Ultrasound Obstet Gynecol 2002; **20**: 281–285

Three-dimensional sonographic and power Doppler characterization of ovaries in late follicular phase

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KEYWORDS: Angiogenesis, Three-dimensional power Doppler, Three-dimensional ultrasound, Transvaginal color Doppler, Transvaginal ultrasound

ABSTRACT

Objective *To determine ovarian blood flow characteristics using three-dimensional power Doppler ultrasound.*

Methods *We examined 30 patients (30 cycles) prior to the start of their* in vitro *fertilization treatment in the late follicular phase using three-dimensional power Doppler ultrasound. The volume, vascularization index, flow index, vascularization flow index, mean grayness and the presence of the dominant follicle were determined for each ovary separately.*

Results *The dominant follicle could be detected in 24 out of 30 cycles (80.0%). The volume of the dominant ovary was 9.9 (standard deviation, 4.0) cm^3 and the volume of the non-dominant ovary 6.8 (standard deviation, 2.8) cm^3 (P < 0.001). Mean grayness in the dominant ovary was 43.3 (standard deviation, 5.0) and in the non-dominant 47.2 (standard deviation, 4.0) (P < 0.001), but no other differences could be observed between dominant and non-dominant ovaries. The shell with a diameter of 2 mm surrounding the dominant follicle had a higher vascularization index (mean, 9.0; standard deviation, 5.9) and vascularization flow index (mean, 4.2; standard deviation, 2.8) than the whole dominant ovary (mean, 5.5; standard deviation, 2.5 and mean, 2.5; standard deviation, 1.3, respectively) (P = 0.003 and 0.002, respectively). In the cycles without a dominant follicle (n = 6), flow index (mean, 50.0; standard deviation, 5.9) and vascularization flow index (mean, 7.3; standard deviation, 6.2) on the left side were higher than on the right side (mean, 40.2; standard deviation, 3.1; mean, 1.5; standard deviation, 1.4;* P*-values 0.013 and 0.046, respectively).*

Conclusion *In the dominant ovary, the volume was higher and mean grayness lower than in the non-dominant ovary. The vascularization index in the shell surrounding the dominant follicle was higher than the average vascularization index in the whole dominant ovary. In addition, there were differences in the vascularization and flow indices between right and left ovaries, which may be related to the anatomical difference in the venous drainage between right and left ovaries.*

INTRODUCTION

In the ovary, angiogenesis, vessel maturation and vessel regression seem to play a critical role in the selection of the dominant follicle, ovulation and corpus luteum function[1,2], and thus affect the total ovarian blood flow during different phases of the menstrual cycle. Color Doppler has enabled the non-invasive follow-up of these physiological phenomena during the menstrual cycle[3–5]. From the clinical point of view, previous studies using color Doppler sonography have shown that higher follicular blood flow velocities appear to predict the development of a healthy oocyte[6,7]. In addition, increased ovarian stromal vascularity may be associated with polycystic ovary[8–10] and the risk of ovarian hyperstimulation syndrome during *in vitro* fertilization (IVF) treatment[11].

A recent development in the field of ultrasound is three-dimensional (3D) power Doppler sonography, which provides the possibility of systemic analysis of blood flow and vascularization of the whole target organ, unlike two-dimensional (2D) color Doppler sonography where the measurement of the vascularization is restricted to the subjective depiction of randomly selected 2D slices[12]. So far, the application of this method has been confined to testing the reproducibility of the technique and quantifying blood flow in adnexal tumors[12]. No studies concerning vascularization in normal ovaries are available.

The aim of our study was to evaluate the characteristics of ovarian blood flow during the spontaneous menstrual cycle prior to IVF treatment using 3D power Doppler sonography.

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Accepted 2-4-02

PATIENTS AND METHODS

Patients

Thirty women who were attending the Infertility Unit at St George's Hospital Medical School, London, in order to undergo IVF treatment, volunteered to take part in the study. The treatment included down-regulation of pituitary and ovarian function using gonadotropin releasing hormone (GnRH)-analogs followed by controlled ovarian hyperstimulation with gonadotropins. Women with fibroids, known endometriosis or endometrioma, single ovary, polycystic ovaries (PCO), salpingectomy, ovarian cystectomy or known contraindications to use GnRH agonists and human menopausal gonadotropin were excluded from the study. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels taken on cycle days 1–5 were measured during the preceding 6 months. Ethical approval was obtained from the hospital's local research ethics committee, and each subject gave their written informed consent before participating in the study.

Procedure

The sonographic examinations for the study took place on cycle days 8–16 before the start of any medication for IVF treatment. If the corpus luteum was visible during the scan, the patient was excluded from the study.

The pelvic ultrasound scans were performed using the Kretz Combison 530D Voluson® (Kretztechnik-Medison, Zipf, Austria) with a transvaginal 3–7-MHz volume transducer, which has a 100° field of view. Identical preinstalled instrument settings (color gain 45.6, pulse repetition frequency 0.5, C-PWR 2, wall motion filter 72, frame rate 4–6) were used in all patients.

The region of interest was first visualized in 2D B-mode. The 'angio mode' was switched on; the mobile sector appeared and was set up to cover only the region of interest. The 3D facility was engaged by switching to 'volume mode'. The volume sector angle was preset to 90° and the fast volume acquisition (low resolution) setting was selected to avoid artifacts. The duration of the scanning procedure depends on the size of the mobile sector (i.e. a larger one taking more time). The duration of the volume acquisition was between 10 and 20 s. Movement of the vaginal probe was avoided during volume acquisition.

Acquired 3D volumes were stored on a removable 540 Mb hard disk cartridge (Nomai, Electronique d2, Avranches France) for later review. The volumes stored on the hard disk were transferred to a personal computer using DICOM (Digital Imaging and Communications in Medicine) connection for subsequent analysis. No compression of the data was used at any time.

The analysis of stored volumes was performed using the Virtual Organ Computer-aided AnaLysis (VOCAL™)-imaging program (version 4.0), which is integrated in Kretztechnik's Voluson® 530D sonography system.

The VOCAL-imaging program was used for volume calculations. A maximum longitudinal section was taken as a reference image and the rotation step was selected as 30°, generating six contours for each ovary. Instead of using the automatic contour mode, we selected the manual mode which enables any irregularity on the surface of the ovary to be outlined. Once a contour was defined in all image planes, the volume of the ovary was obtained.

The VOCAL-imaging program then automatically calculated gray-scale and color-scale values for the defined volume (i.e. for the whole ovary) (Figure 1). The stored ultrasound volume obtained using 3D power Doppler sonography is defined by voxels (smallest unit of volume). Gray-scale voxels contain all 3D gray-scale information grades from black to white, the lowest value (intensity) being 0 and the highest 100 (g0 to g100). A similar scale was used for color values (c0 to c100). According to these values, four indices were calculated: vascularization index (VI), flow index (FI), vascularization flow index (VFI) and mean grayness (MG). The formulas for the indices are shown in (Figure 2).

Vascularization index measures the number of color voxels in the region of interest representing the vessels in the tissue and is expressed as a percentage (%). Flow index, the mean color value in the color voxels, represents the average intensity of flow. Vascularization flow index is the mean color value in all the voxels in the sphere, representing both vascularization and flow. Mean grayness represents the average grayness in the gray voxels of the region of interest.

The volume of the dominant follicles was determined in the same manner as the volume of the ovaries. Following this, VI, FI, VFI and MG were calculated for a shell with a thickness of 2 mm surrounding the follicle.

All the statistical data were analyzed using the Statistical Package for the Social Sciences (Release 10.0, SPSS Inc., Chicago, IL, USA). Departure from a normal distribution was assessed using the Kolmogorov–Smirnov test. Paired *t*-tests were carried out for normally distributed data and the Wilcoxon test for skewed data. $P < 0.05$ was considered statistically significant. All data are given as mean (SD).

RESULTS

A total of 30 women underwent 3D sonography. Data concerning the reason for IVF treatment, ovulation frequency, age, menstrual cycle length, number of previous pregnancies, number of previous IVF treatments, pretreatment FSH and LH levels and LH/FSH ratio and smoking status are shown in Table 1. A dominant follicle of mean volume 2.3 mL ((standard deviation (SD), 1.8 mL)) and mean maximum diameter 17.8 mm (SD, 4.0 mm) indicating imminent ovulation could be detected in 24 out of 30 patients (80.0%).

Comparison between right and left ovaries ($n = 30$) did not reveal any other difference than higher FI on the left side (Table 2). In patients with a dominant follicle on either side ($n = 24$), no difference in the volume or indices between right and left sides could be found (data not shown). In the remaining patients ($n = 6$), when no dominant follicle could be detected, FI and VFI were higher on the left side (Table 3).

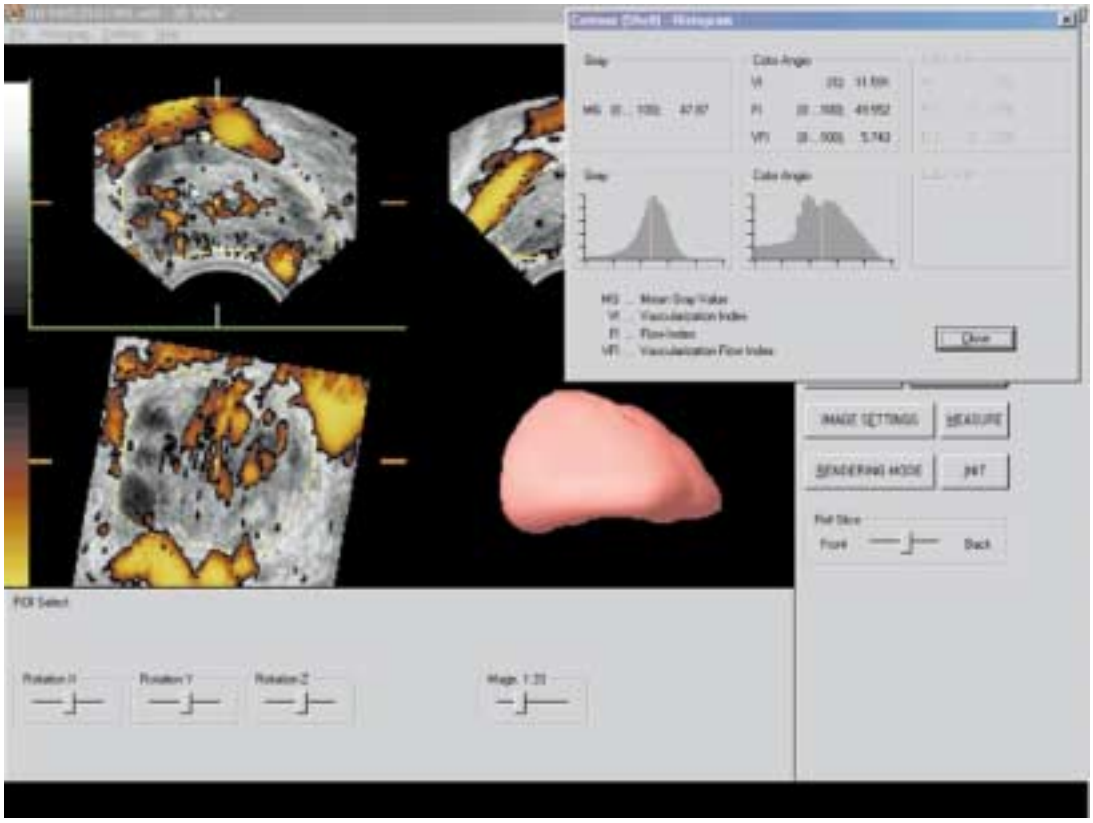

Figure 1 Three-dimensional (3D) power Doppler sonography histogram analysis in an ovary after defining its contour using the VOCAL-imaging program. VI, Vascularization index; FI, flow index; VFI, vascularization flow index; MG, mean grayness.

VI

$$VI = \frac{\sum_{c=1}^{100} hc(c)}{\sum_{g=1}^{100} hg(g) + \sum_{c=0}^{100} hc(c)} \quad (ANGIO)$$

FI

$$FI = \frac{\sum_{c=1}^{100} c \cdot hc(c)}{\sum_{c=1}^{100} hc(c)} \quad (ANGIO)$$

VFI

$$VFI = \frac{\sum_{c=1}^{100} c \cdot hc(c)}{\sum_{g=1}^{100} hg(g) + \sum_{c=0}^{100} hc(c)} \quad (ANGIO)$$

MG

$$MG = \frac{\sum_{g=1}^{100} g \cdot hg(g)}{\sum_{g=1}^{100} hg(g)}$$

Figure 2 Formulas for vascularization index (VI), flow index (FI), vascularization flow index (VFI) and mean grayness (MG). g, Gray-scale value in ultrasound image normalized to 0–100 (1, low intensity; 100, high intensity); c, color value in ultrasound image (ANGIO mode) normalized to 0–100 (1, low intensity; 100, high intensity); $hg(x)$, frequency of gray value x in ultrasound image; $hc(x)$, frequency of color value x in ultrasound image (ANGIO mode).

Table 1 Patient characteristics

Characteristic	*Value*
Number of patients (*n*)	30
Reason for *in vitro* fertilization (*n*, %)	
Male factor	10 (33.3)
Poor responder	2 (6.7)
Tubal factor	6 (20.0)
Unexplained	10 (33.3)
Mixed	2 (6.7)
Dominant follicle/cycle (*n*)	24/30
Right	12
Left	12
Age (years (SD, range))	35.3 (3.4, 25.6–42.3)
Menstrual period (days (SD, range))	28.2 (1.7, 24–32)
Number of previous pregnancies (SD, range)	0.6 (0.8, 0–3)
Number of previous IVF (SD, range)	0.4 (0.7, 0–2)
FSH (IU/L (SD, range))	7.1 (1.9, 4.6–12.0)
LH (IU/L (SD, range))	4.4 (2.6, 1.6–14.0)
LH/FSH ratio (SD, range)	0.6 (0.3, 0.2–1.6)
Smoking (%)	8 (26.7)

IVF, *in vitro* fertilization treatments; FSH, follicle stimulating hormone; LH, luteinizing hormone.

The dominant ovary containing the dominant follicle was larger and had lower MG than the non-dominant one, but there were no differences in VI, FI or VFI (Table 4). The shell surrounding the dominant follicle had higher VI, VFI and MG than the dominant ovary itself (Table 5). The characteristics in the dominant ovary between right and left sides did not reveal any statistical differences (data not shown).

Table 2 Comparison of right and left ovaries in all 30 patients showing mean (SD) ovarian volume, vascularization index, flow index, vascularization flow index and mean grayness

Characteristic	*Right*	*Left*	P-*value*
Volume (cm^3)	8.6 (4.3)	7.9 (3.1)	0.372
Vascularization index	6.2 (4.5)	7.4 (5.8)	0.530
Flow index	43.4 (4.8)	46.5 (6.5)	0.041
Vascularization flow index	2.8 (2.2)	3.7 (3.5)	0.329
Mean grayness	45.8 (5.1)	46.0 (4.6)	0.818

Table 3 Comparison of right and left ovaries in cases where no dominant follicle was detected on either side ($n = 6$) showing mean (SD) ovarian volume, vascularization index, flow index, vascularization flow index and mean grayness

Characteristic	*Right*	*Left*	P-*value*
Volume (cm^3)	8.3 (5.2)	7.6 (2.4)	0.700
Vascularization index	3.7 (3.3)	13.7 (10.1)	0.075
Flow index	40.2 (3.1)	50.0 (5.9)	0.013
Vascularization flow index	1.5 (1.4)	7.3 (6.2)	0.046
Mean grayness	48.2 (3.6)	48.4 (4.6)	0.921

Table 4 Comparison of dominant and non-dominant ovary ($n = 24$) showing the mean (SD) ovarian volume, vascularization index, flow index, vascularization flow index and mean grayness

Characteristic	*Dominant*	*Non-dominant*	P-*value*
Volume (cm^3)	9.9 (4.0)	6.8 (2.8)	< 0.001
Vascularization index	5.5 (2.5)	7.1 (4.6)	0.067
Flow index	44.6 (3.8)	45.1 (7.1)	0.692
Vascularization flow index	2.5 (1.3)	3.4 (2.4)	0.063
Mean grayness	43.3 (5.0)	47.2 (4.0)	< 0.001

Table 5 Comparison of the shell surrounding the dominant follicle and the dominant ovary ($n = 24$). The diameter of the shell was 2 mm; mean values (SD) for ovarian volume, vascularization index, flow index, vascularization flow index and mean grayness are shown

Characteristic	*Dominant follicle shell*	*Dominant ovary*	P-*value*
Volume (cm^3)	2.1 (0.9)	9.9 (4.0)	< 0.001
Vascularization index	9.0 (5.9)	5.5 (2.5)	0.003
Flow index	45.2 (6.7)	44.6 (3.8)	0.376
Vascularization flow index	4.2 (2.8)	2.5 (1.3)	0.002
Mean grayness	48.1 (4.2)	43.3 (5.0)	< 0.001

DISCUSSION

This is the first study to utilize both 3D sonography and power Doppler to evaluate volume, total vascularization and blood flow and mean grayness during the late follicular phase of normally ovulating women. The main difference compared to traditional 2D color Doppler sonography is that this method enables the analysis of total blood flow characteristics in the target organ, including both arterial and venous flow. In 2D color Doppler sonography, the information gained is restricted to arterial flow in a subjectively chosen 2D slice, which represents the target organ. Basically, the information received using 3D power Doppler sonography should measure the total blood perfusion in the target organ better than 2D color Doppler sonography. We are aware that 3D power Doppler imaging, being a form of color imaging, is subject to some of the limitations of the conventional technique[13], and this may affect the assessment of vascularization and flow detection. For example, pulse repetition frequency, wall filter setting, power gain and frame rate must be optimized for 3D display and quantitative analysis; attenuation, vessel detection between nearer and deeper parts of the tissue may be different.

In this study, the right and left sides were compared with one another, and the dominant and non-dominant sides were compared with each other. Patients with PCO were excluded because, according to earlier studies, the stromal vascularity in PCO seems to be different from that in normal ovaries[8–10]. The comparison between right and left sides revealed higher color indices on the left side, and this difference appeared to exist only during non-ovulatory cycles since there was no difference between the sides when the dominant follicle was detected.

The only known difference between the right and left ovary lies in the anatomy of the veins. The left ovarian vein drains to the left renal vein and the right ovarian vein to the inferior vena cava. In males, the venous drainage from the testis corresponds to female ovaries. Varicocele, a collection of large veins in spermatic vein, most often affects the left side (the left side is affected in 70–100% and the right in 0–9% of cases[14]) and is considered to be due to the anatomical difference in the venous drainage. It is possible that the same anatomical difference also causes symptoms in females since there appears to be a connection between chronic pelvic pain and varicosis in the local veins[15–17]. In some studies investigating the differences in function between the right and left ovaries, it was found that the ovulation frequency is higher on the right side[18–20], which may have a clinical impact in terms of embryo implantation[20]. The possibility cannot be excluded that the higher color indices on the left side observed in our study represent an increase of venous flow on this side, suggesting that the anatomical difference in the ovarian veins may also have an effect on the blood flow characteristics in the ovaries.

The dominant ovary was found to have a larger volume than that of the non-dominant one, which is a direct consequence of the existence of the dominant follicle. In addition, the mean grayness in the dominant ovary was lower than that in the non-dominant ovary, and this might also be expected as the gray-scale voxel values for the fluid inside the dominant follicle are low.

The measurements were performed during the late follicular phase, and no differences could be detected in the color indices between the dominant and the non-dominant ovaries. Vascularization index presents the ratio of the number of color voxels to the number of all the voxels. Because the dominant ovary was larger, the total number of voxels was higher than in the non-dominant ovary, which means that the

total number of color voxels was higher also in the dominant ovary, since there was no difference in the VI between dominant and non-dominant ovaries. Neither was there any difference in FI, which measures the mean intensity of the color, suggesting that the mean blood flow in the blood vessels was equal in the dominant and non-dominant ovaries. Accordingly, vascularization increases in the dominant ovary, but the ratio of the amount of vascularization per volume remains constant and similar to that in the non-dominant ovary at this phase of the menstrual cycle.

Inside the dominant ovary, the shell (diameter 2 mm) surrounding the dominant follicle had higher VI and VFI compared to the dominant ovary itself, probably resembling increased perifollicular vascularity. It was previously observed using 2D color Doppler that the perifollicular blood flow velocities gradually increase during the periovulatory period, while the pulsatility index (PI) remains relatively constant, suggesting a marked increase in blood flow at that time[3,4]. Mean grayness in the shell surrounding the follicle was higher than the average MG in the whole dominant ovary, and is probably due to MG in the dominant ovary including the dominant follicle with low value gray-scale voxels.

In summary, our results obtained using 3D power Doppler imaging demonstrate that the dominant ovary was larger than the non-dominant one; with a lower MG and with a higher perifollicular vascularization than the overall vascularization in the dominant ovary. These findings are in concordance with the current knowledge. In addition to these findings, our results suggest that there may be a difference in vascularization and blood flow between ovaries on the right and left sides. Three-dimensional power Doppler imaging appears to be a valuable new technique to evaluate changing flow characteristics in the ovaries of ovulating women.

ACKNOWLEDGMENT

This research (I.Y.J.) was supported by a Marie Curie Fellowship of the European Community Programme 'Quality of Life and Management of living Resources' under contract number QLRI-CT-1999-51230.

REFERENCES

1 Abulafia O, Sherer DM. Angiogenesis of the ovary. *Am J Obstet Gynecol* 2000; 182: 240–6
2 Augustin HG. Vascular morphogenesis in the ovary. *Baillieres Best Pract Res Clin Obstet Gynaecol* 2000; 14: 867–82
3 Campbell S, Bourne T, Waterstone J, Reynolds K, Crayford T, Jurkovic D, Okokon E, Collins W. Transvaginal color blood flow imaging of the periovulatory follicle. *Fertil Steril* 1993; 60: 433–8
4 Sladkevicius P, Valentin L, Marsal K. Blood flow velocities in the uterine and ovarian arteries during the normal menstrual cycle. *Ultrasound Obstet Gynecol* 1993; 3: 199–208
5 Van Blerkom J, Antczak M, Schrader R. The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. *Hum Reprod* 1997; 12: 1047–55
6 Nargund G, Bourne T, Doyle P, Parsons J, Cheng W, Campbell S, Collins W. Associations between ultrasound indices of follicular blood flow, oocyte recovery and preimplantation embryo quality. *Hum Reprod* 1996; 11: 109–13
7 Nargund G, Doyle PE, Bourne TH, Parsons JH, Cheng WC, Campbell S, Collins WP. Ultrasound derived indices of follicular blood flow before HCG administration and the prediction of oocyte recovery and preimplantation embryo quality. *Hum Reprod* 1996; 11: 2512–7
8 Zaidi J, Campbell S, Pittrof R, Kyei-Mensah A, Shaker A, Jacobs HS, Tan SL. Ovarian stromal blood flow in women with polycystic ovaries – a possible new marker for diagnosis? *Hum Reprod* 1995; 10: 1992–6
9 Agrawal R, Sladkevicius P, Engmann L, Conway GS, Payne NN, Bekis J, Tan SL, Campbell S, Jacobs HS. Serum vascular endothelial growth factor concentrations and ovarian stromal blood flow are increased in women with polycystic ovaries. *Hum Reprod* 1998; 13: 651–5
10 Engmann L, Sladkevicius P, Agrawal R, Bekir J, Campbell S, Tan SL. The pattern of changes in ovarian stromal and uterine artery blood flow velocities during *in vitro* fertilization treatment and its relationship with outcome of the cycle. *Ultrasound Obstet Gynecol* 1999; 13: 26–33
11 Agrawal R, Conway G, Sladkevicius P, Tan S, Engmann L, Payne N, Bekir J, Campbell S, Jacobs H. Serum vascular endothelial growth factor and Doppler blood flow velocities in *in vitro* fertilization: relevance to ovarian hyperstimulation syndrome and polycystic ovaries. *Fertil Steril* 1998; 70: 651–8
12 Pairleitner H, Steiner H, Hasenoehrl G, Staudach A. Three-dimensional power Doppler sonography: imaging and quantifying blood flow and vascularization. *Ultrasound Obstet Gynecol* 1999; 14: 139–43
13 Kurjak A, Kupesic S, Breyer B, Sparac V, Jukic S. The assessment of ovarian tumor angiogenesis: what does three-dimensional power Doppler add? *Ultrasound Obstet Gynecol* 1998; 12: 136–46
14 Takihara H, Sakatoku J, Cockett AT. The pathophysiology of varicocele in male infertility. *Fertil Steril* 1991; 55: 861–8
15 Giacchetto C, Catizone F, Cotroneo GB, Cavallaro V, Cammisuli F, Minutolo V, Rodolico G. Radiologic anatomy of the genital venous system in female patients with varicocele. *Surg Gynecol Obstet* 1989; 169: 403–7
16 Giacchetto C, Cotroneo GB, Marincolo F, Cammisuli F, Caruso G, Catizone F. Ovarian varicocele: ultrasonic and phlebographic evaluation. *J Clin Ultrasound* 1990; 18: 551–5
17 Beard RW, Highman JH, Pearce S, Reginald PW. Diagnosis of pelvic varicosities in women with chronic pelvic pain. *Lancet* 1984; 2: 946–9
18 Potashnik G, Insler V, Meizner I, Sternberg M. Frequency, sequence, and side of ovulation in women menstruating normally. *BMJ (Clin Res Ed)* 1987; 294: 219
19 Check JH, Dietterich C, Houck MA. Ipsilateral versus contralateral ovary selection of dominant follicle in succeeding cycle. *Obstet Gynecol* 1991; 77: 247–9
20 Järvelä I, Nuojua-Huttunen S, Martikainen H. Ovulation side and cycle fecundity: a retrospective analysis of frozen/thawed embryo transfer cycles. *Hum Reprod* 2000; 15: 1247–9