Assessment of changes in endometrial and subendometrial volume and vascularity during the normal menstrual cycle using three-dimensional power Doppler ultrasound

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ABSTRACT

Objectives To describe changes in endometrial and subendometrial volume and vascularity during the normal menstrual cycle using three-dimensional (3D) power Doppler ultrasound.

Methods Fourteen healthy volunteers, 24 – 44 years old, with regular menstrual cycles underwent serial transvaginal 3D power Doppler ultrasound examinations of the uterus using a GE Voluson 730 Expert ultrasound system on cycle day 2, 3 or 4, then daily from cycle day 9 until follicular rupture and 1, 2, 5, 7 and 12 days after follicular rupture. Endometrial and subendometrial volume (cm^3), vascularization index (VI), flow index (FI) and vascularization flow index (VFI) were calculated using the VOCAL^{TM} software.

Results Endometrial and subendometrial vascularity indices increased throughout the follicular phase, decreased to a nadir 2 days after follicular rupture and then increased again during the luteal phase. Endometrial and subendometrial volume increased rapidly during the follicular phase and then remained almost unchanged during the luteal phase**.**

Conclusions Substantial changes occur in endometrial volume and vascularization during the normal menstrual cycle. There is potential for 3D power Doppler ultrasound to become a useful tool to assess pathological changes associated with female subfertility and abnormal uterine bleeding.

Introduction

Angiogenesis is essential for growth and development of tissues and organs. Physiological angiogenesis in the endometrium occurs every month, depending on interactions between hormones and growth factors[1]. Spectral Doppler ultrasound has been used to investigate vascular changes in the endometrium and surrounding tissues during the normal menstrual cycle[2,3]. However, spectral Doppler allows investigation of only one selected vessel at a time. This may not properly reflect changes in blood circulation in the whole target organ.

Advances in ultrasound technology and introduction of three-dimensional (3D) power Doppler ultrasound has opened a new possibility to assess vascularization in the whole volume of an organ, e.g. in the uterus or in an ovary. It is reasonable to believe that an analysis of power Doppler signals in a volume better reflects the overall vascularization in an organ than analysis of a two-dimensional (2D) ultrasound image or measurement of blood flow velocity in a single or a few vessels. Using 3D power Doppler ultrasound we can assess both arterial and venous circulation, and computer analysis makes this assessment objective. Therefore, 3D power Doppler ultrasound should be a good tool to assess physiological and pathological changes in blood circulation.

The aim of our study is to describe changes in endometrial volume and in endometrial and subendometrial blood circulation during the normal menstrual cycle using 3D power Doppler.

Subjects and methods

Subjects

The study protocol was approved by the Ethics Committee of the Medical Faculty of Lund University, Sweden. Informed consent was obtained from all participants, after the nature of the procedures had been fully explained.

Sixteen healthy women (members of staff or friends of the authors) with regular menstrual cycles voluntarily participated in our study. The inclusion criteria were: age 20 – 45 years, regular menstrual cycles of 26 – 32 days with 3 – 8 days of bleeding, no more than mild menstrual pain, and no cycle disturbances for at least one year; no hormonal contraception for the last 2 months; no intrauterine contraceptive device; no previous major gynecological surgery; normal ovaries at a baseline scan, and normal baseline values of follicular stimulating hormone (FSH) and estradiol on cycle day 2, 3 or 4. Estradiol and FSH were analyzed by a competitive immunoassay using Technicon Immuno 1® System (Bayer Corporation, Tarrytown, NY, USA) or UniCel™ DxI 800 Beckman Access® Immunoassay system (Beckman-Coulter, Chaska, MI, USA) with normal baseline values of estradiol being 100 – 250 pmol/L and <420 pmol/L, respectively and of FSH being 1 – 13 IU/L and 4 – 9 IU/L, respectively. To confirm a luteinizing hormone (LH) surge, blood samples were drawn daily from cycle day 9 until the day after follicular rupture (day +1) for analysis of LH. To assure that the luteal phase was adequate, a blood sample was taken 7 days after follicular rupture (day + 7) for analysis of serum progesterone. Blood was centrifuged at rotation speed 5000 rotations per minute at 4470 x g for 10 min. The serum was separated and frozen at -20°C until analysis in one batch. LH was measured with a chemiluminescent sandwich immunoassay method and progesterone with a chemiluminescent one step competitive immunoassay. Both analyses were made using UniCel™ DxI 800, Beckman Access® Immunoassay system.

Equipment

All data were acquired using a GE Voluson 730 Expert ultrasound system (General Electrics, Zipf, Austria) equipped with a 2.8 – 10 MHz transvaginal transducer. Identical fixed pre-installed power Doppler ultrasound settings were used in all women: frequency 3 – 9 MHz, pulse repetition frequency 0.6 kHz, gain -4.0, wall motion filter “low 1”.

Study design

This is a prospective longitudinal study. All women underwent a baseline transvaginal ultrasound examination on cycle day 2, 3 or 4, then daily from cycle day 9 until follicular rupture and 1, 2, 5, 7 and 12 days after follicular rupture. The results are expressed in relation to presumed ovulation: the last day when the dominant follicle was visible on the ultrasound screen was called day –1, and the first day when a corpus luteum was visible was called day +1. Thus, ovulation was presumed to have occurred between examination day –1 and +1. The women were examined in the lithotomy position with an empty bladder. The 3D ultrasound probe was introduced into the vagina. Once a longitudinal view of a satisfactory gray scale image of the uterus had been obtained, the uterus was centralized within the 3D sector on the screen, the ultrasound system was switched into the power Doppler mode and then the 3D mode was switched on. The woman was asked to remain as still as possible, and a 3D power Doppler data set of the uterus was acquired. The resultant multiplanar display was examined to ensure that a complete volume of the endometrial and subendometrial area had been captured. Volumes of satisfactory quality and with no artefacts were stored on a hard disk for future analysis.

Analysis of stored ultrasound volumes was done off-line on a personal computer. The VOCAL™ (virtual organ computer-aided analysis) imaging program was used to calculate endometrial volume and endometrial and subendometrial power Doppler flow indices. The acquired volumes yielded multiplanar views of the uterus in the mid-sagittal, axial and

coronal planes. All calculations were done on these multiplanar images. The longitudinal view was used as the reference image. The rotation steps were 30° resulting in the definition of six contours of the endometrium. Endometrial contours were manually drawn in all six sections using the computer mouse. Once all contours had been drawn, the volume of the endometrium was calculated automatically. Using the histogram facility of the VOCAL™ software, three vascular indices were generated: vascularization index (VI), flow index (FI) and vascularization flow index (VFI). VI is the ratio of color voxels to all voxels in the region of interest, expressed as a percentage. It reflects the density of vessels in the volume analyzed. FI is the mean intensity of all the power Doppler voxels in the volume analyzed (the sum of weighted color voxels divided by the number of all color voxels in the region of interest). It reflects the energy reflected from the blood corpuscles in the vessels of the volume, i.e., the more blood corpuscles in the blood vessels, the higher the FI values. VFI is the sum of weighted color Doppler voxels divided by all voxels in the region of interest. It reflects both the density of vessels and the density of blood corpuscles in the vessels[4]. Subendometrial flow indices were obtained by applying the shell function of the VOCAL™, which generates a parallel contour to the originally defined surface. We used a 2 mm shell outside the endometrial contour.

Four examinations performed on cycle day 2, 3 or 4 were excluded from analysis because of power Doppler artefacts explained by moving blood in the endometrial cavity or subendometrial contractions.

Statistical analysis

Statistical calculations were performed using StatView™, version 5 (SAS Institute Inc., Berkely, CA, USA, 1999). Because the data were skewed, we used Spearman rank correlation coefficient and Wilcoxon´s signed rank test for statistical analysis. Two-tailed p-values are given.

Results

Two women were excluded from the study, one because of unruptured follicle and one because of short luteal phase and low serum progesterone in the mid-luteal phase. The mean age of the 14 women included was 28 ± 5.2 (SD) years, range 24 – 44. Nine of them were nulliparous. The fourteen menstrual cycles studied were normal with normal baseline estradiol and FSH values (median estradiol 115 pmol/L, range 75 – 166 and 134 pmol/L, range 125 – 247; median FSH 4.45 IU/L, range 4 – 8.2 and 8.4 IU/L, range 4.9–9.4), sonographic criteria of ovulation (follicle $\geq$ 15 mm replaced by a corpus luteum), an LH peak on the day before follicular rupture (day –1) and adequate serum progesterone levels 7 days after follicular rupture (day + 7) (median LH at peak 45.8 IU/L, range 23.6 – 106.8, median mid-luteal serum progesterone 44.8 nmol/L, range 30 – 80.9).

Changes in endometrial and subendometrial volume and blood flow indices are shown in Figures 2 – 5. The statistical significance of differences is presented in Tables 1 – 4. Endometrial and subendometrial volume increased during the follicular phase and then remained virtually unchanged throughout the luteal phase. Endometrial and subendometrial VI and VFI increased during the follicular phase to reach a maximum 2 days before ovulation (Figure 1a), then decreased to reach a nadir 2 days after ovulation (Figure 1b) and then rose progressively during the remaining part of the luteal phase. Changes in FI were less clear-cut, but both endometrial and subendometrial FI values were highest 2 days before ovulation. FI decreased to reach a nadir 5 days after ovulation in the endometrium and 2 – 5 days after ovulation in the subendometrium, then FI values rose slightly again.

There was no statistically significant correlation between flow indices either in the endometrium/subendometrium on day +7 and progesterone levels on day +7 or between flow indices in the endometrium/subendometrium on days –1 or +1 and LH concentration on day -1.

Discussion

Our results suggest that endometrial-subendometrial vascularization changes markedly during the normal menstrual cycle. It increases throughout the follicular phase, decreases to a nadir 2 days after ovulation and then increases again during the luteal phase. Endometrial and subendometrial volume increase rapidly during the follicular phase and then remain almost unchanged during the luteal phase. The vascular changes in our study agree with those described in one previous 3D power Doppler ultrasound study[5] (the only one we have found in an extensive literature search) and one 2D color and spectral Doppler ultrasound study[2]. In the 2D ultrasound study, blood flow velocity in subendometrial arteries was highest 1 day before ovulation and 7 and 12 days after ovulation, and lowest 2 days after ovulation, and PI was at its highest 2 days after ovulation reflecting maximum resistance to flow soon after ovulation[2]. In the 3D ultrasound study by Raine-Fenning et al, a preovulatory increase in VI and VFI with a peak 3 days before ovulation and a nadir 5 days after ovulation both in the endometrium and subendometrium was found, and FI increased preovulatory and fell postovulatory both in the endometrium and subendometrium[5]. In our study FI reached a nadir 5 days after ovulation in the endometrium and 2 – 5 days after ovulation in the subendometrium. The very small differences in results between our study and that of Rainee-Fenning et al may be explained by differences in study design: we performed ultrasound examinations every day during the periovulatory period, while Raine-Fenning et al examined every second day before ovulation and every fourth day after ovulation. Our study provides more precise information about periovulatory vascular changes. The endometrial VI and VFI in our study were two to three times higher than in those of Raine-Fenning et al. This difference is likely to be explained by our use of an ultrasound system with higher Doppler sensitivity and possibly by the difference in shell size. Raine-Fenning et al arbitrarily chose a 5 mm shell whereas we arbitrarily chose a 2 mm shell. Choosing a 2 mm shell we may have

studied the most vascularized area of the subendometrium. FI values were similar in the two studies, which is explained by FI reflecting the mean density of blood corpuscles in the vessels. Therefore, FI should be less affected by power Doppler sensitivity and shell size. Quite interestingly, Nakai et al using spectral Doppler to measure endometrial blood flow, expressed in ml/min, observed an increase in endometrial blood flow between the day of ovulation and day 3 after ovulation in spontaneous menstrual cycles of infertile women[6]. This is in contrast to our findings and those of Raine-Fenning et al of decreased vascularity in the endometrium a few days after ovulation in normal menstrual cycles in women with no history of infertility.

Our study and the studies cited[2,5] suggest that endometrial vascularization is at its lowest between 1 and 5 days after ovulation. Theoretically the reduction in endometrial blood flow just after ovulation could be explained by increased uterine contractility after ovulation, increased uterine contractility having been shown to coincide with decreased endometrial blood flow[7]. Endometrial cyclical contractions originate in the subendometrial myometrium[8] and are related to estrogen and progesterone cyclical expression in this area[9]. However, the results of studies examining uterine/endometrial contractility during the normal menstrual cycle are conflicting. Only one[10] of four studies[10,11,12,13] reported a change in uterine contractility around the time of ovulation with high frequency low intensity contractions and high basal pressure. Whether uterine contractility has anything to do with the changes in endometrial vascularization that we observed remains uncertain.

The period of the menstrual cycle when endometrial vascularization was at its lowest, i.e., 1 to 5 days after ovulation is the period when morphological changes to prepare the endometrium for blastocyst implantation occur[14]**,** and it is also the period when endometrial receptivity is thought to be maximal[15]. Decreased endometrial vascularization during the days after ovulation may lead to endometrial hypoxia. It has been shown in animal studies that

near-atmospheric oxygen concentration reduces embryo viability and compromises embryo development[16] and that oxygen tension in the uterus is lowest during the implantation period[17]. Decreased oxygen concentration during the implantation window might be necessary for normal embryo development. On the other hand, Tan et al[3] who measured time averaged maximum velocity and pulsatility index in the uterine arteries during the normal menstrual cycle reported a decline in blood flow velocity in the uterine arteries 2 days before ovulation and an increase in blood flow velocity and a decline in resistance during the mid-luteal phase. They thought that these changes indicated optimal vascularity for blastocyst implantation. They did not perform any investigations between 6 hours and 6 days after ovulation.

Endometrial hypoxia stimulates production of vascular endothelial growth factor (VEGF) in endometrial stromal cells[18]. VEGF in turn regulates angiogenesis in the endometrium[19]. Hypoxia stimulating VEGF production could explain the increase of VI and VFI values – presumably reflecting increasing vascularization – in the endometrium and subendometrium after day +2. VEGF production has been reported to increase in the secretory phase of the cycle[20]. A positive correlation between serum VEGF levels and levels of estradiol and progesterone has also been demonstrated[21]. The vascular changes in the endometrium observed in our study – i.e., increasing VI and VFI during the follicular phase, a nadir of VI and VFI in the endometrium and subendometrium 2 days after ovulation and then an increase again during the luteal phase – mirror the changes in plasma estradiol during the menstrual cycle.

Our finding that endometrial volume increased during the follicular phase and then remained virtually unchanged during the luteal phase is in agreement with the findings of Raine-Fenning et al[22].

To sum up, using 3D power Doppler technique we have found substantial changes to occur in endometrial volume and vascularization during the normal menstrual cycle. There is

potential for 3D power Doppler ultrasound to become a useful tool to assess pathological changes associated with female subfertility and abnormal uterine bleeding.

Acknowledgments

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Table 1 Endometrial and subendometrial volume: significance of differences between results obtained on different days of the normal menstrual cycle. P-values are shown.

Endometrial volume											Subendometrial volume									
Day	-12	-5	-4	-3	-2	-1	+1	+2	+5	+7	-12	-5	-4	-3	-2	-1	+1	+2	+5	+7
-5	0.017	X									0.012	X								
-4	0.013	ns	X								0.012	ns	X							
-3	0.008	0.005	ns	X							0.008	0.013	ns	X						
-2	0.008	0.003	0.004	ns	X						0.008	0.004	0.008	0.004	X					
-1	0.005	0.003	0.004	0.019	0.019	X					0.005	0.003	0.004	0.005	ns	X				
+1	0.005	0.003	0.003	0.002	0.034	ns	X				0.005	0.003	0.006	0.003	ns	ns	X			
+2	0.005	0.003	0.013	0.010	ns	ns	0.048	X			0.005	0.021	0.023	0.021	ns	ns	ns	X		
+5	0.005	0.004	0.003	ns	ns	ns	ns	ns	X		0.005	0.003	0.003	ns	ns	ns	ns	ns	X	
+7	0.005	0.006	ns	ns	ns	0.045	0.006	ns	0.036	X	0.005	0.013	0.021	ns	ns	ns	0.012	ns	ns	X
+12	0.012	0.012	0.036	ns	ns	ns	ns	ns	ns	ns	0.012	0.036	0.025	ns	ns	ns	ns	ns	ns	ns

Days –12 to –1 represent the number of days before follicular rupture and days +1 to +12 represent the number of days after follicular rupture. Because of differences in length of the follicular phase, day –12 represents days –17 to –9; ns, non-significant ($p > 0.05$)

Table 2 Vascularization index (VI) in the endometrium and subendometrium: significance of differences between results obtained on different days of the normal menstrual cycle. P-values are shown.

VI in the endometrium											VI in the subendometrium									
Day	-12	-5	-4	-3	-2	-1	+1	+2	+5	+7	-12	-5	-4	-3	-2	-1	+1	+2	+5	+7
-5	ns	X									ns	X								
-4	ns	ns	X								0.049	ns	X							
-3	ns	ns	ns	X							ns	ns	ns	X						
-2	0.008	ns	ns	0.004	X						0.008	0.028	ns	0.003	X					
-1	0.037	ns	ns	ns	ns	X					0.028	ns	ns	ns	ns	X				
+1	0.013	ns	ns	ns	0.046	ns	X				0.017	ns	ns	ns	0.016	ns	X			
+2	ns	ns	0.013	0.007	0.002	0.005	ns	X			ns	ns	ns	0.019	0.002	0.011	0.03	X		
+5	ns	ns	ns	ns	0.013	ns	ns	ns	X		ns	ns	ns	ns	0.009	0.048	ns	ns	X	
+7	0.037	ns	ns	ns	ns	ns	ns	0.016	ns	X	ns	ns	ns	ns	0.019	ns	ns	ns	ns	X
+12	0.025	0.018	ns	ns	ns	ns	ns	0.008	ns	ns	0.012	0.063	ns	ns	ns	ns	ns	0.033	0.013	0.041

Days –12 to –1 represent the number of days before follicular rupture and days +1 to +12 represent the number of days after follicular rupture. Because of differences in length of the follicular phase, day –12 represents days –17 to –9; ns, non-significant ($p > 0.05$)

Table 3 Flow index (FI) in the endometrium and subendometrium: significance of differences between results obtained on different days of the normal menstrual cycle. P-values are shown.

FI in the endometrium											FI in the subendometrium									
Day	-12	–5	-4	-3	-2	-1	+1	+2	+5	+7	-12	–5	-4	-3	-2	-1	+1	+2	+5	+7
-5	ns	X									ns	X								
-4	0.025	0.047	X								0.017	ns	X							
-3	ns	0.013	ns	X							0.011	ns	ns	X						
-2	ns	0.017	ns	ns	X						0.008	0.013	ns	0.006	X					
-1	ns	ns	ns	ns	ns	X					0.009	ns	ns	ns	ns	X				
+1	ns	ns	ns	ns	0.016	ns	X				0.028	ns	ns	ns	0.007	ns	X			
+2	ns	ns	0.021	ns	0.002	0.048	ns	X			0.028	ns	0.041	0.039	0.005	0.030	ns	X		
+5	ns	0.028	0.008	0.019	0.002	0.019	ns	ns	X		0.037	ns	0.021	ns	0.013	ns	ns	ns	X	
+7	ns	ns	ns	ns	0.007	ns	ns	ns	ns	X	ns	ns	ns	ns	0.005	ns	ns	ns	ns	X
+12	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.016	0.017	ns	ns	ns	0.037	ns	ns	ns	ns	ns

Days –12 to –1 represent the number of days before follicular rupture and days +1 to +12 represent the number of days after follicular rupture. Because of differences in length of the follicular phase, day –12 represents days –17 to –9; ns, non-significant ($p > 0.05$)

Table 4 Vascularization flow index (VFI) in the endometrium and subendometrium: significance of differences between results obtained on different days of the normal menstrual cycle. P-values are shown.

VFI in the endometrium											VFI in the subendometrium									
Day	-12	–5	-4	-3	-2	-1	+1	+2	+5	+7	-12	–5	-4	-3	-2	-1	+1	+2	+5	+7
-5	ns	X									ns	X								
-4	ns	ns	X								0.036	ns	X							
-3	ns	ns	ns	X							0.038	ns	ns	X						
-2	0.008	ns	ns	0.004	X						0.008	0.032	ns	0.003	X					
-1	0.037	ns	ns	ns	ns	X					0.028	ns	ns	ns	ns	X				
+1	0.021	ns	ns	ns	0.025	ns	X				0.017	ns	ns	ns	0.016	ns	X			
+2	ns	ns	0.013	0.006	0.002	0.006	ns	X			ns	ns	ns	0.013	0.002	0.013	0.038	X		
+5	ns	ns	ns	ns	0.011	ns	ns	ns	X		ns	ns	ns	ns	0.016	0.048	ns	ns	X	
+7	ns	ns	ns	ns	0.016	ns	ns	0.016	ns	X	ns	ns	ns	ns	0.016	ns	ns	ns	ns	X
+12	0.017	0.028	ns	ns	ns	ns	ns	0.008	ns	ns	0.018	0.063	ns	ns	ns	ns	ns	0.041	0.013	ns

Days –12 to –1 represent the number of days before follicular rupture and days +1 to +12 represent the number of days after follicular rupture.

Because of differences in length of the follicular phase, day –12 represents days –17 to –9; ns, non-significant ($p > 0.05$)

Legends

Figure 1 Multiplanar display of the uterus obtained by three-dimensional ultrasound. Longitudinal section through the uterus in the upper left quadrant, transverse section in the upper right quadrant and coronal section in the lower left quadrant. The resultant vascular tree in the endometrium is shown in the lower right quadrant a) 2 days before follicular rupture (day –2) b) 2 days after follicular rupture (day +2). Both images are from the same woman.

Figure 2 Changes in endometrial and subendometrial volume during the menstrual cycle. The filled boxes represent the endometrium and the open circles the subendometrium. Median, 10^{th} and 90^{th} percentiles are shown. The figures in brackets denote the number of women examined. Days –12 to –1 represent the number of days before ovulation and days +1 to +12 represent the number of days after ovulation. Because of differences in the length of the follicular phase, day –12 represents days –17 to –9.

Figure 3 Changes in vascularization index (VI) in the endometrium and subendometrium during the menstrual cycle. The filled boxes represent the endometrium and the open circles the subendometrium. Median, 10^{th} and 90^{th} percentiles are shown. The figures in brackets denote the number of women examined. Days –12 to –1 represent the number of days before ovulation and days +1 to +12 represent the number of days after ovulation. Because of differences in length of the follicular phase, day –12 represents days –17 to –9.

Figure 4 Changes in flow index (FI) in the endometrium and subendometrium during the menstrual cycle. The filled boxes represent the endometrium and the open circles the subendometrium. Median, 10^{th} and 90^{th} percentiles are shown. The figures in brackets denote the number of women examined. Days –12 to –1 represent the number of days before

ovulation and days +1 to +12 represent the number of days after ovulation. Because of differences in length of the follicular phase, day –12 represents days –17 to –9.

Figure 5 Changes in vascularization flow index (VFI) in the endometrium and subendometrium during the menstrual cycle. The filled boxes represent the endometrium and the open circles the subendometrium. Median, 10^{th} and 90^{th} percentiles are shown. The figures in brackets denote the number of women examined. Days –12 to –1 represent the number of days before ovulation and days +1 to +12 represent the number of days after ovulation. Because of differences in length of the follicular phase, day –12 represents days –17 to –9.

Figure 1a

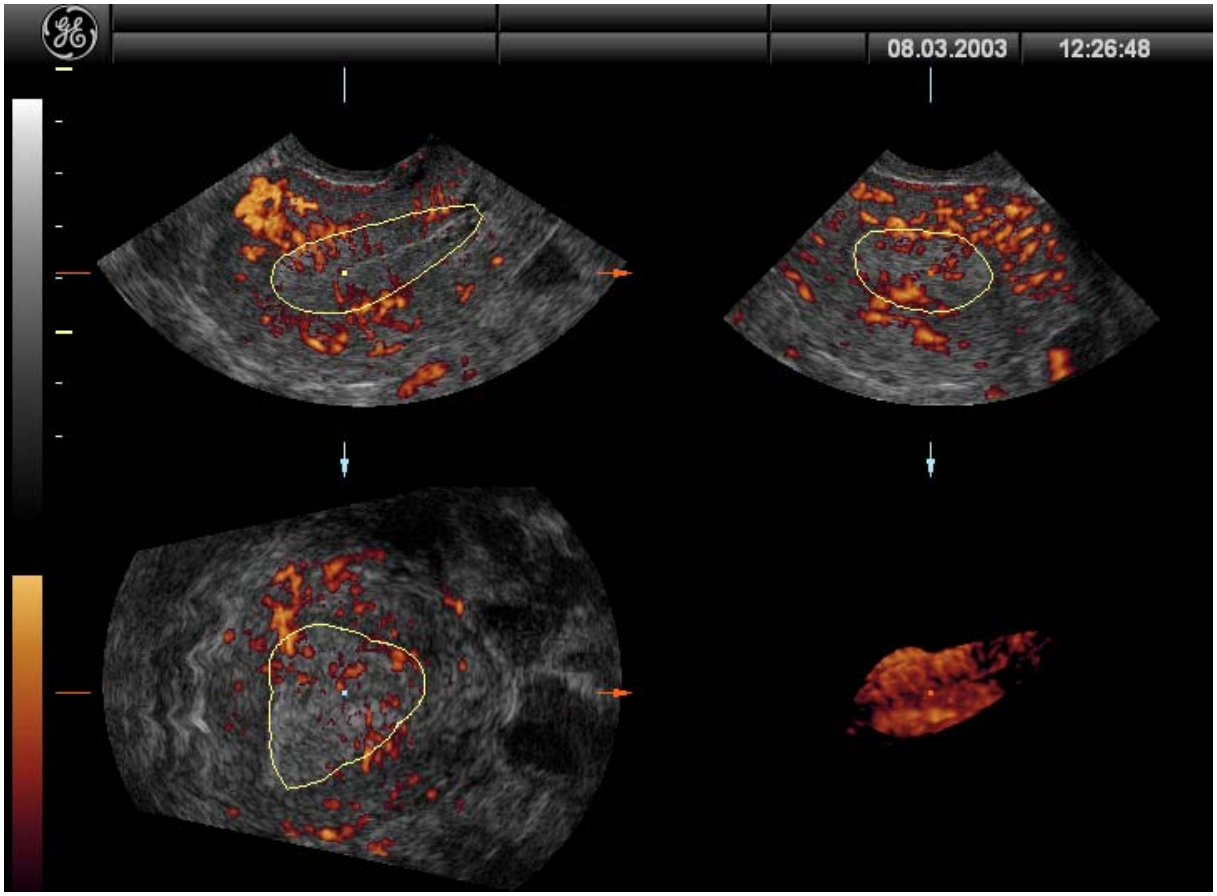


Figure 1b

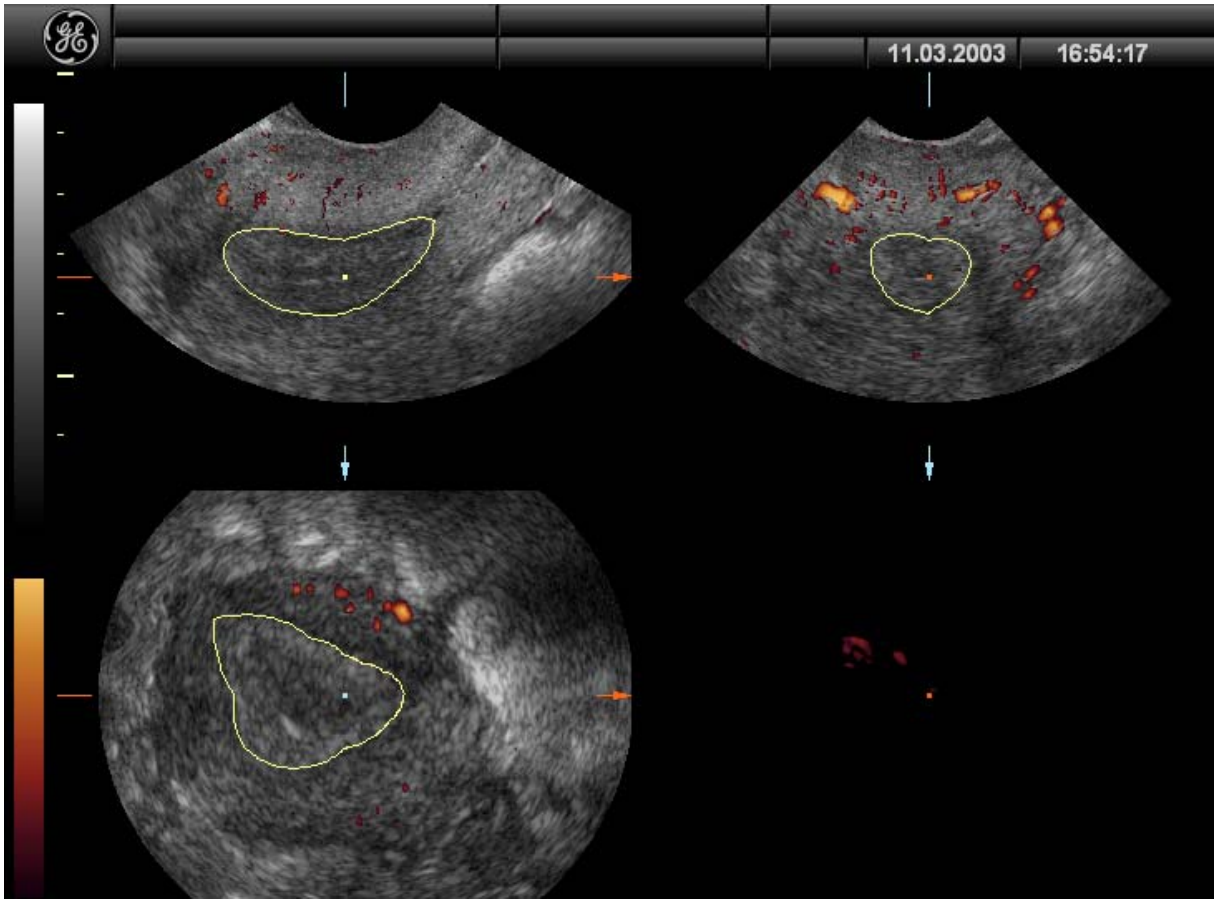


Figure 2

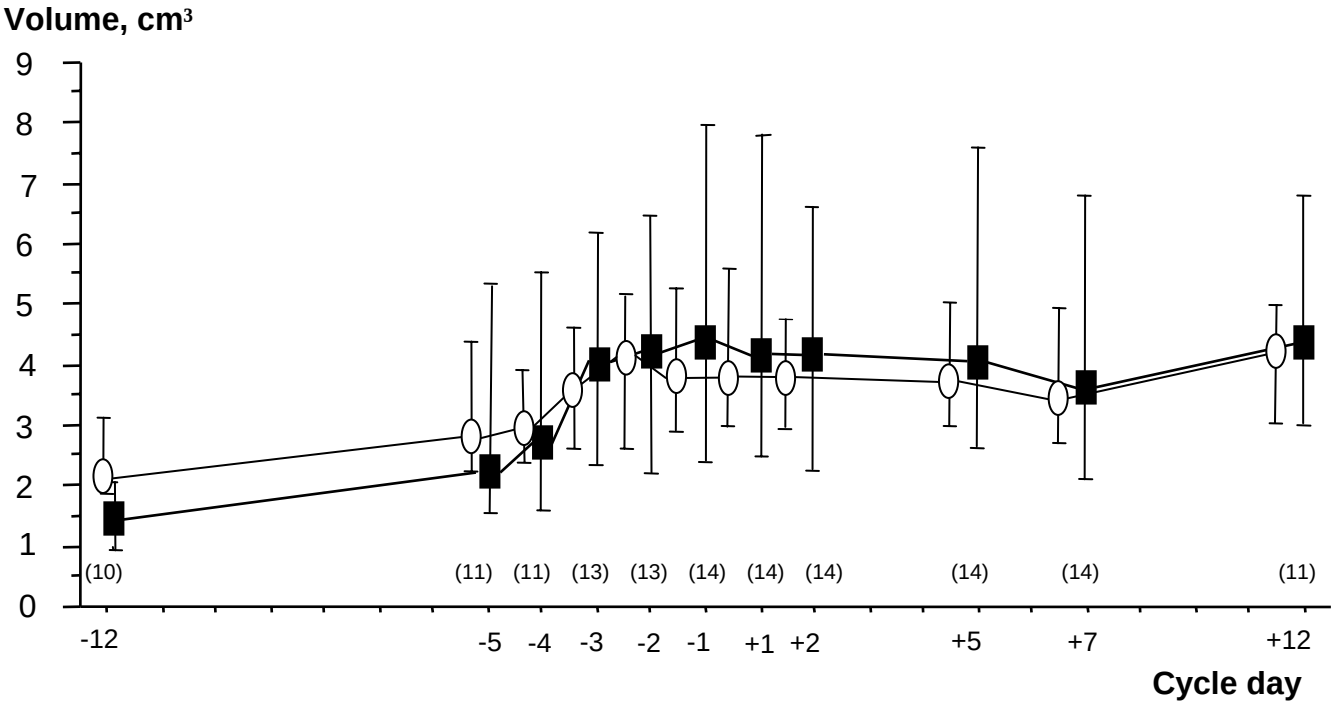

Volume, cm³
9
8
7
6
5
4
3
2
1
0
(10)
(11)
(11)
(13)
(13)
(14)
(14)
(14)
(14)
(14)
(11)
-12
-5
-4
-3
-2
-1
+1
+2
+5
+7
+12
Cycle day

Figure 3

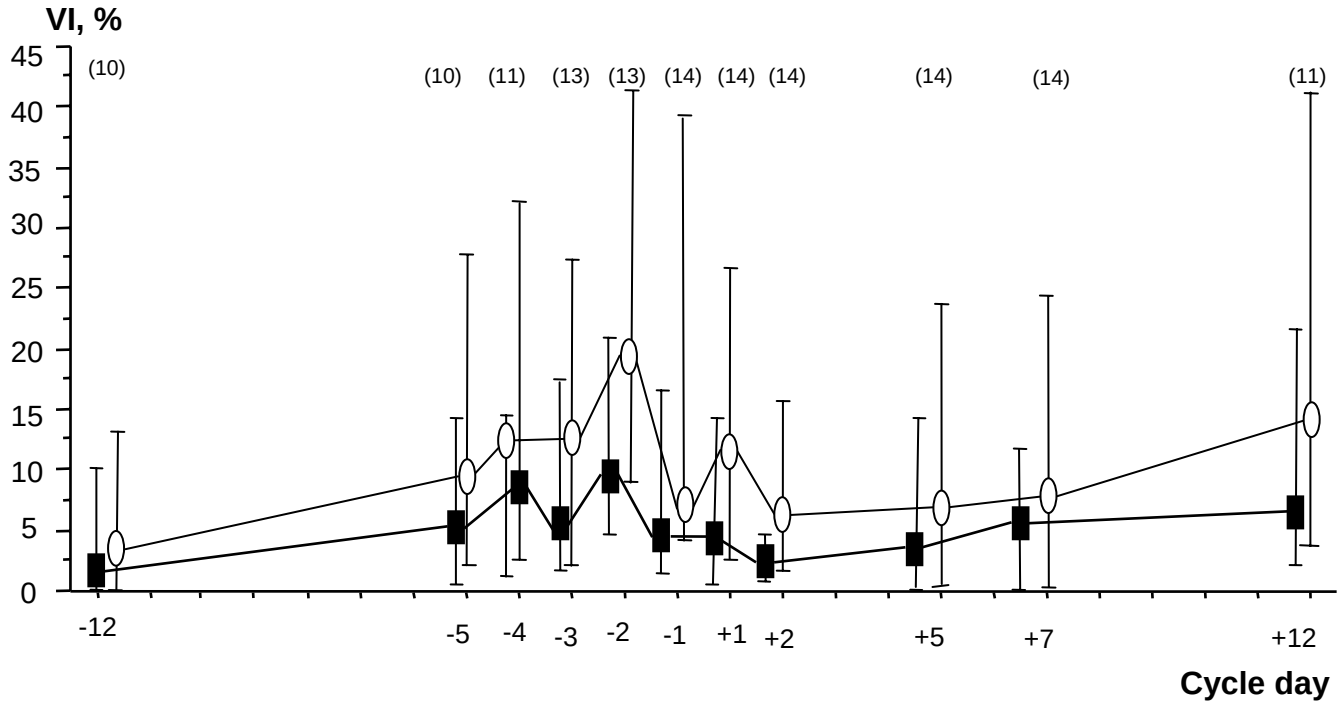

VI, %
45
40
35
30
25
20
15
10
5
0
(10)
(10)
(11)
(13)
(13)
(14)
(14)
(14)
(14)
(14)
(11)
-12
-5
-4
-3
-2
-1
+1
+2
+5
+7
+12
Cycle day

Figure 4

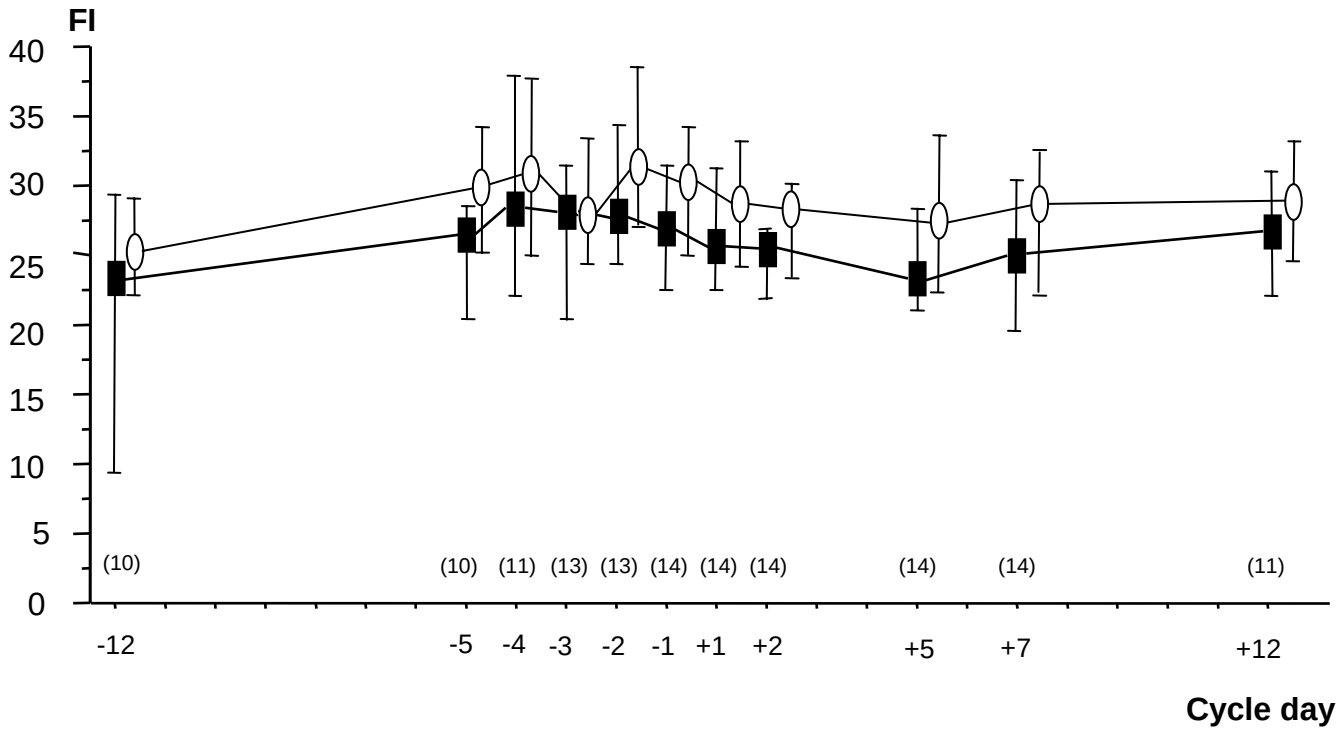
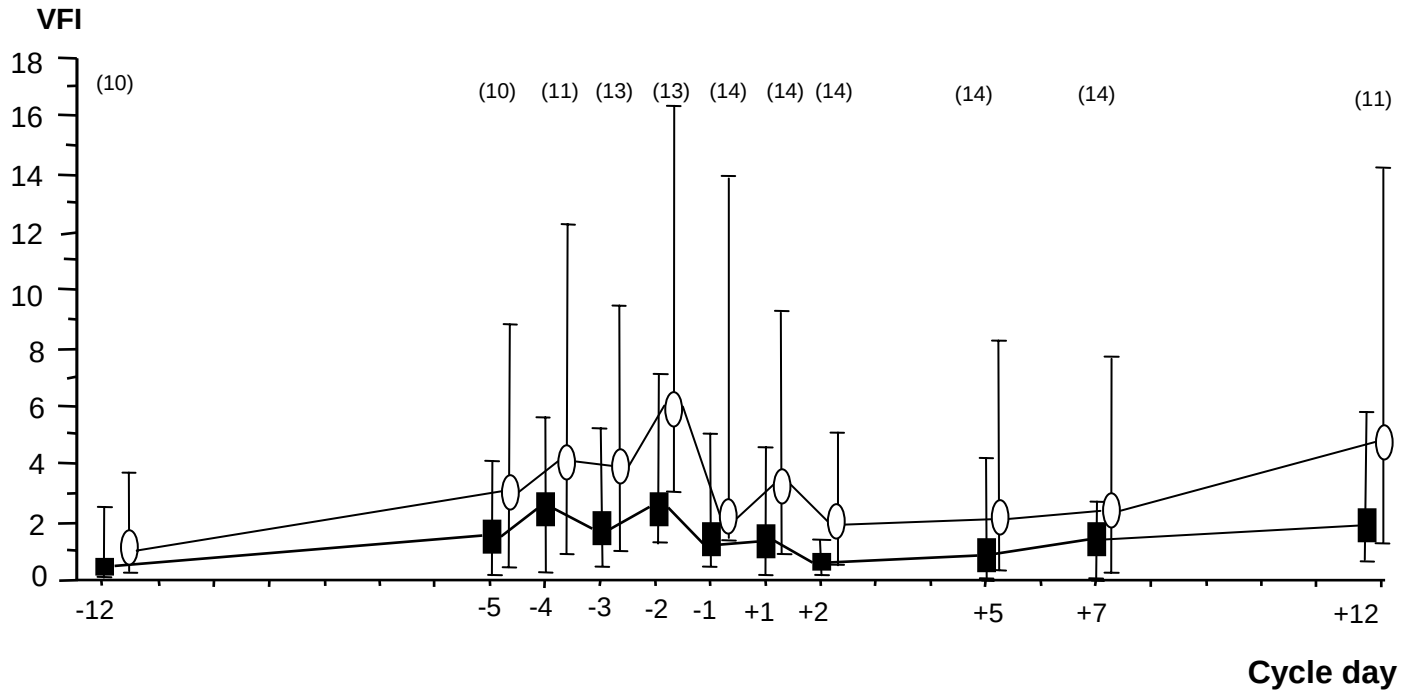

FI
40
35
30
25
20
15
10
5
0
(10)
(10)
(11)
(13)
(13)
(14)
(14)
(14)
(14)
(14)
(11)
-12
-5
-4
-3
-2
-1
+1
+2
+5
+7
+12
Cycle day

Figure 5


VFI
18
16
14
12
10
8
6
4
2
0
(10)
(10)
(11)
(13)
(13)
(14)
(14)
(14)
(14)
(14)
(11)
-12
-5
-4
-3
-2
-1
+1
+2
+5
+7
+12
Cycle day