Follicular vascularity—the predictive value of transvaginal power Doppler ultrasonography in an in-vitro fertilization programme: a preliminary study

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The aim of this prospective study was to investigate the ability of transvaginal power Doppler ultrasonography to assess the relationship between follicular vascularity and outcome in women undergoing in-vitro fertilization. Each of 38 subjects underwent a single transvaginal power Doppler ultrasound scan on the day of oocyte collection, where the vascularity of individual ovarian follicles was assessed, using a subjective system, and graded 1 to 4. In addition, conventional pulsatility indices (PI) of the uterine and intra-ovarian (stromal) arteries were calculated, which showed no significant differences between the pregnant and non-pregnant groups. Using power Doppler ultrasonography, a total of 188 follicles was studied. The follicular vascularity grade was found to be independent of follicular size and there was no significant difference in fertilization rates with different degrees of vascularity, although there was a trend towards higher fertilization rates with higher grade vascularity. There were 10 pregnancies, giving a pregnancy rate of 26.3% per embryo transfer. Pregnancies were confined to those women whose embryos were derived from follicles with grade 3 and 4 vascularity (pregnancy rates per embryo transfer of 12.5 and 61.5% respectively), with only those from grade 4 follicles resulting in livebirths. This preliminary study suggested that high grade follicular vascularity is associated with increased pregnancy rate and that there is a possible link between follicular vascularity and implantation potential.

Key words: follicular vascularity/in-vitro fertilization/transvaginal power Doppler

Introduction

Transvaginal ultrasound during assisted conception therapy has significantly enhanced the accuracy of monitoring folliculogenesis. Follicular diameters of >18 mm are generally accepted as a criterion of pre-ovulatory status, but ultrasound assessment of spontaneous cycles has shown ovulation to occur with a leading follicle of 15–29 mm (Trounson et al., 1981). In recent years, uterine, ovarian and follicular blood flow have been assessed using Doppler ultrasound in an attempt to identify conception cycles. However, at present, the results are inconclusive (Long et al., 1989; Battaglia et al., 1990; Scholtes et al., 1990; Kupesic and Kurjak, 1993).

Nevertheless, many authors agree that there is generalized decrease in impedance in the ovarian vascular bed during the luteal phase of the menstrual cycle which is thought to be due to neovascularization in the corpus luteum (Steer et al., 1990; Fleischer, 1991). Indeed, we have previously demonstrated changes in overall ovarian vascularity in both natural and stimulated cycles in women with unexplained infertility using colour Doppler imaging (CDI) (Chui et al., 1995). The results of these initial studies suggested an association between ovarian vascularity and outcome, but there was a significant overlap between conception and non-conception cycles. However, anecdotal evidence suggested an increase in follicular vascularity on CDI in the pregnant group. Indeed, it has been demonstrated that there is a significant increase in peri-follicular blood flow in the peri-ovulatory follicle, although this is not related to a decrease in vascular resistance (Campbell et al., 1993).

CDI can be used to assess follicular vascularity (Campbell et al., 1993) but attempts to quantify the vascularity would be limited by the fact that any flow at 90° to the probe would not be displayed. The recent introduction of power Doppler imaging overcomes this problem. Power Doppler is a new modality, which unlike conventional CDI, maps the amplitude of the frequency shift to produce a colour image. As a result, power Doppler is more sensitive than the conventional CDI and enables flows with lower volumes and velocities to be displayed. In addition, power Doppler is not affected by aliasing, which so often hampers clear images of CDI, and can display perfused regions where the mean velocity is zero (Ishrak et al., 1994; Meire and Farrant, 1994; Twining, 1994). Clearly these factors would improve visualization of small vessels.

The purpose of this study was to assess the follicular vascularity in patients undergoing in-vitro fertilization (IVF) treatment using power Doppler imaging, with a view to establishing the role of follicular vascularity assessment as a predictor of treatment outcome.

Materials and methods

A total of 38 patient volunteers undergoing standard IVF treatment were recruited into the study. The study was approved by the South Glamorgan Local Research Ethics Committee. A detailed explanation, both verbal and written, was given to the patients prior to recruitment. Consent to participate in the study was obtained from each patient.

A standard ovarian stimulation protocol was adopted. This consisted...
of menstrual delay using 5 mg twice daily of norethisterone from
day 21 of the preceding cycle until 2 days prior to the predetermined
day 1 of the treatment cycle. The gonadotrophin-releasing hormone
(GnRH) analogue, buserelin (Suprefact; Hoechst UK Ltd, Hounslow,
UK) 50 μgm (0.5ml) was administered s.c. on days 2, 3 and 4, and
225 units of human menopausal gonadotrophin (HMG) (Pergonal;
Seroni Laboratories, Welwyn Garden City, UK) or urofollitropin
(Metrodin High Purity; Seroni) was commenced daily from day 3 until
appropriate response was obtained. Human chorionic gonadotrophin
(10 000 IU HCG, Profasi; Seroni Laboratories, Woking, UK) was
given i.m. when at least two follicles were ≥18 mm in mean diameter.
Transvaginal follicular aspiration was performed 35 h after HCG
administration.

Approximately 2–3 h before oocyte collection, each subject under-
grew a single transvaginal Doppler ultrasound scan using a Toshiba
SSA-270A unit which was equipped with a 6 MHz curvilinear colour
Doppler probe (PVF-621VT). During the scanning procedure, the
flow velocity waveforms from the main uterine artery and the intra-
ovidian (stomatal) arteries were obtained on both sides. In addition,
the vascularity of the developing follicles with the highest flow was
studied. A varying number of follicles of different diameters were
examined depending on the patient’s ovarian response. Due to time
constraints, a maximum of eight follicles were studied for each patient.
The vascularity of each study follicle was subjectively graded by
the experienced operator (D.K.C.Chui and N.D.P.) at the time of
the scan using power Doppler image. The grading system devised
consisted of assessing the percentage of the follicular circumference
in which most flow was identified from a single cross-sectional slice.
The grading system was as follows: F1 = <25% of follicular circumference in which flow was identified (Figure 1a); F2 =
26–50% of follicular circumference in which flow was identified
(Figure 1b); F3 = 51–75% of follicular circumference in which flow was identified (Figure 1c); F4 = 76–100% of follicular circumference
in which flow was identified (Figure 1d).

In addition, the mean follicular diameter and the position within
the ovary of each studied follicle were recorded. A hard copy of
the print of the follicular position was obtained in order to assist in the
identification of the follicle at the time of oocyte collection.

At oocyte collection, all study follicles were identified and each
follicle aspirated in the conventional manner. Each oocyte of the
study follicles was individually processed. Embryological processing
was carried out without prior knowledge of the vascularity grading.
The embryos were selected for transfer according to the standard
protocol, based on morphology and cleavage rates. However, embryos
from study follicles with the highest vascularity grading were trans-
ferred if there was no difference in grade or quality of the embryos
compared with the non-study embryos, i.e. embryos derived from
follicles where the vascularity had not been assessed. A varying
number of embryos, between one and three, were transferred 48 h
after oocyte aspiration. Subsequently all patients received 100 mg
i.m. progesterone (Gestone; Paines and Byrne Ltd, West Byfleet, UK)
daily from day of oocyte collection for 16 days. Serum βHCG
estimation was carried out 14 days after embryo transfer. If serum
βHCG was positive, a clinical pregnancy was confirmed by ultrasound
scan 6 weeks and i.m. gestone was continued for the first trimester.

Statistical analysis
This was performed using a χ² test for the comparison of pregnancy
rate and unpaired Student’s t-test for mean values, with P <0.05
being regarded as significant.

Results
A total of 188 follicles was studied in 38 patients. There was
no significant difference in age, number of follicles >16 mm,
plasma oestriadiol concentration on the day of HCG administra-
tion, number of oocytes aspirated, fertilization rate, and number
and quality of embryos transferred between those women who conceived and those who did not (Table I).

The mean pulsatility index (PI) values of the left and right
uterine arteries in the non-pregnant and pregnant groups on
the day of oocyte collection is shown in Table II. No significant
difference was found between the left and right PI values or
between the PI of the two groups. Similarly, the mean PI
values of the intra-ovarian flow measured from within the
stromal vessels of the left and right ovaries did not demonstrate
any significant difference between the two sides or between
the women who conceived and those who did not (Table II).

Of the 188 follicles studied using power Doppler, 12 follicles
were graded F1; 78 as F2; 59 as F3 and 39 as F4. The results demonstrated that follicular vascularity grading was
independent of follicular size and oocyte retrieval rate. The
fertilization potential of oocytes, however, appeared to improve
with increased vascularity (Table III) but this was not statisti-
cally significant. It was noted, however, that oocytes derived
from follicles with poor vascularity resulted in a significantly
higher proportion of triploid embryos (25–30% for F1 and F2
follicles) than those of good vascularity (<6% for F3 and F4
follicles).

There were 10 clinical pregnancies giving a pregnancy rate
per embryo transfer of 26.3%. The breakdown of pregnancy
rate and follicular vascular grading is shown in Table IV. No
patient where the maximum follicular blood flow was grade 1
or 2 became pregnant. In the group of women whose transferred
embryos were derived from follicles with blood flow not
exceeding grade 3, the pregnancy rate was 12.5%. For women
with one or more embryos derived from follicles with grade
4 blood flow, the pregnancy rate was 61.5%. Thus the pregnancy
rate for women in the grade 4 group was significantly higher
than for women whose maximum follicular blood flow was of
grade 3 or less (P <0.01). Further detailed analysis of the 10
pregnancies revealed that only patients with embryos resulting
from follicles with grade 4 vascularity achieved livebirths
(Table V). There were a further four biochemical pregnancies
in which the serum βHCG concentrations 14 days after embryo
transfer were raised (>100 IU/l) but with no detectable
intrauterine or extrauterine gestational sac. All these occurred
in the group with grade 3 follicular vascularity. These latter
pregnancies were not included in the calculation of preg-
nancy rate.

Discussion
Transvaginal pulsed Doppler has been used quite extensively
to assess uterine and ovarian blood flow patterns in both
natural and stimulated cycles (Long et al., 1989; Scholtes
et al., 1990; Kupesic and Kurjak, 1993). At present, there is
little agreement as to whether the flow velocity waveforms
obtained from uterine or particularly ovarian circulation can
be used to predict the outcome of IVF programmes.

In the uterine circulation, several authors have shown a
significant difference in the mean value of uterine artery PI
between those women who conceived and those who did not,
The predictive value of Doppler ultrasonography in IVF

Table I. Clinical and laboratory data of all subjects in accordance with outcome of in-vitro fertilization (IVF)

<table>
<thead>
<tr>
<th></th>
<th>Pregnant (n = 10)</th>
<th>Non-pregnant (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (range)</td>
<td>32.0 (25–38)</td>
<td>33.0 (24–40)</td>
</tr>
<tr>
<td>No. follicles &gt; 16 mm (mean ± SD)</td>
<td>3.7 ± 2.0</td>
<td>3.8 ± 2.6</td>
</tr>
<tr>
<td>Plasma oestradiol concentration (nmol/l)</td>
<td>5.60 ± 3.0</td>
<td>5.97 ± 3.3</td>
</tr>
<tr>
<td>(day of HCG administration; mean ± SD)</td>
<td></td>
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<tr>
<td>No. oocytes aspirated (mean ± SD)</td>
<td>8.1 ± 2.9</td>
<td>7.5 ± 2.8</td>
</tr>
<tr>
<td>No. embryos transferred (mean ± SD)</td>
<td>72.7 ± 25.4</td>
<td>70.8 ± 24.5</td>
</tr>
<tr>
<td>Percentage grade A embryos (mean ± SD)</td>
<td>2.8 ± 0.6</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>Percentage grade A embryos (mean ± SD)</td>
<td>57.2 ± 33.2</td>
<td>66.7 ± 37.3</td>
</tr>
</tbody>
</table>

Differences not significant.
HCG = human chorionic gonadotrophin.

following IVF treatment. On the day of embryo transfer, Steer et al. (1992) were able to show a significantly lower uterine artery PI in women who became pregnant. Similarly, Sterzik et al. (1989) and Strohmer et al. (1991) were able to show significantly lower PI values in women who conceived, either on the day of follicular aspiration or on the day of HCG injection respectively. In the study by Coulam et al. (1995), although they were unable to demonstrate any predictive value of uterine artery PI in their IVF programme, they showed significantly lower PI value in conception cycles than non-conception cycles in their frozen–thawed embryo-transfer programme. The same investigators (Bustillo et al. 1995) later suggested that achievement of uterine artery PI <3.4 in recipient women may be beneficial prior to embryo transfer in oocyte donation treatment. This was in agreement with the data of Steer et al. (1992). Conversely, Favre et al. (1993) were unable to demonstrate any significant difference in uterine artery PI measured on the day of embryo transfer. Tekay et al. (1995) also failed to predict the outcome of pregnancy in 30 IVF patients, even though the patients were examined on six separate occasions throughout the menstrual cycle. In the present study, we were unable to show any significant difference in uterine artery PI in those women who conceived compared with those who did not when measured on the day of oocyte collection. In addition, the mean value of PI in the pregnant

Figure 1. Power Doppler scans showing grading system used to assess follicular vascularity. (a) Shows <25% circumferential flow (grade F1); (b) 26–50% flow (F2); (c) 51–75% flow and (d) >75% flow (F4).
Table II. Pulsatility indices of the left and right uterine and intra-ovarian arteries in the pregnant and non-pregnant groups (on day of oocyte collection)

<table>
<thead>
<tr>
<th></th>
<th>Uterine artery</th>
<th>Intra-ovarian artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>Left (mean ± SD)</td>
<td>3.42 ± 1.89</td>
<td>2.82 ± 1.27</td>
</tr>
<tr>
<td>Right (mean ± SD)</td>
<td>3.02 ± 1.02</td>
<td>2.88 ± 0.66</td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>3.22 ± 1.46</td>
<td>2.85 ± 0.97</td>
</tr>
</tbody>
</table>

Differences not significant.

Table III. Clinical and embryological data in relation to follicular vascularity grading

<table>
<thead>
<tr>
<th>Follicular vascularity grading</th>
<th>F1 (n = 12)</th>
<th>F2 (n = 78)</th>
<th>F3 (n = 59)</th>
<th>F4 (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular diameter (mm) (mean ± SD)</td>
<td>19.8 ± 3.2</td>
<td>19.8 ± 3.0</td>
<td>19.2 ± 3.0</td>
<td>18.7 ± 3.1</td>
</tr>
<tr>
<td>Oocyte retrieval rate (%)</td>
<td>7/12 (58.3)</td>
<td>63/78 (80.8)</td>
<td>47/59 (79.7)</td>
<td>28/39 (71.8)</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>4/7 (57.1)</td>
<td>39/63 (61.9)</td>
<td>34/47 (72.3)</td>
<td>22/28 (78.6)</td>
</tr>
<tr>
<td>Triploid rate (%)*</td>
<td>1/4 (25)</td>
<td>12/39 (30.8)</td>
<td>2/34 (5.9)</td>
<td>1/22 (4.5)</td>
</tr>
</tbody>
</table>

*P < 0.01 when combined rate of F1 and F2 is compared with that of F3 and F4.

F1, F2, F3, F4 grades correspond respectively to <25%, 26–50%, 51–75% and 76–100% of follicular circumference in which flow was identified.

Table IV. Breakdown of pregnancy rate and follicular vascularity grading

<table>
<thead>
<tr>
<th>Follicular vascularity grading</th>
<th>F1 (n = 12)</th>
<th>F2 (n = 78)</th>
<th>F3 (n = 59)</th>
<th>F4 (n = 39)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pregnancies (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (12.5)</td>
<td>8 (61.5)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>No. of patients</td>
<td>2</td>
<td>7</td>
<td>16</td>
<td>13</td>
<td>38</td>
</tr>
</tbody>
</table>

Pregnancy rate for F4 significantly higher than for F1, F2 or F3 (P < 0.01).

Table V. Outcomes of pregnancy in relation to follicular vascularity grading

<table>
<thead>
<tr>
<th>Follicular vascularity grading</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twins</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Single</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Ectopic</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>(Biochemical, not included in the analysis)</td>
<td>(4)</td>
<td>(–)</td>
</tr>
</tbody>
</table>

group was >3.0 which was suggested by Steer et al. (1992) as the maximum value for a successful outcome.

The present study also failed to demonstrate any significant difference in the intra-ovarian PI between those women who conceived and those who did not. This is in accordance with the study of Tekay et al. (1995) but disparate with other workers (Barber et al. 1988; Weiner et al., 1993). This finding is not totally unexpected since although there is a decrease in impedance in the ovarian vascular bed during the luteal phase of the menstrual cycle, the results of various studies are contradictory. Scholtes et al. (1990) were able to show a lower PI in the ovarian artery supplying the ovary bearing the dominant follicle during natural cycle and Kurjak et al. (1991) demonstrated a significant decrease in the resistance index obtained from an intra-ovarian artery after ovulation compared to the pre-ovulatory values in a group of 100 infertile women during natural and stimulated cycles. In a later study, Kupesic and Kurjak (1993) once again demonstrated a significant reduction in the resistance index pre- and post-ovulation, with no significant difference between spontaneous or stimulated cycles. Conversely, neither Sterzik et al. (1989) nor Tekay et al. (1995) were able to demonstrate any significant alterations in the ovarian vascular resistance during the course of the menstrual cycle of women undergoing ovarian stimulation. The conflicting results might be explained by the fact that some of the studies examined intra-ovarian blood flow (Kupesic and Kurjak, 1993; Tekay et al., 1995), whereas others assessed ovarian artery blood flow (Sterzik et al., 1989; Scholtes et al., 1990). Locating the ovarian artery is very difficult, and tubal artery signals might erroneously be detected during transvaginal scanning (Aleem et al. 1994). In addition, in the IVF studies, different treatment protocols, with varying effects on blood flow, were adopted in different studies (Barber et al., 1988; Weiner et al., 1993; Tekay et al., 1995).

From the above discussion, it is clear that whilst uterine and ovarian blood flow does change during the menstrual cycle, current pulsed Doppler methods fail to predict reliably the outcome of pregnancy in fertility programmes. Another approach is to use colour Doppler ultrasound to assess changes in ovarian and follicular vascularity. Studies of ovarian morphology in animal models have shown increased follicular vascularization during the course of the menstrual cycle (Zeleznik et al., 1981; Kranzfelder and Maurer-Schultze, 1989; Tanaka et al., 1989) and recent transvaginal colour Doppler studies have demonstrated changes in blood flow of maturing follicles...
(Campbell et al., 1993; Balakier and Stronell, 1994). To date, these studies have concentrated on using colour Doppler to locate follicular vessels whilst pulsed Doppler has been used to assess the velocity profiles. The results have shown a general increase in intra-follicular blood flow over the peri-ovulatory period (Campbell et al., 1993) and increasing peak systolic velocity with increasing follicular size (Balakier and Stronell, 1994). Indeed, Balakier and Stronell (1994) were able to show a significant elevation of peak velocity following HCG injection.

CDI can also be used to assess the degree and distribution of vascularity, and has been found to be useful in describing pathological circulation in malignant tumours (Kurjak and Predanic, 1992; Ajayi et al., 1992; Timor-Tritsch et al., 1993; Holcombe et al., 1995). Subjective assessment of ovarian vascularity has previously been attempted by Zaidi et al. (1995) who found increased intensity of demonstrable blood flow in the ovarian stroma of women with polycystic ovaries. In the present study, we have been able to develop a subjective grading system for follicular vascularity using power Doppler similar to that utilized in the assessment of tumour vascularity.

We have found that power Doppler ultrasonography improves visualization of follicular vascularity and it is possible to easily and reliably classify vascularity on the basis of percentage circumference demonstrating visible flow. We have shown that follicular vascularity is a possible new dimension in the assessment of folliculogenesis and is unrelated to conventional clinical and embryological parameters, e.g. follicular size and fertilization rate. It is possible, however, that with the continuation of this study and greater numbers of follicles examined the apparent trend towards improved fertilization rates with increased follicular vascularity may prove to be statistically significant.

It is significant that poor follicular blood flow (grade 1 and 2 vascularity) is associated with poor outcome and that pregnancies only occurred in those women with good flow (grade 3 and 4 vascularity). More detailed analysis revealed that successful pregnancies were confined to the group of women whose embryos were derived from follicles of grade 4 vascularity. The results thus suggest that, not only is good follicular blood flow necessary for conception to occur, but it is also essential in implantation and possibly maintenance of pregnancy. There is evidence that the status of the ovary at oocyte collection, measured by the activity of the ovarian cell in vitro, may provide a predictor of outcome in IVF. Thus, Michael et al. (1993) demonstrated an association between the expression of 11β-hydroxysteroid dehydrogenase by granulosa lutein cells in vitro and failed IVF implantation, while Gregory et al. (1994) similarly reported an association between the failure of cumulus cells to proliferate in vitro and failed implantation. Cumulus cells contribute to the intra-follicular environment of the developing oocyte (Gregory and Leese, 1996) and have been shown to express ‘vascular endothelial growth factors’ (VEGF) which have been implicated in the neovascularization of the follicle and implantation site (Shweiki et al., 1993; Dissen et al., 1994). The observed differences in follicular vascularity may, therefore, reflect the activity of the cumulus complex. Whatever the mechanism underlying this apparent link between follicular vascularity and outcome, the implication in IVF programmes is far-reaching, since pregnancy rates may well be increased by selecting for transfer those embryos derived from follicles with the highest grade of vascularization. Equally it may be appropriate to counsel patients as to the advisability of proceeding with oocyte collection in cycles where the follicular vascularity is universally poor.

In conclusion, the present study has shown that follicular vascularity may represent a new parameter in the assessment of folliculogenesis and a possible predictor of the outcome of assisted conception therapy. The mechanism controlling the varying degrees of blood flow is unknown, as is the link between vascularity and outcome. Further work is necessary to confirm and elucidate these findings.

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