Associations between ultrasound indices of follicular blood flow, oocyte recovery and preimplantation embryo quality

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The aim of this study was to elucidate possible relationships between ultrasound indices of follicular blood flow, oocyte recovery and the subsequent production and morphological quality of preimplantation embryos. A total of 27 women with bilateral tubal occlusion, undergoing treatment for infertility by in-vitro fertilization and embryo transfer, contributed data from 29 cycles. Transvaginal ultrasonography with colour Doppler imaging and pulsed Doppler spectral analysis was used to obtain indices of blood flow for each follicle immediately before it was aspirated. The main outcome measures for each follicle were the pulsatility index, peak systolic velocity, recovery or non-recovery of an oocyte and the subsequent production or non-production of an embryo. A total of 126 follicles were studied, 102 oocytes were recovered and 58 embryos (49 at grades I or II) were produced. There were six clinical pregnancies (pregnancy rate 27.3% per embryo transfer, 22.2% per patient). There was a significant correlation ($P < 0.0001$, Student’s $t$-test) between whether or not follicular blood flow was detected and whether or not an oocyte was recovered. The sensitivity of a test based on the presence of detectable blood flow and the subsequent recovery of an oocyte was 74% and the positive predictive value was 93%. The peak systolic velocity (PSV, measured in cm/s, mean ± SD) in follicles with detectable blood flow was significantly higher in follicles that were associated with the production of a preimplantation embryo ($17.2 ± 10.8$ cm/s) compared with those that were not ($9.9 ± 5.3$, $P < 0.0001$, Student’s $t$-test). There was a 70% chance of producing a grade I or II embryo if the follicular blood velocity was $≥10$ cm/s, compared with 14% if the PSV was $<10$ cm/s, or 18% if no blood flow was detected. We conclude that there is a physiological relationship between follicular blood velocity, oocyte recovery and the production of a high-grade preimplantation embryo, which may form the basis of a useful clinical test.

Key words: colour Doppler imaging/follicular blood flow/IVF/oocyte recovery/transvaginal ultrasonography

Introduction

The complex interrelated series of morphological, cellular and molecular events which culminate in mammalian ovulation have been reviewed (Bomsel-Helmreich et al., 1979, 1988; Morikawa et al., 1989). The process has many features which resemble those of an acute inflammatory reaction (Espey, 1980, 1994). The advent of transvaginal ultrasonography with pulsed Doppler spectral analysis (Baber et al., 1988; Deutinger et al., 1989) and colour Doppler imaging (Bourne, 1991) has provided a minimally invasive method to study morphological and vascular changes within the ovary. There have been detailed reports of changes in follicular blood flow over the peri-ovulatory period in apparently normal ovaries (Bourne et al., 1991; Collins et al., 1991; Campbell et al., 1993; Sladkevicius et al., 1993), and during the development and demise of the corpus luteum (Sladkevicius et al., 1993, 1994; Bourne et al., 1995).

There have also been reports on the concentration of oxygen within the ovarian follicle (Gosden and Byatt-Smith, 1986) and the possible association between changes in oxygenation with the processes which lead to ovulation (Fischer et al., 1992). Other workers have shown that oxygen is consumed during the development of cultured oocytes and blastocysts (Zeilmaker et al., 1972; Magnusson et al., 1986). Oxygen is required for oxidative phosphorylation and the concentration of ATP in spent media has been shown to be a useful index for predicting oocyte and embryo development (Van Blarom et al., 1995). The follicular concentration of oxygen is probably related to blood flow. Consequently, the aims of our study were to investigate possible relationships between indices of follicular blood flow, oocyte recovery and preimplantation embryo quality. The recruitment of patients being treated for infertility by in-vitro fertilization (IVF) and embryo transfer provides a range of follicles and oocytes at different stages of development. It was anticipated that the data obtained might reveal some information about the physiological role of blood flow changes within the pre-ovulatory follicle, and thus form the basis of new tests to maximize the chances of successful treatment.

Materials and methods

The aim was to study at least 100 follicles ($≥14$ mm mean maximum diameter) on two occasions, and subsequently oocyte recovery and preimplantation embryo quality during the treatment of infertility by IVF and embryo transfer. The study was approved by the Research Ethics Committee of King’s College Hospital. The women were recruited from the Assisted Conception Units at King’s College Hospital, London, UK and...
the Thomson Medical Centre, Singapore. Identical methods, equipment and clinical report forms were used at both centres after the implementation of common training and quality control procedures.

**Treatment regimen for IVF and embryo transfer**

All women received buserelin acetate (Suprefact; Hoechst UK Ltd., Hounslow, UK) from day 1 of menses (500 μg/day, s.c.) for 14 days, or until the ovaries and uterus appeared to be quiescent by pelvic ultrasonography. Human menopausal gonadotrophin (HMG, Pergonal i.m.; Serono Laboratories Ltd., Welwyn Garden City, UK) and buserelin were then administered daily. The dose of HMG was determined according to the woman's age, previous response to treatment, and serum concentration of follicle stimulating hormone (FSH; on days 2–4 of a previous menstrual cycle). Follicular and endometrial growth were monitored by pelvic ultrasonography. The dose of HMG was increased if necessary. Human chorionic gonadotrophin (HCG, Profasi 10 000 IU i.m.; Serono) was administered when the mean diameter of the leading follicle reached 18 mm. Transvaginal ultrasound-directed follicle aspiration was performed 34–36 h after the injection of HCG (Waterstone and Parsons, 1992). Methods for the culture of oocytes, spermatozoa and embryos have been described (Bolton et al. 1989). A maximum of three embryos were transferred 2 or 3 days after oocyte retrieval and IVF. Progesterone pessaries (200 mg Cyclogest; Hoechst) were given vaginally twice daily for 16 days from the day of follicle aspiration.

**Inclusion criteria for study**

All women recruited for the research study had a maximum of six follicles in either ovary immediately before the administration of HCG. Their previous medical history must have indicated regular menstrual cycles (25–33 days), a baseline concentration of FSH of <8 IU/l, and bilateral tubal occlusion. The current partner of each woman must have had normal spermiograms, with a sperm concentration >20×10⁶/ml semen, >60% motility and >30% normal forms on two separate occasions.

**Transvaginal ultrasonography**

The first examination with transvaginal colour Doppler imaging and pulsed Doppler spectral analysis was performed on the day of, but prior to, HCG administration (before HCG). The second was undertaken immediately before transvaginal ultrasound-directed follicle aspiration (before aspiration). All scans were performed by the same operator (G.N.) and checked by a colleague. The transducer was orientated so that the ultrasound beam was in the transverse plane. During the first scan the follicles were numbered 1 to 6 and photographed to aid subsequent identification. An ATL-Ultramark 9, HDI machine (Advanced Technology Laboratories, Bothell, WA, USA) with a 5 MHz transvaginal probe was used for 96% of the scans. The spatial peak temporal average intensity for B-mode and Doppler imaging was <80 mW/cm², which is well within the safety limits recommended by the Bioeffects Committee of the American Institute of Ultrasound in Medicine (Gill, 1982). The filter for spectral analysis was set at 50 Hz.

The sample volume size ranged from 1.5 to 1.0 mm. All follicles were examined for the presence of colour signals indicative of vascularity. Care was taken to ensure that signals assigned to one follicle were not close to another. A pulsed Doppler range gate was placed over the vessels of interest. Flow velocity waveforms (FVW) obtained from these vessels were used for spectral analysis. Indices of blood flow for each follicle were recorded from those vessels with the highest peak systolic velocity (PSV). A double-channel needle was used to puncture and aspirate each follicle. Residual fluid in the needle and tubing was displaced into the collecting tube by injecting 1.5 ml of medium into the follicle. This procedure allowed the source of each oocyte to be determined with confidence. The follicular fluid was stored for subsequent analysis (to be reported elsewhere). Ultrasound images of a stimulated ovary with multiple follicular development are shown in Figure 1. The pulsed Doppler range gate is positioned over the most significant area of vascularity for follicle no. 2 in the upper image and for follicle no. 4 in the lower image. The FVW in this example show that the velocity is not necessarily related to the area of colour observed.
Follicular vascularity and IVF treatment

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**End points**

The maximum transverse \( (D_1) \), anteroposterior \( (D_2) \) and longitudinal \( (D_3) \) diameters of all follicles were measured (cm) and the volumes (ml) estimated with the formula for a modified prolate ellipsoid: volume = \( (0.523 \times D_1 \times D_2 \times D_3) \). Blood flow impedance was expressed as the pulsatility index (PI), which was calculated from curves fitted to FVW over three cycles according to the formula: \( PI = (S - D)/M \), where \( S \) is the peak systolic shifted frequency, \( D \) is the maximum end diastolic frequency, and \( M \) is the time-averaged maximum frequency over the whole cardiac cycle. A reduction in PI is thought to reflect a decrease in impedance distal to the point of sampling, which may indicate an increase in blood flow. As the direction of the vessels was not apparent, the probe was angled until a FVW with the maximum peak systolic shifted frequency was obtained, and the PSV was then measured. It is generally accepted that for each follicle at least one vessel will be at a low or zero angle so that reproducible measurements of PSV can be made. A one-way analysis of variance of replicate data (3–5 measurements by the same operator) from each of 13 women (previously attending the ovarian screening clinic) with a pre-ovulatory follicle or a corpus luteum gave a coefficient of variation of 7.6% for the PI and 4.4% for the PSV. The absence of FVW around a given follicle was reported as no blood flow detected.

The following data were recorded by follicle: the recovery or non-recovery of an oocyte, the subsequent presence or absence of fertilization, the number of pronuclei (i.e. 0, 1, 2, 3 or >3) on day 1 after oocyte recovery, the number of cells per preimplantation embryo and the morphological grade (from I, good to IV, poor) on day 2 or 3 according to the criteria of Bolton et al. (1989), except that the numbering system was reversed. The number of treatment cycles per patient, embryo transfers and clinical pregnancies (the presence of an active fetal heart by pelvic ultrasonography) were also recorded. The data on follicle volume, all variables before HCG administration, and the changes before follicle aspiration will be the subjects of separate publications.

**Statistical analysis**

Since women could have had more than one follicle examined, the variance of blood flow velocity values between and within women was assessed using standard analysis of variance methods. Velocity was analysed as both a categorical and a continuous variable. Significance of differences between two categorical variables was assessed by applying the \( \chi^2 \) test, and the difference between groups for continuous variables was assessed with Student’s \( t \)-test. Receiver operating characteristic curves for continuous variables were used to assess the potential value of follicle function tests. The Statistical Package for the Social Sciences (SPSS) was used for all analyses.

**Results**

A total of 27 women (aged 29–43 years) fulfilled the criteria for entry into the study, of whom 10 were attending King’s College Hospital, and 17 the Thomson Medical Centre. A total of 29 treatment cycles and 126 follicles were studied. Of the

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**Table I. Association between detection of follicular blood flow immediately before ultrasound-directed follicle aspiration and oocyte recovery**

<table>
<thead>
<tr>
<th>Blood flow detected</th>
<th>Oocytes recovered</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>75 (60)</td>
<td>6 (5)</td>
</tr>
<tr>
<td>No</td>
<td>27 (21)</td>
<td>18 (14)</td>
</tr>
<tr>
<td>All</td>
<td>102 (81)</td>
<td>24 (19)</td>
</tr>
</tbody>
</table>

\( P < 0.0001, \chi^2 \) test.

**Table II. Mean pulsatility index and follicular peak systolic blood velocity immediately before ultrasound-directed follicle aspiration with regard to subsequent production of a preimplantation embryo after in-vitro fertilization**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Embryo produced*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pulsatility index</td>
<td>46</td>
<td>29</td>
</tr>
<tr>
<td>PSV (cm/s)</td>
<td>46</td>
<td>29</td>
</tr>
</tbody>
</table>

\( * \)In those follicles where blood flow was detected and an oocyte was aspirated.

**Table III. Association between indices of follicular blood flow immediately before ultrasound-directed follicle aspiration and the non-production or morphological grade of preimplantation embryos**

<table>
<thead>
<tr>
<th>Index of follicular blood flow</th>
<th>No oocyte and/or embryo produced</th>
<th>Oocyte and embryo produced</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Grades III &amp; IV n (%)</td>
<td>Grades I &amp; II n (%)</td>
</tr>
<tr>
<td>Absence</td>
<td>32 (71.1)</td>
<td>5 (11.1)</td>
<td>8 (17.8)</td>
</tr>
<tr>
<td>PSV &lt;10 cm/s</td>
<td>22 (78.6)</td>
<td>2 (7.1)</td>
<td>4 (14.3)</td>
</tr>
<tr>
<td>PSV ≥10 cm/s</td>
<td>14 (26.4)</td>
<td>2 (3.8)</td>
<td>37 (69.8)</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>9</td>
<td>49 (126)</td>
</tr>
</tbody>
</table>
women, 21% had one or two study follicles, 41% had three or four, and 38% had five to eight. The mean maximum follicle diameter was 22 mm (range 18–31). The oocyte recovery rate per follicle was 81%. A total of 58 preimplantation embryos were produced and 22 transfers (of 1–3 embryos) were attempted. The clinical pregnancy rate was 20.7% per cycle, 27.3% per embryo transfer and 22.2% per patient. The variance of follicle blood velocity between women was not significantly different from that within women ($F = 1.25, P = 0.24$). Accordingly, in the following analysis, values for PSV were treated as independent measurements.

**Oocyte recovery**

There was a significant association ($\chi^2$ test) between whether or not FVW were detected immediately before follicle aspiration and whether or not an oocyte was recovered (Table I). The sensitivity of a test based on the presence of follicular blood flow for predicting the recovery of an oocyte in these data would be $75/102 \times 100 = 74\%$. The positive predictive value of the test in this setting was $75/81 \times 100 = 93\%$.

**Preimplantation embryo quality**

Of the oocytes obtained from follicles with detectable blood flow, 45 were fertilized (56%) and 41 formed grade I or II embryos. Of the 27 oocytes recovered from follicles with no detectable blood flow, 13 (48%) went on to form an embryo (of which $50\%$ were grades I or II). In all, 45 embryos (78%) were derived from oocytes that came from follicles with a measurable PSV. There was a significant difference in means between the PSV immediately before follicle aspiration and the subsequent production of an embryo after IVF ($P < 0.0001$) (Table II). There was no corresponding significant difference in the PI. A receiver operating characteristic curve for PSV is shown in Figure 2. A threshold value $\geq 10$ cm/s for the PSV would predict the production of embryos in 90% of cases, with a 0.4% false positive rate. The corresponding results for a threshold value of $\geq 15$ cm/s would be $71$ and $18\%$ respectively. The association between indices of follicular blood flow with the non-production or morphological grade of embryo is shown in Table III. There is $\sim 29\%$ chance of an embryo being produced if the follicular blood flow is not detectable. Conversely, there is a $70\%$ chance of producing a grade I or II embryo if the follicular PSV is $\geq 10$ cm/s, compared with 14% if the PSV is $< 10$ cm/s.

**Discussion**

The potential role of colour Doppler imaging in the overall assessment of folliculogenesis in patients undergoing IVF and embryo transfer has been studied previously (Balakier and Stronell, 1994). In that study, patients with a variety of causes for their infertility were recruited and treated according to conventional procedures. Mean values for the intra-ovarian resistance index (RI) and the PSV were calculated for each patient. There was no significant difference in the values for these indices between groups of women categorized by age, or by whether or not they became pregnant during the treatment cycle. These findings may be explained by the experimental design, which would tend to minimize the between-women differences in values for both indices of ovarian blood flow. Similarly, a longitudinal study of blood flow changes in the uterus and ovarian vasculature of patients undergoing IVF and embryo transfer revealed that the maximum PSV recorded from a study of both ovaries in each subject before oocyte recovery was similar in women who subsequently became pregnant to those who did not (Tekay et al., 1995).

We have attempted to study the relationship between follicular blood flow and the production of morphologically normal embryos in more detail. Accordingly, the protocol was designed to study individual follicles, oocytes and preimplantation embryos. Patients were selected who had a limited number of developing follicles, to facilitate the technical difficulty of measuring the indices of blood flow and relating each oocyte recovered to the appropriate follicle of origin. The embryologist was unaware of the ultrasonographer’s findings prior to embryo transfer. The oocyte recovery rate (81%) was consistent with our reported experience (Waterstone and Parsons, 1992). The selection of patients who had a limited number of developing follicles also provided a useful working range for all indices of blood flow. The outcome measures used in our study did not involve the number of embryos transferred to the uterus, or the variable role of endometrial factors involved in implantation. We do not know if the approach could be applied to the evaluation of ovaries with more than six follicles, or what the results would have been if the more elaborate scoring system of Veeck (1988) had been used to describe oocyte and preimplantation embryo status.

The results of our study immediately before follicle aspiration showed that there was a significant relationship between follicular PSV (but not PI) and the ability to recover an oocyte, and the subsequent production of morphologically normal embryos. The findings are consistent with previous studies on the relationship between PSV and PI in unstimulated ovaries and the times of follicular rupture, i.e. presumed ovulation (Campbell et al., 1993) and the luteinizing hormone surge (Sladkevicius et al., 1993). The relationship between ultrasound indices of follicular blood flow and the onset of atresia during spontaneous ovarian cycles is unknown. The consistent finding of an increase in the maximum follicular blood velocity prior to rupture without an associated decrease in indices of impedance may reflect the presence of spatially distributed biochemical processes within the pre-ovulatory follicle. The current findings are consistent with some studies on the association between oxygen tension, ATP concentration and embryo development (Fischer et al., 1992; Van Blerkom et al., 1995). There is convincing evidence, however, that the formation of reactive oxygen species might have a regulatory effect on folliculogenesis and ovulation (Behrman et al., 1993) and inhibit embryogenesis in vitro (Johnson and Nasr-Esfahani, 1993). In addition, there may be an interesting relationship between low blood velocity and the development of a luteinized unruptured follicle (Zaidi et al., 1995).

The data from the present study may have use in the future management of some patients being treated by IVF and embryo transfer. The knowledge that 72% of follicles with a PSV $\geq 10$ cm/s can lead to the production of a grade I or II
preimplantation embryo could be useful in the selection of oocytes for fertilization. There is also the possibility that the information could be used to optimize the dose of drugs required for ovarian stimulation in subsequent treatment cycles. The data may also be useful for selecting embryos for transfer into the uterus (i.e. those originating from follicles with a good blood supply). Conversely, the technique could be used to identify patients with only a small number of ischaemic follicles in a given treatment cycle. These women might not benefit from ultrasound-directed follicle aspiration until a subsequent treatment cycle. Prospective clinical trials are required to test these possibilities.

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References


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