Color Doppler Study of the Subendometrial Area (Junctional Zone) Of the Uterus in Unexplained Infertility

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ABSTRACT:

Background/Aim: A case control study aiming to study endometrial perfusion and receptivity in unexplained infertility as it is simple and is available in many radiological clinics.

Patients & Methods: Thirty-five cases of unexplained infertility and 16 fertile females were submitted, in the midluteal phase of the menstrual cycle; to Color Doppler study of the subendometrial area which is the inner most part of the myometrium, 5 mm outside the echogenic basal layer of the endometrium.

Results: Study of vascularization of this area showed that, blood flow was detected in 51.4% of the group of unexplained and 93.8 % of the control group. The difference is statistically significant. Mean pulsatility index (PI) in the subendometrial arteries, if detected by color flow mapping was 2.65 ± 0.37 in unexplained infertility and 1.95 ±0.24 in control group. The difference is statistically significant. The mean peak systolic velocity (PSV) in the subendometrial arteries in unexplained infertility was 0.29 ± 0.02 m/second and in the control group was 0.42 ± 0.03 m/second. The difference is statistically significant. Our color Doppler study of the uterine arteries showed also that PI, resistance index (RI) are higher and PSV was lower in unexplained infertility than in control fertile group. Our study showed that in midluteal phase of the menstrual cycle in patients with unexplained infertility blood perfusion of the subendometrial area was impaired.

Conclusion: Blood perfusion of the endometrium was also impaired as the blood supply to it passes through the subendometrium area. A possible treatment of these patients is uterine perfusion enhancers as sildenafil (Viagra), low dose aspirin and glyceryl trinitrate

Key words: Color Doppler, Subendometrial Area of the Uterus, Unexplained Infertility

INTRODUCTION

Up to 30% of couples who are unable to conceive are determined to have unexplained infertility (1). Traditionally this diagnosis is made only after the basic infertility evaluation fails to reveal an obvious abnormality. The basic evaluation should provide evidence of ovulation, adequate sperm production and potency of the fallopian tubes. However, even the most sophisticated diagnostic assessment cannot reveal all possible abnormalities. It is supposed that unexplained infertility appears to represent either the lower extreme of the normal distribution of fertility or it arises from a defect in fecundity that cannot be detected by the routine infertility evaluation (2).

Couples in the unexplained infertility suffer from both diminished and delayed fecundity. In review of studies of unexplained infertility, the average cycle fecundity in the untreated control
group was 1.8% in 11 non-randomized studies and 3.8% in 6 randomized studies (3). Pregnancy rates are lower with increasing age of the female partner and duration of infertility (9).

In absence of a connectable abnormality, the therapy of unexplained infertility is by default, empiric. Proposed treatment regimens include intrauterine insemination (IUI), ovulation induction with clomiphene citrate or gonadotropin, combination of IUI with ovulation induction and assisted reproductive technologies (ART). The pregnancy rate after treatment of unexplained infertility that relies on level 1 evidence from randomized clinical trials is small except with ART which is considerably more costly than other lines of treatment and is associated with adverse effects as multiple pregnancy and ovarian hyperstimulation. Consequently a search for a cause of unexplained infertility should continue to perfect the results of treatment (5).

From the female perspective, unexplained infertility 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 to be a key determinant in successful implantation (6). The current assessment of the endometrium is restricted to a simplistic measurement of its thickness and description of its appearance (7). These parameters are important in that conception is less likely to occur in patients with thinner endometrium or in those with aberrant echogenic patterns, but there are not specific and their value as prognostic indicators of implantation is limited (8).

Doppler ultrasound assessment of uterine blood supply appears more informative. Pulsed Doppler waveform indices of high resistance to flow within the uterine artery have been repeatedly linked to poor outcomes during ART (9-12). Controlled ovarian stimulation itself, however, has a significant effect on uterine perfusion (3) making information derived from patients undergoing assisted reproduction not necessarily representative of women with explained infertility.

Although pregnancy outcome tended to be poor in patients with higher mean uterine arterial impedance indices (RI) or pulsatility index (PI), their value in assessing endometrial respectively seems to be limited (8,10). One of the explanations is that the major compartment is the myometrium and not the endometrium and thus most of the blood passing through the uterine arteries never reaches the endometrium. A more logical approach would be to evaluate the vascularization around the endometrium directly in an attempt to assess endometrial receptivity in unexplained infertility since visualization of the blood supply to the endometrium is difficult by conventional color Doppler (5). Moreover, quantification of power Doppler has not been established till now (14). Moreover, endometrial blood flow studies, either with conventional color Doppler sonography or the newer techniques of power or three dimensional (3D) power Doppler sonography have failed to demonstrate any significant difference between conventional color Doppler two dimensional (2D) sonography and power Doppler 3-D sonography for the study of endometrial blood flow and receptivity (15,16).

Blood flow to the endometrium comes from the radial artery, which divides before the myometrial–endometrial junction to form the basal arteries that supply the basal portion of the endometrium and the spiral arteries that continue upward to the endometrial surface. At the myometrial endometrial junction, a specific subendometrial area can be identified as a thin hypoechoic layer between the echogenic endometrium and the myometrium on ultrasound examination (19-21). It has been described as the subendometrial halo (area or region) or the junctional zone. Histological studies have confirmed that the subendometrial halo surrounding the endometrium represents the inner most layer of the myometrium and compared with the outer myometrium it consists of a distinct compartment of more highly packed muscle cells with increased vascularity (20,21). Many studies have shown that interactions between the subendometrial area and the...
endometrium may play an important role in the implantation process or endometrial receptivity[7,22]. Considering that blood supply to the endometrium must go through the subendometrial area, vascularization within this area may be related to endometrial perfusion and ultimately endometrial function and receptivity. All previous studies on endometrial perfusion were done on patients that were submitted to ART with ovarian hyperstimulation that raised serum estradiol to > 800 pg/ml which increased the endometrium perfusion and consequently endometrial vascularization can be assessed in these patients by conventional color Doppler. This is not the case in unstimulated endometrium in natural cycles. Kurjak et al (15) found that endometrial perfusion could not be detected by conventional color Doppler in normal, atrophic and 92% of hyperplastic endometrium. So study of the endometrial perfusion in natural cycle can be done by either power 3-D Doppler or through the study of the vascularization of the subendometrial area by conventional color Doppler. We preferred the last one to study endometrial perfusion and receptivity as it is simple and is available in many radiological clinics.

**MATERIAL AND METHODS**

Research Design: Case control study i.e. two groups of patients based on the presence or absence of an outcome.

Two groups of patients: one group 35 patients suffering from unexplained infertility and a control group of 16 fertile females. Both groups were submitted to the study of the presence or absence of blood flow in the subendometrial areas in the midluteal phase of the menstrual cycle using color Doppler sonography. Both groups were recruited through outpatient clinic of the Department of Obstetrics and Gynecology, Tanta University Hospital.

Inclusion criteria:

The inclusion criteria required ultrasonographic study of the pelvis, ovulatory as proved by folliculometry. Ovulation was confirmed by the disappearance of the preovulatory follicle or reduction in the diameter of the follicle of > 4 mm, irregular contour of the follicle and the presence of intrafollicular echoes with or without the presence of fluid in the pelvic cavity and midluteal phase serum progesterone of ≥10ng/ml, had satisfactory ovarian reserve (on day 3 of the menstrual cycle, serum FSH was < 10 m IU/ml, ovarian volume was ≥ 3 cm³ and contained > 5 antral follicle), tubes were patent as demonstrated by hysterosalpingography and/or laparoscopy and semen fluid analysis results were within the WHO guidelines. Endometrial thickness in the midluteal phase was ≥ 10 mm with normal texture.

Exclusion criteria:

It includes: age > 35 years, history of pelvic and/or abdominal surgery, previous pelvic inflammatory disease and clinical features suggestive of endometriosis including heavy, painful period and deep dyspareunia. women with pelvic pathology including ovarian cysts, polycystic ovary syndrome, endometrial polyps and fibroids.

Control group: (16 cases) were selected from nulliparous nulligravidous patients who attended the infertility clinic < 6 months after marriage. If they were gynecologically free, their menstrual pattern was regular and husband’s semen fluid analysis was normal, the patients were examined by color Doppler ultrasonography and blood flow in the subendometrial areas identified. They were then allowed for 3-6 cycles of timing of fertility oriented intercourse before being submitted to investigation and treatment. Those who become pregnant spontaneously during this period of fertility oriented intercourse were included in the study as control fertile cases. All women enrolled in the study of both groups have no hormonal therapy for at least three months before the start of the study.

Ultrasound investigation: on the day of midluteal phase (day 21-22 of the menstrual cycle), women in both groups were scanned transvaginally using a 6.5-MHz transvaginal transducer (Siemens Onmia Medison, 9900) with color Doppler facility between 1.00 and 3.00 PM.
with the patients in the lithotomy position over a period of minutes to ensure that the analysis spanned several cardiac cycles and to exclude circadian effect of blood flow. All women rested for at least 20 minutes before being scanned to minimize the effect of exercise on uterine blood flow (23). When a longitudinal view of the uterus was obtained, the region of interest was the subendometrial area. The layer compromising the subendometrial myometrium could be documented as a sonographically hypoechoic band (halo) of 5 mm width that regularly encircled the hyperechogenic margin (basal part) of the endometrium at the level of the uterine cavity. The pulse repetition was chosen for color velocity range of 3 cm/second and the color gain was adjusted to 80% ±2% to optimize detection of blood flow in the small vessels. Identical color Doppler setting was used in all patients of the study including the control group to standardize the examination.

Color flow mapping was used to determine the presence or absence of blood flow in the subendometrial areas. Spectral waveforms were obtained from vessels with the highest color intensity within the subendometrial area. The insonation angle was kept at 0° because the cause of the small spiral arteries could not be determined. After confirming that waveforms were continuous, an average of three to five cardiac cycles was selected for calculation of PI and maximum peak systolic blood flow velocity (PSV). The vessels with the lowest PI were considered for further statistical analysis. Uterine circulation was assessed simultaneously in each examination; bilateral uterine arteries were sampled lateral to the cervix near the internal os. Angle of insonation of the uterine arteries was less than 30°, the critical cutoff level for the high pass filter suppressing low level frequency Doppler signals caused by vessel wall movements were set at 20 cm/second for uterine arteries and 2.5 cm/second for subendometrial arteries. Estimation of average RI, PI and PSV of both uterine arteries was then performed.

STATISTICAL ANALYSIS

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 12. For quantitative variables, the range, mean and standard deviation were calculated. The difference between two means was statistically analyzed using the students (t) test. For qualitative variables, the number and percent distribution was calculated. Chi square was used as a test of significance. Significance was adopted at p<0.05 for interpretation of results of tests of significance (23).

RESULTS

The average age of cases of unexplained infertility was 25.14±3.42 years and in control group 24.44±3.42. The difference is not statistically significant (P = 0.537), table (1).

The blood flow was present in the subendometrial area in 18 cases of unexplained infertility (51.4%) and absent in 17 cases (48.6%). The blood flow was present in the subendometrial area in 15 cases of the control group (93.8%) and absent in one case (6.3%). The difference between the two groups is statistically significant (P = 0.001), table (2).

The average PI and PSV of the subendometrial arteries in cases of unexplained infertility were 2.65±0.37 and 0.29±0.02 m/second respectively. In control group they were 1.95±0.2 and 0.42±0.03 cm/second the difference between the two groups is statistically significant (P = 0.001), table (3).

The average PI, PSV and RI of the uterine arteries in cases of unexplained infertility was 2.67±0.19, 0.29±0.02 m/second and 0.86±0.01 respectively. In the control group these indices were 2.00±0.2, 0.42±0.03 m/second and 0.80±0.02 respectively. The difference between the indices in the two groups are statistically significant (P=0.001), table (4). Figure (1) shows the delineation of the endometrial area. Figure (2) shows no detectable blood flow in the subendometrial area in a case of unexplained infertility. Figures (3) and (4) show detectable flow in the subendometrial area in a fertile patient and a patient of unexplained infertility.
Table (1): Comparison of mean age between studied groups

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Cases</th>
<th>Control</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
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<tr>
<td>Range</td>
<td>20-36</td>
<td>19-28</td>
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<td></td>
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<tr>
<td>Mean</td>
<td>25.14</td>
<td>24.44</td>
<td>0.622</td>
<td>0.537</td>
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<tr>
<td>S.D.</td>
<td>3.90</td>
<td>3.42</td>
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Table (2): Comparison of subendometrial blood flow between studied groups

<table>
<thead>
<tr>
<th>Subendothelial blood flow</th>
<th>Cases</th>
<th>%</th>
<th>Control</th>
<th>%</th>
<th>X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>18</td>
<td>51.4</td>
<td>15</td>
<td>93.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>17</td>
<td>48.5</td>
<td>1</td>
<td>6.3</td>
<td>8.612</td>
<td>0.003*</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100.0</td>
<td>16</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant

Table (3): Comparison of pulsatility index and peak systolic velocity (m/second) of subendometrial artery between studied groups

<table>
<thead>
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<th>Control</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsatility index:</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Range</td>
<td>2.2-3.48</td>
<td>1.7-2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Mean</td>
<td>2.65</td>
<td>1.95</td>
<td>6.579</td>
<td>0.001*</td>
</tr>
<tr>
<td>• S.D.</td>
<td>0.37</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak systolic velocity:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Range</td>
<td>0.25-0.32</td>
<td>0.33-0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Mean</td>
<td>0.29</td>
<td>0.42</td>
<td>13.333</td>
<td>0.001*</td>
</tr>
<tr>
<td>• S.D.</td>
<td>0.02</td>
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<td></td>
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</table>

*Statistically significant

Table (4): Comparison of pulsatility index and peak systolic velocity (m/second) and resistance index of uterine artery between studied groups

<table>
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<th>Control</th>
<th>t</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Pulsatility index:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Range</td>
<td>2.3-2.9</td>
<td>1.8-2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Mean</td>
<td>2.67</td>
<td>2.00</td>
<td>11.590</td>
<td>0.001*</td>
</tr>
<tr>
<td>• S.D.</td>
<td>0.19</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak systolic velocity:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Range</td>
<td>0.25-0.35</td>
<td>0.35-0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Mean</td>
<td>0.29</td>
<td>0.42</td>
<td>16.196</td>
<td>0.001*</td>
</tr>
<tr>
<td>• S.D.</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistance index:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Range</td>
<td>0.83-0.88</td>
<td>0.78-0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Mean</td>
<td>0.86</td>
<td>0.80</td>
<td>10.817</td>
<td>0.001*</td>
</tr>
<tr>
<td>• S.D.</td>
<td>0.01</td>
<td>0.02</td>
<td></td>
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</table>

*Statistically significant
Fig. (1): Longitudinal ultrasonography of the uterus delineating the subendometrial area (arrows) extending for 5 mm from the basal echogenic border of the endometrium through the myometrium.

Fig. (2): Color flow mapping of the uterus showing absence of blood flow in the subendometrial area (arrows) in a case of unexplained infertility.

Fig. (3): Color flow mapping of the uterus showing presence of blood flow in subendometrial area (arrows) in a fertile patient PI 1.9.

Fig. (4): Color flow mapping of the uterus showing presence of blood flow in the subendometrial area (arrow) in a case of unexplained infertility PI 2.8.
DISCUSSION

The condition of the uterus is critical to the process of embryo implantation and among uterine conditions; endometrium development is the most important. Endometrial vasculature has been shown to play a prominent role in the early endometrial response to the implanting blastcysts and vascular changes may contribute to uterine receptivity. Moreover, recent developments in ultrasonography have presented new opportunities for assessing endometrial perfusion with non-invasive methods [26].

Zaidi et al., [26] assessed the presence or absence of subendometrial and intraendometrial blood flow velocimetry on the day of HCG administration and related to pregnancy rate. They found that the absence of subendometrial and intraendometrial vascularization was associated with failure of implantation in stimulated cycles in vitro fertilization and embryo transfer (IVF and ET). Yange et al. [27] used the so called intraendometrial power Doppler Area (EDPA) defined as vascular signals seen within the endometrial myometrial interface (subendometrial area) as measured by power Doppler sonography. They found that patients with higher vascular signals in this area had a better chance of pregnancy than patients with a lower EPPA in IVF and ET.

Schild et al. [17] investigated the role of 3D power Doppler of the subendometrial area on the first day of ovarian stimulation in predicting the outcome of IVF program and found that endometrial thickness; uterine artery blood flow and vascularity of the subendometrial area were correlated to the pregnancy rate.

Kupesic et al. [28] compared the 2-D with conventional color Doppler (CCD) and 3-D in the power Doppler (CPD) ultrasonographic scoring systems by combined parameters including endometrial thickness, volume, echogenicity and subendometrial blood flow and found that the two systems (2-D CCD and 3-D CPD) had similar efficiencies in predicting endometrial receptivity and pregnancy outcome.

A study of Noe et al. [29] by immuno-cytocchemistry revealed that the subendometrial myometrium, also called junctional zone or aschi myometrium, exhibits a cyclic pattern of estrogen and progesterone receptor expression that parallels that of the endometrium and the vessels of this region exhibit also the same cyclic receptor expression as that of the endometrium. Thus the endometrium and the subendometrium form a functional unit with various cyclic reproductive functions including the blood perfusion which reaches the endometrium through the subendometrial area.

In fact, the responsiveness of the subendometrial region had been shown to be associated with implantation success during IVF-ET treatment. The subendometrial area became significantly thicker at ET in pregnant group, in contrast, changes in this area were less pronounced in patients who did not conceive [22]. This may explain the relation between endometrial receptivity and perfusion of the subendometrial region.
Many investigators had noted the correlation of subendometrial myometrium contractions, which require a computer assisted image analysis to detect them and endometrial receptivity and pregnancy outcome in natural cycles. Assisted reproduction with stimulated cycles showed the same correlation. The 3 teams of investigators found that less active subendometrial myometrium was associated with higher pregnancy rate. This will help to explain the absence of vascularization in this region in unexplained infertility as myometrial contraction may obstruct the blood flow.

Kupesic et al showed that the resistance to blood flow was more indicative than the presence or absence of blood flow alone in the subendometrial area. Chien et al found that Doppler indices of subendometrial blood flow velocity did not correlate with pregnancy, but the presence or absence of blood flow in both endometrial and subendometrial area correlated well with pregnancy rate. Edmond et al found that the presence of blood flow in the subendometrial area as well as low Doppler indices of its arteries correlated well with endometrial receptivity and pregnancy rate. This is in agreement with the findings in our study as we found in unexplained infertility the subendometrial blood flow was present in 51.4% of cases while in fertile group it was present in 93.8% of cases. Also we found that average PI and PSV subendometrial arteries in 18 cases in whom subendometrial blood flow was present was 2.65±0.37 and 0.29±0.20 m/second respectively. In contrast in the control fertile group average PI and PSV of the subendometrial arteries in 15 cases in whom blood flow was detected was 1.95±0.24 and 0.42±0.03 m/second receptivity. The differences between the two groups were statistically significant.

Edmond et al using conventional color Doppler found that in natural unstimulated cycles, arterial blood flow was detected in more than 80% of fertile patients in the subendometrial area in different phases of these cycles and in more than 95% of fertile women at the midluteal phase. This is in agreement with the results of our study as blood flow was detected in 93.8% of the fertile group in the subendometrial area at the midluteal phase but was present in this area at this phase in 51.4% of patients in unexplained infertility.

There is no consensus of opinions about the value of the color Doppler study of the uterine arteries in different phases of the menstrual cycle and pregnancy outcome. Sterzik et al and Steer et al believed that high resistance to flow in the uterine arteries is linked to poor pregnancy outcome during ART. Tekay et al and Friedler et al stated that although pregnancy outcome tended to be poor in patients with high PI and RI of uterine arteries but the value of PI and RI of uterine arteries in assessing uterine receptivity is limited. In our study we found that PI and RI were higher in group of unexplained infertility at the mid-luteal phase than in fertile group and the differences between the two groups was significant (table 4).

The rate of endometrial blood flow during the normal female reproductive cycle has been correlated with increased expression of angiogenic factors, predominantly of vascular endothelial growth factors (VEGF). They are members of a family of heparin binding proteins that act directly on the endothelial cells. They induce proliferation and angiogenesis. A possible additional effect of VEGF on the vasculature is that of vasodilatation mediated by the release of prostacyclin and nitric oxide by the endothelial cells.

Donnez et al found that women with unexplained infertility had a significantly lower immuno-histochemical expression of VEGF in the euotopic secretory endometrium. It was demonstrated that there is a significant positive correlation between circulating estradiol level and VEGF in the endometrial cells. Since estradiol levels are normal in patients with unexplained infertility, this may be due to endometrial estradiol resistance or increased expression of progesterone B receptors which oppose the effect of estradiol in these patients.

Our study showed that in patients with unexplained infertility, there is impaired perfusion of the subendometrial area in the midluteal phase of natural cycles. The perfusion may be increased by treating these cases with
uterine perfusion enhancers such as sildenafil (viagra) which had improved uterine blood flow in women undergoing ART with reasonable effect. There are several other perfusion enhancers including aspirin (37), glyceryl trinitrate and angiogenic growth factors (38). If patients with unexplained infertility prove to have poor uterine perfusion by color Doppler sonography, they may be given perfusion enhancers if they proved to be safe.

REFERENCES


