Endometrial and subendometrial perfusion are impaired in women with unexplained subfertility

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BACKGROUND: We used three-dimensional power Doppler angiography (3D-PDA) to examine the periodic changes in endometrial development and subendometrial vascularity during the normal menstrual cycle in 29 women with unexplained subfertility and 19 controls. METHODS: 3D-PDA was performed on alternate days from day 3 of the cycle until ovulation and then every 4 days until menses. VOCAL™ (Virtual Organ Computer-aided Analysis) and shell-imaging were used to define and to quantify the power Doppler signal within the endometrial and subendometrial regions producing indices of their relative vascularity. RESULTS: Women with unexplained subfertility demonstrated significant changes with time (P < 0.001) in the indices of vascularity within the endometrium and subendometrium during the menstrual cycle characterized by a pre-ovulatory peak and post-ovulatory fall. These changes mirrored those observed in the control group but were significantly reduced in the endometrium and subendometrium during the mid-late follicular phase and early luteal phase. There were no differences in endometrial thickness or volume between the groups or in the plasma concentrations of estradiol or progesterone. CONCLUSIONS: Endometrial and subendometrial vascularity are significantly reduced in women with unexplained subfertility during the mid-late follicular phase irrespective of estradiol or progesterone concentrations and endometrial morphometry.

Key words: endometrial perfusion/menstrual cycle/power Doppler/three-dimensional ultrasound/unexplained subfertility

Introduction

The aetiology of infertility is diverse. Although the majority of couples will be found to have an identifiable male or a female factor explaining their inability to conceive, 10–25% will be labelled as having ‘unexplained infertility’ (Hull et al., 1985). Within this cohort of patients there is a spectrum of disorders ranging from those patients with a reduced fecundity that will conceive with time to those in whom conception is very unlikely with current medical practice. From the female perspective subfertility may reflect a diminished ovarian reserve, a disorder of oogenesis or suboptimal endometrial receptivity.

Whilst there are several clinically useful tests available that may uncover ovarian dysfunction, the endometrial requirements for implantation remain an enigma. The endometrium is likely to be a key determinant in successful implantation but its individual contribution relative to that of the developing embryo is an area of continued debate (Schwartz et al., 1997). This partly reflects the current assessment of the endometrium, which is invariably restricted to a simplistic measurement of its thickness and description of its appearance (Turnbull et al., 1995). These parameters are important in that conception is less likely to occur in patients with thinner endometria or in those with aberrant echogenic patterns, but they are not specific and their value as prognostic indicators of implantation following embryo transfer is limited (Friedler et al., 1996).

Doppler ultrasound assessment of the uterine blood supply appears more informative. Pulsed wave Doppler waveform indices of high resistance to flow within the uterine artery have been repeatedly linked to poor outcomes during assisted reproduction treatments (Sterzik et al., 1989; Steer et al., 1992; Coulam et al., 1994; Levi-Setti et al., 1995). Controlled ovarian stimulation itself, however, has a significant effect on uterine perfusion (Kupesic and Kurjak, 1993), making information derived from patients undergoing assisted reproduction not necessarily representative of women with unexplained subfertility as a whole. Surprisingly there is a relative paucity of work specifically looking at pelvic blood flow in these women during a spontaneous menstrual cycle. Evidence suggests that the observed increase in impedance to uterine artery blood flow during ovarian stimulation exists prior to treatment (Goswamy et al., 1988; Kurjak et al., 1991; Tinkanen et al., 1994). However, blood flow characteristics within the uterine artery may not be representative of endometrial perfusion, and whereas pulsed wave Doppler may be used to examine the smaller downstream radial and spiral arteries it reveals information from single vessels
rather than from the endometrium as a whole. Recent evidence suggests that different information is obtained from different uterine sampling sites, thereby limiting the value of such studies even further (Hsieh et al., 2000).

Colour Doppler ultrasound may be used to examine the uterine vasculature as a whole through the demonstration of blood flow as a colour map. Subjective analysis of vessel distribution has shown that the absence of subendometrial and intra-endometrial colour Doppler signals is associated with non-conception cycles (Applebaum, 1995; Zaidi et al., 1995). Power Doppler is better suited to the study of endometrial perfusion as it is more sensitive to low flow and thus overcomes the problems of angle dependence and background noise associated with both colour and pulsed wave Doppler (Rubin and Adler, 1993; Fortunato, 1996). In addition, whereas colour Doppler provides qualitative information, the power Doppler signal can be subsequently analysed to produce quantitative information through one of several computer software packages (Amso et al., 2001; Jun et al., 2002).

In combination with three-dimensional ultrasound, power Doppler offers a tool with which one may not only demonstrate but also quantify total endometrial and regional uterine blood flow (Fleischer, 2001; Raine-Fenning et al., 2003). In the present study we used the technique of three-dimensional power Doppler angiography to examine the (3D-PDA) hypothesis that endometrial development and subendometrial blood flow are impaired in women with unexplained subfertility.

Materials and methods

Experimental design

The design was that of a prospective, longitudinal observational study. Subjects were seen on alternate days from day 3 of the menstrual cycle until ovulation, confirmed both ultrasonographically and endocrinologically following demonstration of the pre-ovulatory surge in LH, and then every 4 days until the next menstrual period. Approval was given by the hospital ethical committee following assessment of the study by the Research and Development Department in accordance with local practice.

Patient selection

Thirty women with unexplained subfertility were recruited through the fertility clinic and via advertisement in the local media. The inclusion criteria required ultrasonographic and endocrinological demonstration of ovulation, confirmation of tubal patency and seminal fluid analysis results within the World Health Organization (1999) guidelines. Tubal patency was determined by either laparoscopy and hystrotubation or hysterosalpingo-contrast-sonography (HyCoSy) with the positive contrast agent Echovist® (Campbell et al., 1994). Women with a history of pelvic or abdominal surgery, previous pelvic inflammatory disease and clinical features suggestive of endometriosis including heavy, painful periods and deep dyspareunia were excluded as were women with pelvic pathology including ovarian cysts, polycystic ovary syndrome, endometrial polyps and fibroids. Assessment was undertaken by an experienced clinician (N.J.R.F.) who determined the patients’ eligibility for the study.

The results of the study group were compared with those derived from a control group whose results have been previously reported (Raine-Fenning et al., 2004a). Briefly, this consisted of 27 women of reproductive age without a history of subfertility or menstrual dysfunction who were not using any medication that could potentially interfere with the pelvic blood supply and who had regular menstrual cycles. Of these 27 patients, eight were current smokers and the data from these individuals have been excluded since this was found to exert a significantly negative effect on measurements of subendometrial vascularity, leaving a control group of 19 patients for comparison in this study. Parity was not considered in the recruitment criteria and of these 19 women, five were nulliparous and 14 parous. The median duration from the last delivery in the parous women was 34 months (range 11–94).

Data acquisition and analysis

The patients in this study were examined in an identical manner to those women recruited in the preliminary study designed to define normal uterine blood flow during the menstrual cycle (Raine-Fenning et al., 2004a). In summary, an automated 7.5 MHz transvaginal three-dimensional transducer (Voluson 530D™; GE Kretz, Austria) was used to acquire power Doppler information from the uterus using identical settings (pulse repetition frequency 1.0, power 4.0, colour gain 38.4, wall motion filter 75, rise 0.2, persistence 0.8, reject 82 and with the central frequency set to mid) and technique in all cases. The data were initially stored on magnetic optical disks before being sent to a personal computer via a dedicated DICOM link (Digital Imaging and Communications in Medicine). Volumetric and vascular measurements were then undertaken using Virtual Organ Computer-aided Analysis (VOCAL™, GE Kretz) imaging programme. This allows the user to manually define the volume of interest and then apply a shell of variable thickness that parallels the originally defined surface contour (Raine-Fenning et al., 2003). These techniques allowed for the definition of the endometrium, through the delineation of the myometrial-endometrial border, and the subendometrium within 5 mm of this border, through the application of a surface shell, respectively, and the subsequent quantification of the power Doppler signal within these regions. The vascularization index (VI) represents the relative proportion of power Doppler data within the defined volume, the flow index (FI) the mean signal intensity of this power Doppler information and the vascularization flow index (VFI) a combination of both. These parameters, which have been suggested as representative of vascularity and flow intensity, are unitless with the exception of the VI that is expressed as a percentage and will be referred to as ‘indices of vascularity’ (Pairleitner et al., 1999). Having confirmed our own interobserver reliability of both the acquisition and the quantification of 3D power Doppler data from the endometrium, we chose to acquire a single data set from each patient and subsequently conducted two serial measurements of all resultant data sets whose mean value was recorded (Raine-Fenning et al., 2004b, 2003).

Blood was collected into heparinized tubes at each visit, the plasma separated through centrifugation and stored at −20°C. Steroid hormone measurements were made by radioimmunoassay as described previously (Raine-Fenning et al., 2004a). The sensitivity, intra- and inter-coefficients of variation were 50 pmol/l, 10.6 and 13.5% for plasma estradiol measurements, 175 pmol/l, 8.6 and 14.5% for plasma progesterone measurements and 1.3 IU/l, 6.6 and 12.8% for LH respectively.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Release 10.1.4; SPSS Inc., USA). A general linear model with repeated measures was used to determine differences with time within each group and the interaction between
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Results

Of 64 women interviewed with reputed ‘unexplained subfertility’, only 34 met the recruitment criteria. The remaining 30 subjects failed to meet the inclusion criteria mainly because of histories suggestive of potential pelvic disease or because they had not been adequately investigated to support a diagnosis of ‘unexplained subfertility’. Of the 34 women who met the inclusion criteria, four were lost on their initial ultrasound scan, three with polycystic-appearing ovaries and one with a subendometrial leiomyoma. The remaining 30 women were entered into the study and data acquired accordingly. However, the compounding effect of smoking was not accounted for in the original exclusion criteria and further to the results obtained in the control group we subsequently excluded women currently smoking. This resulted in the loss of a single patient from the study group, giving a final study group of 29 women, with a mean duration of failure to conceive of 28.14 months, whose characteristics are shown in Table I alongside those of the 19 control women. Of these women, 82.8% (24/29) had undergone laparoscopy as part of their fertility assessment.

Whilst there were no differences in age or body mass index between the two groups, gravidity (P < 0.05) and parity (P < 0.01) were significantly lower in the subfertile population with 11.1% (5/19) of the control patients being nulliparous compared to 58.6% (17/29) of the subfertile population. A higher proportion of the subfertile group also gave a history of previous miscarriage (P < 0.05). There were no differences in cycle length overall or between the relative duration of the follicular and luteal phases (Table I).

Endometrial morphometry

There were no significant differences between the two groups in either endometrial thickness or volume throughout the menstrual cycle, although both measurements tended to be higher in the subfertile group at every time-point (Figure 1). Both parameters demonstrated significant changes with time (P < 0.01), endometrial growth being restricted to the proliferative phase. Growth remained relatively static during the secretory phase where the mean endometrial thickness was 9.11 mm in the control patients and 10.24 mm (P > 0.05) in the subfertile group with corresponding values of 3.48 and 4.51 cm³ for endometrial volume (P > 0.05) in each group respectively.

Endometrial vascularity

Women with unexplained subfertility demonstrated significant changes with time (P < 0.001) in the indices of vascularity within the endometrium (Figure 2) and subendometrium during the menstrual cycle (Figure 3). The variation was characterized by a pre-ovulatory peak in each index followed by a fall reaching a nadir ~5 days after ovulation before a final increase during the transition from the mid-late luteal phase. These changes mirrored those observed in the control group but were significantly reduced in the women with unexplained subfertility.

The endometrial VI (P < 0.001) and FI (P < 0.05) were significantly reduced in the women with unexplained subfertility (Figure 2a and b). Assessment of individual time-points throughout the menstrual cycle confirmed that these differences were restricted to the mid-late follicular phase and very early luteal phase with no differences evident in any index during the early follicular phase or mid-late luteal phase. Although the difference did not reach statistical significance there was a distinct trend to a similar reduction in the endometrial VFI (P = 0.058) between the groups (Figure 2c).

A similar pattern of impaired vascularity in the group with unexplained subfertility was seen at the level of the subendometrium (Figure 3). In this region, however, it was the subendometrial VI (P < 0.001) and VFI (P < 0.01) that were significantly reduced (Figure 3c). Again there was a clear trend in the remaining index, the subendometrial FI (P = 0.088), which just failed to reach significance (Figure 3b).

Sex steroids

Whilst the variation in each index paralleled the changes in estradiol during the follicular phase and progesterone during the luteal phase, there were no differences in either steroid between the two groups (Figure 4).

Discussion

This is the first study to use 3D-PDA to identify a deficit in endometrial and subendometrial ‘perfusion’ in a cohort of women with unexplained subfertility. These differences were not constant but dependent upon the stage of the menstrual cycle and restricted to the mid-late follicular phase. This was concomitant chronologically with the peak in serum estradiol that occurred 3 days prior to ovulation. However, the differences between the two groups cannot be explained by lower estradiol levels in the subfertile population, as there was no significant difference between the plasma concentrations throughout the menstrual cycle. Another possible explanation relates to the well-defined transient increase in myometrial basal tone and uterine contractility seen during the peri-ovulatory period (Lyons et al., 1991; de Ziegler et al., 2001).

Table I. Demographics and cycle characteristics for the two groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n = 19)</th>
<th>Subfertile group (n = 29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.3 ± 1.47</td>
<td>35.4 ± 0.80</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.64 ± 0.81</td>
<td>25.95 ± 0.95</td>
<td>NS</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1.48 ± 0.29</td>
<td>0.87 ± 0.09</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Parity</td>
<td>1.30 ± 0.27</td>
<td>0.23 ± 0.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>0.07 ± 0.06</td>
<td>0.63 ± 0.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Follicular phase (days)</td>
<td>15.33 ± 0.64</td>
<td>14.28 ± 0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Luteal phase (days)</td>
<td>13.78 ± 0.38</td>
<td>14.13 ± 0.59</td>
<td>NS</td>
</tr>
<tr>
<td>Total cycle length (days)</td>
<td>29.11 ± 0.47</td>
<td>27.93 ± 0.32</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
NS = not significant.
This has been associated with a temporary increase in resistance to uterine blood flow (Scholtes et al., 1989; Steer et al., 1990; Collins et al., 1991; Sladkevicius et al., 1993) but is unlikely to account for the differences between the two groups, which occurred prior to ovulation. Hypothetically, therefore, the differences in endometrial and subendometrial 'perfusion' are more likely to reflect aberrancies at a local level and the end organ effect of estradiol on blood vessels than abnormal ovarian function or myometrial activity.

If we accept that the reduced power Doppler signal reflects a difference between the two groups at a local level, this may be due either to suboptimal endometrial angiogenesis or to dynamic vascular changes such as vasoconstriction or reduced vasodilatation in the subfertile patients relative to the control group. Based on our knowledge of endometrial histology (Ferenczy et al., 1979; Peek et al., 1992) and angiogenesis (Gargett and Rogers, 2001) it is more likely that the differences reflect dynamic events or relative vascular density than variation in vessel number due to temporal changes in the rate of vessel formation. The vascular indices generated through the quantification of the power Doppler signal within a defined volume are thought to represent the degree of vascularity and perfusion within this three-dimensional area (Pairleitner et al., 1999; Jarvela et al., 2003). This reflects the knowledge that power Doppler demonstrates blood flow by encoding the power in the Doppler signal rather than its mean frequency shift and is dependent upon the number of erythrocytes which act as backscatterers of the ultrasound pulses (Rubin et al., 1994). Assuming a minimal variation in haematocrit between individuals, the power Doppler signal will be seen to increase in areas containing more vessels or larger vessels. This is why we have expressed our findings in terms of a variance in 'vascularity', 'perfusion' or the 'power Doppler signal' itself rather than 'flow rate' or 'blood flow'. Whilst there are no in vitro phantom data to address how the three-dimensional
Figure 2. Endometrial perfusion. The three graphs illustrate the variation in the power Doppler signal within the endometrial cavity as assessed by the vascularization index (VI; a), flow index (FI; b) and vascularization flow index (VFI; c) between the two groups. Data from the subfertile population are illustrated by circles joined with a bold black line and control data by triangles joined by a grey line. The midpoint represents the mean value at each time-point and the error bars 1 SEM.
Figure 3. Subendometrial perfusion. The three graphs illustrate the variation in the power Doppler signal within the subendometrium as assessed by the vascularization index ($VI_a$), flow index ($FI_b$) and vascularization flow index ($VFI_c$) between the two groups. Data from the subfertile population are illustrated by circles joined with a bold black line and control data by triangles joined by a grey line. The mid-point represents the mean value at each time-point and the error bars 1 SEM.
power Doppler indices equate to true blood flow characteristics and vascular distributions, there are sufficient biophysical data to support the hypothesis that our findings do equate with vessel number and size (Meyerowitz et al., 1996; Donnelly et al., 2001). Our own studies (in progress) have shown that all three vascular indices are significantly affected by flow rate, erythrocyte density and vessel number but in different ways and to different degrees.

We may speculate further that the ultrasound findings in both groups offer new information on what constitutes both normal and abnormal vascular change throughout the menstrual cycle at the level of the endometrium and subendometrium. The most surprising observation is that of a period of relatively reduced perfusion in the immediate post-ovulatory period, extending to the time when implantation would occur in spontaneous conception cycles. Such vascular changes may be associated with local hypoxia and there is evidence to support a beneficial role for this during implantation in the human. Relatively low oxygen values are present around the blastocyst as it implants (Graham et al., 2000) and the expression of vascular endothelial growth factor (VEGF), an endothelial cell-specific mitogen, is up-regulated by hypoxia irrespective of estradiol or progesterone levels in vitro (Sharkey et al., 2000). Hypoxia may also promote implantation by inducing expression of urokinase-type plasminogen activator (uPAR) through a haem protein-dependent pathway rather than through a VEGF-dependent mechanism (Graham et al., 1998). Low levels of oxygen have been shown to stimulate trophoblast invasion by increasing uPAR expression at the leading edge of the invading cell, where proteolytic activity is required for degradation of the extracellular matrix (Graham et al., 1998). Though the exact contribution of the endometrium to implantation has yet to be established, there is sufficient evidence to conclude that it plays more than a passive role in the overall process (Yaron et al., 1994; Lessey, 2000). It is generally accepted that an adequate endometrial blood supply is required for conception to occur following embryo transfer as part of assisted reproduction treatment. Contrary to this concept it may be that a period of relative endometrial ischaemia and hypoxia associated with the peri-ovulatory fall in estradiol is a more important determinant of endometrial receptivity. We observed the lowest power Doppler signals during this phase of the cycle in all subjects regardless of their apparent

Figure 4. Sex steroid plasma concentration profiles. The differences between the two groups are shown for estradiol (a) and progesterone (b).
fecundity and there were no differences between the two study groups at this stage or during any aspect of the luteal phase. However, the control group of women experienced a greater relative fall in endometrial and subendometrial ‘perfusion’ because they reached a higher pre-ovulatory level than the women with unexplained subfertility. This raises the possibility that it is the degree of change in endometrial perfusion, and hypothetically the degree of local hypoxia, from the late follicular phase through to the early luteal phase that is the determining factor in defining endometrial receptivity. This may equally reflect the simultaneous changes in ovarian vascularity and steroid production with the endometrium acting as a surrogate marker of ovarian function. This seems less likely considering the similarity in serum sex steroid levels between the groups and implicates aberrant endometrial vascular change as a key factor in unexplained subfertility.

Our findings cannot be directly compared with the current consensus on uterine blood flow throughout the normal menstrual cycle, which is largely derived from conventional pulsed wave Doppler studies and expressed in terms of the resistance to flow and absolute velocities throughout the cardiac cycle. These studies almost universally report a gradual yet continuous increase in blood flow velocity in association with a reduction in the resistance to flow through the menstrual cycle from the early follicular phase, maximal at the time of implantation (Goswamy and Steptoe, 1988; Scholtes et al., 1989; Battaglia et al., 1990; Steer et al., 1990; Santolaya-Forgas, 1992; Sladkevicius et al., 1993; Bourne et al., 1996; Tan et al., 1996; Zaidi, 2000). In addition, most groups have investigated blood flow within the uterine artery rather than at the endometrial level although there is some evidence that the two have similar flow characteristics (Sladkevicius et al., 1993; Achiron et al., 1995; Bourne et al., 1996). Nevertheless, regardless of the level assessed, the results relate to blood flow characteristics within individual vessels rather than to total uterine or endometrial blood flow. Three-dimensional ultrasound facilitates the acquisition of power Doppler data from the whole endometrium and power Doppler is ideally suited to assessment of blood flow at this level as it is more sensitive to low flow velocities and essentially angle-independent (Rubin and Adler, 1993).

The suggestion that women with unexplained subfertility may have an impaired uterine blood supply was first raised by Goswamy et al. (1988). Using transabdominal pulsed wave Doppler, they described increased impedance to blood flow within the uterine artery in women with no apparent cause for their subfertility. A subsequent study also suggested that unexplained infertility may be associated with aberrant uterine artery blood flow and intermittently absent end-diastolic flow (Kurjak et al., 1991). It is somewhat surprising therefore that since these initial studies, the vast majority of imaging-based investigations have concentrated upon women undergoing ovarian stimulation as part of assisted reproduction treatment (Sterzik et al., 1989; Fleischer, 1991; Steer et al., 1992; Coulam et al., 1994; Levi-Setti et al., 1995). This must be questioned when one considers that ovulation induction itself has a negative effect on the uterine blood supply (Kupesic and Kurjak, 1993) and that it is difficult to identify a ‘control’ group of patients under such circumstances. Pre-treatment assessment of suboptimal uterine perfusion in women with unexplained subfertility would appear to offer more scope in terms of diagnostic capability and treatment modalities. Sildenafil citrate (Viagra™), for example, has recently been used to improve uterine blood flow in women undergoing assisted reproduction treatment with good effect (Sher and Fisch, 2000, 2002) and there are several other potential alternatives including aspirin (Rubinstein et al., 1999), glyceryl trinitrate and angiogenic growth factors (Azrin, 2001). If a group of women with poor uterine perfusion can be readily identified, these women may be offered such treatments, subject to demonstration of their safety, prior to more intensive and invasive regimens as a first line therapy in an attempt to increase natural fecundity.

In our first study of endometrial perfusion in ‘apparently fertile’ controls we demonstrated a significant effect of age, parity and smoking (Raine-Fenning et al., 2004a). We were able to account for the effect of nicotine by excluding the single smoker from the subfertile group and comparing the remainder to the non-smoking controls only. However, whilst the mean ages were not different between the groups (controls 33.3 ± 6.42 versus subfertiles 35.4 ± 4.33 years), there was a significantly lower parity in the subfertile group (0.23 ± 0.51 versus 1.30 ± 1.17 respectively, \( P < 0.01 \)). This was to be expected from the study design and can only be addressed by comparing nulliparous patients or those women with secondary subfertility, both of whom may have different aetiologies. We had originally decided to study both groups of women, as this was reflective of the patients seen in the infertility clinic and treated within our assisted conception unit. However, in our first study parity was shown to be associated with a higher FI in the subendometrium only with no difference evident within the endometrium or between the VI and VFI at either level. In this comparative study we demonstrated differences both within the endometrium and subendometrium and in all three indices at one level or another. In the preliminary study, a negative effect of age on the subendometrial FI appeared to outweigh the positive effect of parity and it is possible that the effect of subfertility has even more powerful weighting. However, numbers were too small to allow a detailed subgroup analysis and further work is required to quantify these potentially confounding variables. We also noted a significantly higher rate of miscarriage in the subfertile group and it would be worthwhile to study patients with recurrent miscarriage as a separate entity.

One other limitation of this work relates to the study groups. Certain assumptions were made at recruitment in terms of the fertility of the control subjects and the normality of the subfertile women. Whereas we did not restrict recruitment to those women with laparoscopic evidence of pelvic normality, we did exclude women with a history of pelvic or abdominal surgery, previous pelvic inflammatory disease and clinical features suggestive of endometriosis. This was assessed in the initial recruitment interview by an experienced
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Gynaecologist (N.J.R.F.) and accounted for 21 subjects (62%, 21/34) not being recruited. Of those subjects who met the recruitment criteria, 82.8% (24/29) had undergone normal laparoscopic investigation within the last 36 months. Of the nine nulliparous women in this study considered ‘potentially fertile’, and accepting an incidence of female factor subfertility of ~7%, it is possible that at least one patient may not be representative of a truly ‘normal’ control group. If we have inadvertently included subfertile women in our control group, the number is therefore likely to be small and this would have presumably reduced the differences seen between the groups rather than increased them.

In conclusion, women with unexplained subfertility demonstrate a significant reduction in endometrial and subendometrial perfusion, as defined by three-dimensional power Doppler angiography, prior to ovulation. This correlates with the time of the peak in estradiol but does not relate to the absolute plasma concentrations, which were similar to that of controls.

The exact relationship of these indices of vascularity to true blood flow and vessel characteristics remains to be determined. Future work should focus upon correlating such ultrasound findings with both histological and molecular information from the endometrial and subendometrial vascular beds.

Pre-treatment assessment of women with unexplained subfertility with 3D-PDA may prove useful as a diagnostic tool or facilitate the development of alternate, less invasive and ultimately more affordable management strategies.

References


Raine-Fenning NJ, Campbell BK, Kendall NR, Clewes JS and Johnson IR (2004a) Quantifying the changes in endometrial vascularity throughout the normal menstrual cycle with three-dimensional power Doppler angiography. Hum Reprod 19,330–338.


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