Ultrasound studies of vascular and morphological changes in the human uterus after a positive self-test for the urinary luteinizing hormone surge

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The aim of the study reported here was to establish complementary data for changes in uterine size, echogenicity and vascularity during the menstrual cycle relative to a positive self-test for urinary luteinizing hormone (LH) and day 1 of next menses. Thirteen volunteers (aged 23–32 years) with apparently regular menstrual cycles were recruited from the nursing staff. The plan was to examine all women by transvaginal ultrasonography with colour Doppler imaging on day 11 of the menstrual cycle. A urinary LH self-test was to be used daily until a positive result was obtained and the women were to be re-scanned daily until the dominant follicle had ruptured. All women were then to be scanned at least every 48 h (within ±2 h of the same time of day) until day 6 of the next menstrual cycle. Matched samples of peripheral blood were taken at the time of each scan for hormone analysis. The main outcome measures were the times of follicular rupture, a positive test result for urinary LH and the start of menses, uterine volume, cavity length, endometrial thickness and grade, pulsatility index (PI), and time-averaged and peak systolic maximum velocities in uterine and radial arteries and in subendometrial vessels. Nine women fulfilled the criteria for an ovulatory cycle, and seven provided data over the complete study. The principal changes relative to a positive urinary LH test were (i) a continued rise in endometrial thickness to days 3 and 4 (this index then remained relatively constant, but the layered appearance was lost) and (ii) a gradual decrease in the uterine arterial PI. There was a significant rise in uterine volume, cavity length and uterine arterial PI around the time of the next menses, and a fall in endometrial thickness and blood velocity in the uterine and radial arteries and subendometrial vessels. The data may have implications for the assessment of reproductive status and the design of future studies on disorders of implantation or menstruation.

Key words: colour Doppler imaging/endometrium/LH test/transvaginal ultrasonography/uterine arteries

Introduction

The uterus undergoes characteristic changes in form, blood flow and function during menstrual and conceptional cycles. Transabdominal ultrasonography with B mode imaging is one of the least invasive methods for monitoring changes in endometrial thickness, consistency and volume (Sakamoto and Nakano, 1982; Hackeloer, 1984; Fleischer et al., 1986a,b; Giorlandino et al., 1987). The use of transvaginal ultrasonography produces clearer images (Timor-Tritsch et al., 1986; Randall et al., 1989; Bakos et al., 1993), and the additional facilities of colour Doppler imaging and pulsed Doppler spectral analysis provide information on blood flow in the main uterine arteries (Hata et al., 1988; Scholtes et al., 1989; Steer et al., 1990; Kupesic and Kurjak, 1993; Sladkevicius et al., 1993, 1994) and subendometrial vessels (Sladkevicius, 1993).

There is still a need, however, for a comprehensive, longitudinal study of uterine size, form and blood flow during the secretory phase of the menstrual cycle in relation to instant, external reference points of ovarian and uterine function. This information would be of value in the assessment of reproductive status and the design of investigations into disorders of implantation or menstruation. Accordingly, our aim was to obtain complementary data on ultrasound-derived indices of uterine physiology in relation to a positive result from a simple self-test for urinary luteinizing hormone (LH), and day 1 of next menses. We are reporting data from the same study on serial changes in the corpus luteum and the concentration of serum progesterone (Bourne et al., 1996) relative to the same reference points.

Materials and methods

Transvaginal ultrasonography with attachments for colour Doppler imaging and pulsed Doppler spectral analysis was used to scan healthy women in the semi-recumbent position over the peri-ovulatory period and during the secretory phase of the menstrual cycle. Examinations for each woman were performed at the same time each day (±2 h). A matched sample of peripheral venous blood was taken at the time of each scan for subsequent hormone analysis. The first scans were performed on day 11. The women tested their urine each morning with a commercial urinary LH dipstick from day 11 until a positive result was obtained (Clear Plan One Step; Unipath Ltd., Bedford, UK). The women then underwent daily scans until the dominant follicle had ruptured, and every other day thereafter. The time of next menses was recorded and the women were studied until day 5 or 6 of the next menstrual cycle. Morphological and blood flow data from each scan were recorded on colour prints. Qualitative and quantitative data relating to ovarian morphology and blood flow were entered into a database using a commercial software package installed on a personal computer. The protocol was approved by the local Ethics Committee of Sahlgrenska Hospital, Göteborg, Sweden.
Subjects
Thirteen nurses employed at the hospital (mean age 28 years, range 23–32) volunteered to take part in the study. All had regular menstrual cycles (length 26–32 days) and had not used any form of hormone treatment for at least 6 months. Subsequently, four women withdrew from the study; two because they could not comply with the minimum number of scans required (at least four), and two because they had not developed a dominant follicle by day 18 of the menstrual cycle. Therefore data from nine women were recorded and analysed.

Pelvic ultrasonography
An Aloka SSD 2000 scanner was used for all scans with a translational probe for B-mode, pulsed and colour Doppler imaging (Aloka Co. Ltd., Tokyo, Japan). The vaginal probe was equipped with a 5 MHz transducer for both B-mode and Doppler functions. Methods for examining uterine morphology, vascularity and blood flow in semirecumbent women have been described previously (Bourne, 1991). The maximum longitudinal diameter (length) of the uterus \( D_1 \) was measured in cm from the internal os to the fundus, and the largest diameters in the transverse \( D_2 \) and anterior/posterior \( D_3 \) planes. The volume (ml) was estimated as \( V = D_1 \times D_2 \times D_3 / 2 \). Cavity length (cm) was measured from the internal cervical os to the surface of the endometrium at the fundus. The total maximum endometrial thickness was measured in the longitudinal plane from one myometrial–endometrial interface to the other across the lumen of the cavity. Endometrial appearance was classified into one of three grades, namely, grade 1, triple layered, grade 3, uniformly hyperechogenic and grade 2, intermediate between grades 1 and 3. Peristaltic activity was recorded as the number of apparent endometrial movements/10 s.

Colour Doppler imaging was used to identify the uterine artery as it ascends just lateral to the internal os of the cervix. The left and right branches of the artery were located at points where the angle of the ultrasound beam approached zero. A pulsed Doppler range gate was placed over each vessel in turn to generate flow velocity waveforms with the highest peak systolic velocity (PSV). The dominant uterine artery was defined as the artery on the same side as the largest follicle or corpus luteum. Indices of blood flow in a mid-fundal radial artery and in subendometrial vessels were determined after the probe had been positioned to produce waveforms with the highest PSV. Subendometrial flow was defined arbitrarily as waveforms emanating from an area \( < 3.0 \) mm from the apparent basal layer of the endometrium. It is generally accepted that enough vessels will be at a low or zero angle to the path of insonation so that reproducible measurements can be made. Blood flow impedance was expressed as the pulsatility index (PI) to allow for the possible absence of diastolic flow. The PI was calculated electronically from smooth curves fitted to waveforms over three cardiac cycles according to the formula 

\[
PI = \frac{S - D}{TAMXV},
\]

where \( S \) is the peak systolic Doppler shifted frequency, \( D \) is the minimum diastolic Doppler shifted frequency and \( TAMXV \) is the time-averaged maximum velocity over the cardiac cycle. A reduction in PI is thought to reflect a decrease in impedance distal to the point of sampling. A previous one-way analysis of variance of replicate data (3–5 measurements) taken from the uterine arteries of 10 women gave a coefficient of variation of 7.6% for PI and 12.0% for PSV (Bourne, 1991). The TAMXV (cm/s) and the PSV (cm/s) were recorded from the uterine and radial arteries and from a subendometrial vessel.

Hormone assay
Serum concentrations of oestradiol, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured by direct automated immunoassay using an enzyme label and a fluorescent end point (IMX; Abbott Scandinavia AB, Stockholm, Sweden). Serum progesterone was measured by a direct competitive binding immunoassay using time-resolved fluorescence to determine the end point (Delphia; Pharmacia, Uppsala, Sweden).

Definitions
The urinary LH test result was classified (according to the manufacturer's instructions) as positive if the blue line in the larger window of the dipstick was more intense than the corresponding line in the smaller window (i.e. the end point was based on the concentration of LH exceeding a threshold value). The day of follicle rupture was the day on which ultrasonography showed a marked reduction (>80%) in the volume of follicular fluid. Day 1 of menses was defined as the first day of blood loss through the vagina requiring sanitary protection.

A study menstrual cycle was considered to be normal if the following criteria were fulfilled retrospectively: (i) the length was between 25 and 33 days, (ii) the time of the positive test for urinary LH was associated with elevated serum concentrations of LH, (iii) the interval from the positive urinary LH test to day 1 of next menses minus 1 was between 8 and 18 days, (iv) there was ultrasonic evidence of follicle rupture and (v) the highest concentration of serum progesterone was \( \geq 15 \) nmol/l.

Statistical analysis
The day and time of the positive test result for urinary LH were recorded and the maximum time to follicle rupture was calculated. The data for each end point were plotted for each individual and subsequently pooled for the whole group in 2-day intervals relative to the day of the positive LH test (for days 1–14), and also to the first day of the next menses (day −4 to day 4). The distribution of pooled values for each end point at all time points was assessed. The data were expressed as the mean and standard deviation. The Pearson coefficient of linear correlation \( r \) was calculated to assess the relationship between end points for paired values.

Results
Some characteristics of the nine study cycles are summarized in Table 1. Seven women completed the whole study (10–12 scans); and two were studied up to day 6 or 7 of the secretory phase (four scans). All indices of ovarian function (derived by ultrasonography and immunoassay) were consistent with normal ovulatory cycles. Figure 1 is an example of an oblique transverse colour Doppler image of the uterus 2 days after a positive urinary LH test. The triple-layered appearance of the endometrium (grade 1) can be seen above blood flow in a radial artery. Figure 2 shows an example of the uterine appearance on day 6 after a positive urinary LH test. The endometrium appears uniformly hyperechogenic (grade 3) above a network of blood vessels. Occasionally the diastolic component of the derived flow velocity waveforms was not continuous with the systolic component, but the pattern was consistent for a given vessel and scan. Seven (78%) of the endometria were grade 3 by day 4 after a positive urinary LH test (all nine by day 8). The earliest return of grade 1 was day 2 of next menses; all seven women providing data at this time were grade 1 by day 6. The number of peristaltic movements (mean \( \pm \) SD) was constant up to the day of a positive LH test plus 4 (2.4 \( \pm \) 0.3) and then continued at a lower frequency to day of LH plus 10 (1.6 \( \pm \) 0.2).
Table I. Characteristics of the nine menstrual cycles studied

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle length (days)</td>
<td>27</td>
<td>27 (1)</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>Day of positive urinary LH test</td>
<td>12</td>
<td>12 (1)</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Maximum time to follicle rupture (h)*</td>
<td>25</td>
<td>35 (22)</td>
<td>7</td>
<td>77</td>
</tr>
<tr>
<td>Secretory phase length (days)*</td>
<td>14</td>
<td>14 (1)</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>No. of ultrasound scans</td>
<td>11</td>
<td>9 (3)</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

*From time of positive luteinizing hormone (LH) test.

Figure 1. Colour Doppler oblique transverse image of the uterus showing blood flow in a radial artery (day 2 from a positive urinary LH test).

Figure 2. Colour Doppler oblique transverse image of the uterus showing subendometrial blood flow (day 6 from a positive urinary LH test).
Uterine morphology

The changes in uterine volume (mean ± SE) relative to day 1 of a positive test for urinary LH and to day 1 of next menses are shown in Figure 3. The volume (mean ± SD) on days -2 and -1 (50.1 ± 14.4 ml) relative to days 1 and 2 of menses (37.7 ± 15.1 ml) was significantly higher (P < 0.05, Student's paired t-test). The length of the cavity (mean ± SD) on days -2 and -1 (4.01 ± 0.50 cm) relative to days 1 and 2 of menses (3.28 ± 0.48 cm) was also significantly higher (P < 0.5, Student's t-test). The mean change in endometrial thickness is shown in Figure 4. The values (mean ± SE) increased until days 3 and 4 after a positive test for urinary LH, and started to decrease on days -2 and -1 relative to day 1 of next menses.

Uterine arterial blood flow

Mean changes in the PI and PSV in the dominant uterine artery relative to day 1 of a positive test for urinary LH and to day 1 of next menses are shown in Figure 5. There was a tendency for the PI to decrease from the time of the LH test to days 11–14. In contrast, the mean PSV tended to fall until days 7 and 8 and then increased. The PI tended to increase and the PSV to reduce on day -2 to +2 of menses. There was no overall significant difference in PI or PSV between the dominant and non-dominant uterine arteries, but the trends for the PI and PSV were more apparent for mean values derived from the dominant artery (data not shown).

Subendometrial blood flow

Mean changes in the TAMXV and PSV in subendometrial vessels relative to day 1 of a positive test for urinary LH and to day 1 of next menses are shown in Figure 6. There was an initial decrease in both indices of blood velocity on days 3 or 4 after the positive LH test with a subsequent rise. The value for both indices tended to fall over days -2 to +4 of menses. There was no apparent change in PI during the secretory phase of the cycle (data not shown).

Comparison of blood flow indices

Values for the PI (mean ± SD) in the dominant uterine and radial arteries and in subendometrial vessels in relation to the time of a positive test for urinary LH are shown in Table II. The values were significantly lower in the radial compared with the dominant uterine artery. The values tended to be lowest in the subendometrial vessels. The mean percentage
Table II. Mean changes (±SD) in the minimum pulsatility index (PI) in various uterine vessels in relation to the time of a positive self-test for urinary luteinizing hormone (LH)

<table>
<thead>
<tr>
<th>Blood vessel</th>
<th>Days from positive urinary LH test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 and 2</td>
</tr>
<tr>
<td>Dominant uterine artery</td>
<td>3.3 (1.4)</td>
</tr>
<tr>
<td>Radial artery</td>
<td>1.4 (0.4)</td>
</tr>
<tr>
<td>Subendometrial</td>
<td>1.1 (0.3)</td>
</tr>
</tbody>
</table>

Table III. Mean changes (±SD) in time-averaged maximum velocity (TAMXV) in various uterine vessels in relation to the time of a positive self-test for urinary luteinizing hormone (LH)

<table>
<thead>
<tr>
<th>Blood vessel</th>
<th>Days from positive urinary LH test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 and 2</td>
</tr>
<tr>
<td>Dominant uterine artery</td>
<td>12.4 (4.0)</td>
</tr>
<tr>
<td>Radial artery</td>
<td>4.4 (2.3)</td>
</tr>
<tr>
<td>Subendometrial</td>
<td>3.5 (0.9)</td>
</tr>
</tbody>
</table>

Table IV. Mean changes (±SD) in peak systolic velocity (PSV) in various uterine vessels in relation to the time of a positive self-test for urinary LH

<table>
<thead>
<tr>
<th>Blood vessel</th>
<th>Days from positive urinary LH test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 and 2</td>
</tr>
<tr>
<td>Dominant uterine artery</td>
<td>39.4 (8.0)</td>
</tr>
<tr>
<td>Radial artery</td>
<td>8.2 (3.6)</td>
</tr>
<tr>
<td>Subendometrial</td>
<td>4.8 (1.4)</td>
</tr>
</tbody>
</table>

reduction from the dominant uterine arterial to subendometrial vessels ranged from 56.5 to 66.7. Values for the TAMXV (mean ± SD) in the same selection of uterine vessels in relation to the time of a positive test for urinary LH are shown in Table III. The values were significantly lower in the radial compared with the dominant uterine artery. There was a trend for the values to be lower in subendometrial vessels. The mean percentage reduction from dominant uterine arterial to subendometrial vessel ranged from 71.2 to 81.6. Values for the PSV (mean ± SD) in the same selection of uterine vessels in relation to the time of a positive test for urinary LH are shown in Table IV. The values were significantly lower in radial (compared with the dominant uterine arteries) and subendometrial vessels (compared with the radial arteries). The mean percentage reduction from dominant uterine arterial to subendometrial vessel ranged from 83.5 to 89.9. There was a good correlation between the TAMXV and the PSV in the dominant uterine arteries (r = 0.80), the radial arteries (r = 0.85) and the subendometrial vessels (r = 0.73). There was no correlation between any index of uterine blood flow and the concentration of serum progesterone or oestradiol.

Discussion
To the best of our knowledge this is the first study to relate physiological changes in uterine morphology and blood flow to the time of a practical self-test for urinary LH. We have already reported data from the same study on changes in the serum concentration of LH, FSH, progesterone and oestradiol, and in ultrasound indices of corpus luteum structure and blood flow (Bourne et al., 1996). There have been previous reports on the use of transvaginal ultrasonography to determine uterine size and echogenicity, and the additional use of colour Doppler imaging to derive indices of blood velocity and impedance to flow, which are relatively independent of the continuity of blood flow throughout the cardiac cycle. The data from these studies have either been related to the peak plasma or urinary LH concentration as determined retrospectively by laboratory techniques (Randell et al., 1989; Steer et al., 1990; Bakos et al., 1993), the time of follicle rupture according to ultrasonography (Sladkevicius et al., 1993), or the time of menstruation (Sladkevicius et al., 1994). One study focused on the peri-ovulatory period (Sladkevicius et al., 1993), while others obtained longitudinal data for one or more indices of uterine morphology (Randall et al., 1989; Bakos et al., 1993) or blood flow (Steer et al., 1990) at defined times, or regular intervals, throughout the menstrual cycle.

We sampled at least every 48 h throughout the secretory phase and the next menstruation. Our results showed that endometrial thickness remained relatively constant after days 3 and 4 from the positive test for urinary LH. The triple-layered appearance of the endometrium, however, had disappeared by day 4 from the positive LH test in seven of the nine women, and from all endometria by day 8. These data are consistent with those reported by Bakos et al. (1993). In addition, the number of endometrial movements was higher up to day 4 from the time of the positive test for urinary LH. These findings may be relevant to ovum and sperm transport in the
female reproductive tract, and to the appearance of an embryo in the uterus of a conception cycle. It is also of interest that there appears to be a physiological increase in the size of the uterus (i.e., volume and cavity length) before menstruation. Both of these indices and endometrial thickness decreased during days -2 and -1 relative to day 1 of the next menses. The endometria of all seven women had returned to grade 1 by day 6 of the menstrual cycle. This finding may have some implications for the onset of the next period of potential fertility.

The changes in PI in the dominant uterine artery were similar to those observed by Steer et al. (1990) for the mean values of both arteries and included the presence of a small peak on days 3 and 4 from the positive urinary LH test. This finding is reassuring in view of the different reference point for the data analysis that was used and the smaller number of women in our current study. The changes in PI at certain times appeared to be more pronounced in the dominant uterine artery, so these data were shown. Nevertheless, there was no significant difference in values over the whole of the secretory and menstrual phases. We took measurements at approximately the same time of day for each woman because a circadian rhythm has been found in uterine artery blood flow during the follicular phase of the menstrual cycle (Zaidi et al., 1995). The results from a previous study have shown that the PI at the mid-luteal phase tends to be higher in infertile women (Steer et al., 1994). There was a correlation between values for the PSV and the TAMXV. Both indices tended to rise towards the end of the secretory phase and may reflect impending menstruation.

The values for PI, PSV and TAMXV tended to remain constant in the subendometrial vessels throughout the secretory phase. This finding suggests that a good blood supply is required if a potential menstrual cycle is to turn into a conception cycle after coitus during the fertile period. The results of studies to compare ultrasound indices of uterine function in menstrual cycles with those of conception cycles after in-vitro fertilization (IVF) and embryo transfer have shown that conception only occurs if the endometrial thickness is above a lower limit of ~5-6 mm (Abdalla et al., 1994; Coulam et al., 1994; Noyes et al., 1995) and the mean uterine arterial PI is below an upper limit of ~3.3 (Steer et al., 1992). There does not, however, appear to be a significant difference between endometrial thickness or the resistance index (RI) on the day of embryo transfer between conception and non-conception cycles after IVF and embryo transfer (Bassin et al., 1995). Similarly, the number of peristaltic movements does not seem to have any predictive value for pregnancy (Narayan and Goswamy, 1994). A good correlation has been reported between ultrasound-derived indices and histological aspects of normal and abnormal endometrial form (Fleischer et al., 1986a), and between uterine artery PI and immunohistochemical markers of endometrial receptivity in down-regulated infertile patients receiving exogenous oestradiol and progesterone (Steer et al., 1995). There is, however, a need for comparative studies of the value of various indices of uterine structure, blood flow and function for predicting pregnancy in subfertile women with irregular menstrual cycles. The data of Dickey et al. (1994) suggest that assessments of uterine blood flow in subfertile women should also include the effect of posture.

Our data extend previous findings by indicating that the optimal time for uterine receptivity to an embryo, or for an ultrasound scan, might be selected on the basis of serial self-testing with a dipstick for urinary LH. Our results also show that the marked variation in blood flow seen in the uterine arteries is dampened by the distal uterine vasculature, leading to a relatively constant blood supply to the presumed target tissue, i.e. the endometrium. It is of interest that we found that the PSV and TAMXV were the most sensitive markers of physiological function for small vessels, such as those adjacent to the endometrium, whereas an index of impedance seemed more appropriate for large vessels such as the uterine artery. This finding is consistent with our studies of the corpus luteum (Bourne et al., 1995) and ovarian masses (unpublished observation). Nevertheless, the TAMXV does reach a maximum at the most likely time of implantation and thus may reflect an important feature of the reproductive process. There is also a profound change in vascularity in relation to the end of the secretory phase and the onset of menstruation. Impedance to flow increases markedly in the uterine arteries and there is a marked decrease in PSV in the subendometrial vessels. Hence, disorders of menstruation may be in part a function of vascular spasm, leading to relative tissue ischaemia and pain. The use of vasoactive drugs (e.g. nitric oxide donors) would be of interest, and transvaginal colour Doppler imaging (TVCDI) could be used to monitor their effects in vivo.

Acknowledgements

We thank the Swedish Medical Research Council for financial support for the project (grant no. B95-17x-11237-01A) and for the Fellowship to T.H.B. (grant no. 2873). We are also grateful to Berner Medicintechnik, Sweden and Aloka Co. Ltd., Tokyo, Japan for the use of their ultrasound equipment, and to Unipath, Lund, Sweden for supplying the self-tests for urinary LH (Clear Plan One Step).

References


Uterine morphology and vascularity


Received on August 10, 1995; accepted on November 2, 1995