concentrations, may be fundamental to the aetiopathogenesis of PCOS. Its measurement, along with Doppler blood flow studies of ovarian blood vessels, may provide an index for the risk of the development of OHSS.

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