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Intraobserver and interobserver variability of ovarian volume, gray-scale and color flow indices obtained using transvaginal three-dimensional power Doppler ultrasonography

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KEYWORDS: Doppler ultrasound; inter-CC; intra-CC; limits of agreement; repeatability coefficient

ABSTRACT

Objective *To assess intraobserver and interobserver variability in ovarian volume and gray-scale and color flow index measurements using transvaginal, three-dimensional, power Doppler ultrasonography.*

Methods *Eleven women (22 ovaries) were examined on day 8 of controlled ovarian hyperstimulation therapy, which was part of their* in vitro *fertilization treatment protocol. The patients were examined twice by the first observer and once by the second observer. The acquired volume datasets were analyzed using the VOCAL™ imaging program, enabling the assessment of ovarian volume, vascularization index (VI), flow index (FI), vascularization flow index (VFI) and mean grayness (MG). For these parameters the intraclass (intra-CC) and interclass (inter-CC) correlation coefficients, within-observer and between-observers repeatability coefficient (*r*) and limits of agreement were calculated.*

Results *Both intraobserver and interobserver repeatability of ovarian volume measurements were considered very good with an intra-CC value of 1.00 and inter-CC value of 0.99, respectively. Also VI, FI, VFI and MG measurements were repeatable by a single observer, the intra-CC ranging from 0.82 to 0.91. The interobserver reproducibility was also good for VI, VFI and MG measurements (inter-CC values 0.73, 0.70 and 0.81, respectively), but for FI measurements the reproducibility was poor (inter-*CC *= 0.29,* r *= 7.87).*

Conclusions *In general, the intraobserver reproducibility was better than interobserver reproducibility for all parameters. The volume assessments were reproducible both by one observer and by two separate observers. The intraobserver and interobserver variabilities were acceptable for VI, VFI and MG, whereas for FI the interobserver reproducibility was poor. Our results suggest that measurement of gray-scale and color Doppler flow indices is reproducible thus allowing them to be used in clinical practice and research. Copyright © 2003 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Power Doppler imaging is a relatively new mode of Doppler ultrasonography. It appears to have several advantages over conventional color Doppler ultrasonography in that it does not alias, it is relatively angle-independent and it is more sensitive than color Doppler imaging at detecting low velocity flow and hence improves the visualization of small vessels[1–3]. Despite this, the use of power Doppler imaging as an assay in clinical settings has been restricted, the major problem being its quantification. There are now several options in two-dimensional (2D) power Doppler research for quantification which include subjective[4], semiquantitative[5] and objective quantitative methods[6].

The latest technical achievement in the field of ultrasonography is three-dimensional (3D) imaging combined with power Doppler. Theoretically it provides the possibility to assess the volume and quantify the power Doppler signal in the whole target organ, unlike 2D ultrasonography, where information on vascularization and blood flow is restricted to one subjectively chosen

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two-dimensional plane. In addition to power Doppler measurements, it is possible to quantify the ultrasonographic brightness or grayness in the region of interest. To date, endometrial[7,8], ovarian[9] and ovarian tumor vascularity[10] have been assessed quantitatively using 3D power Doppler ultrasonography.

Before the impact of 3D power Doppler ultrasonography in clinical practice and research can be established, its reproducibility must be determined. This study aimed to document the intraobserver and interobserver variability in ovarian volume and gray-scale and color flow indices measurements using transvaginal 3D power Doppler ultrasonography.

METHODS

Subjects

The study protocol was approved by the Ethics Committee of the Medical School. We recruited 11 women who all consented to participate. The patients were examined on day 8 of controlled ovarian hyperstimulation therapy, which was part of their *in vitro* fertilization treatment protocol.

Ultrasound equipment

Examinations were performed by two trained observers (I.Y.J. and P.S.). A Kretz Combison 530D Voluson (Kretztechnik-Medison, Zipf, Austria) equipped with a transvaginal 3–7-MHz volume transducer, which has a 100° field of view, was used. 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.

Three-dimensional power Doppler ultrasonography and variability measurements

The patients were examined twice by the first observer (I.Y.J.) and once by the second observer (P.S.), the latter taking place between the two examinations made by the first observer. On each occasion both ovaries were scanned.

After visualizing the ovary in 2D B-mode, the mobile sector for angio mode was switched on and set up to cover only the region of interest (ROI). 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 volume acquisition was between 15 and 25 s, depending on the dimensions. The vaginal probe was kept steady during the volume acquisition. The acquired 3D volumes were transferred immediately to a personal computer using a DICOM (Digital Imaging and Communications in Medicine) connection. No compression of the data was used at any time.

All the stored volumes were analyzed using the VOCAL™ imaging program (Virtual Organ Computer-aided AnaLysis) version 4.0, which is integrated into Kretztechnik's Voluson® 530D ultrasound system. In order to account for any surface irregularity of the ovary, the contour mode in the VOCAL program was set to manual. The longitudinal view was used as a reference image and the rotation step was selected as 30°, resulting in the definition of six contours for each ovary. Once a contour was defined in all image planes, the volume of the ovary was obtained.

The stored ultrasound volume obtained using 3D power Doppler is defined by voxels (smallest unit of volume). The total number of the voxels is the sum of the number of gray-scale voxels and color-scale voxels. 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...g100). The range for the values of color-scale voxels can vary similarly between 0 and 100 (c0...c100).

Once the contour was defined, the VOCAL program automatically calculated indices for gray-scale and color-scale voxels. According to these values four indices were calculated: vascularization index (VI), flow index (FI), vascularization flow index (VFI) and mean grayness (MG).

VI measures the ratio of the number of color voxels to the number of all the voxels in the defined contour. VI is thought to represent the presence of blood vessels in the tissue (vascularization) and is expressed as a percentage value. FI (scale 0...100), the mean value of the color voxels, is thought to express the average intensity of flow in the vessels. VFI measures the ratio of the mean value of color voxels and all the voxels in the defined contour, and is a feature of both vascularization and flow[10]. MG (scale 0...100) in gray voxels corresponds to FI in color voxels, i.e. it is the mean value of the gray voxels. MG expresses the gray-scale brightness or echogenicity of the tissue.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Release 10.0; SPSS Inc., Chicago, IL, USA). Departure from a normal distribution was assessed using the Kolmogorov–Smirnov test. If the data were skewed, a logarithmic transformation was performed before statistical analysis.

The intraobserver and the interobserver variation were assessed by calculating the intraclass correlation coefficient (intra-CC), interclass correlation coefficient (inter-CC) and repeatability coefficient (r). The components of variance were estimated by analysis of variance (ANOVA) tables. In addition, limits of agreements (LOA) for each parameter were assessed for VI, FI, VFI and MG.

The intra-CC estimates the overall correlation between all possible pairs within the subject taken by the same observer and is defined by:

$$1 - [s_w^2/s_b^2 + s_w^2] = 1 - [s_w^2/s^2],$$

Table 1 The descriptive statistics for volume, vascularization index, flow index, vascularization flow index and mean grayness

Parameter	*Observer*	*Measurement*	*Minimum*	*Maximum*	*Mean*	*SD*
Volume (cm^3)	1	1	3.69	78.06	26.54	21.82
Volume (cm^3)	2	2	4.50	81.69	27.50	21.82
Volume (cm^3)	1	3	4.61	76.94	27.12	22.15
Vascularization index (%)	1	1	2.17	19.95	9.72	4.64
Vascularization index (%)	2	2	2.66	17.44	9.67	4.01
Vascularization index (%)	1	3	3.22	23.76	10.95	5.61
Flow index	1	1	39.19	56.21	47.00	4.51
Flow index	2	2	39.74	58.45	47.21	4.70
Flow index	1	3	41.53	52.32	47.33	3.12
Vascularization flow index	1	1	0.90	9.78	4.64	2.39
Vascularization flow index	2	2	1.06	9.87	4.61	2.05
Vascularization flow index	1	3	1.34	12.08	5.28	2.91
Mean grayness	1	1	32.92	53.48	44.85	5.71
Mean grayness	2	2	37.64	53.47	45.82	4.71
Mean grayness	1	3	30.20	54.70	45.17	6.22

where s_w^2 is the within-subject variance, s_b^2 is the between-subject variance and the s^2 is the total variance.

The inter-CC for observations by different observers was calculated using the following formula:

$$s_b^2/[s_b^2 + s_o^2 + s_b^2 + s_w^2],$$

where s_b^2 is the between-subject variance, s_o^2 is the variance due to the observers and s_b^2 is the heterogeneity.

The 95% confidence intervals (CIs) for the intra-CC correlation coefficient values were calculated according to the methods described by Scheffe[11]. The CIs for the inter-CC values defining the interobserver variation are not given, since two observers are too few to give useful estimates[12].

The repeatability coefficient (r) is the maximum difference that is likely to occur between repeated measurements and can be defined as $1.96 \times \sqrt{2}s_w^2$.

In the assessment of interobserver agreement, mean differences between the observers, LOA[13] and their 95% CIs were calculated and plotted. The LOA indicate the range within which 95% of the disagreement between the observers is likely to fall. They were defined as the mean difference $\pm\, t_{n-1}$SD, where t_{n-1} is the probability point of the t distribution with $n - 1$ degrees of freedom, and SD is the standard deviation of the mean difference.

RESULTS

All 11 women participating in the study had intact ovaries, which both were detected each time. The first observer (I.Y.J.) examined the 22 ovaries twice and the second observer (P.S.) once. Table 1 presents the minimum, maximum, mean and SD values for ovarian volume, VI, FI, VFI and MG. Because the volume data were skewed, a logarithmic transformation was performed before statistical analysis. After logarithmic transformation normality was achieved.

Ovarian volume

The range of ovarian volumes was between 3.69 and 81.69 cm^3 (Table 1). The volume assessments had high intra-CC and inter-CC values (Tables 2 and 3) indicating excellent repeatability. Because the volume data were logarithmically transformed, the repeatability coefficient (r) here expresses the ratio of higher to lower volume

Table 2 The intraobserver variation (repeatability) of the volume, vascularization index, flow index, vascularization flow index and mean grayness values for ovaries

Parameter	*Ovaries* (n)	*Measurements* (n)	*Intra-CC (95% CI)*	r
Volume (*ln*)	22	44	1.00	1.12
VI (%)	22	44	0.89 (0.87–0.97)	3.70
FI	22	44	0.82 (0.74–0.93)	3.97
VFI	22	44	0.90 (0.88–0.97)	1.83
MG	22	44	0.91 (0.90–0.98)	3.64

95% CI, 95% confidence interval; FI, flow index; intra-CC, intraclass correlation coefficient; *ln*, logarithmic transformation; MG, mean grayness; *r*, repeatability coefficient; VFI, vascularization flow index; VI, vascularization index.

Table 3 The interobserver variation (repeatability) of the volume, vascularization index, flow index, vascularization flow index and mean grayness values for ovaries

Parameter	*Ovaries* (n)	*Measurements* (n)	*Inter-CC*	r
Volume (*ln*)	22	66	0.99	1.23
VI (%)	22	66	0.73	5.69
FI	22	66	0.29	7.87
VFI	22	66	0.70	3.12
MG	22	66	0.81	5.41

FI, flow index; *ln*, logarithmic transformation; MG, mean grayness; *r*, repeatability coefficient; VFI, vascularization flow index; VI, vascularization index.

values obtained from the same ovary. At all times the ratio between two measurements taken from the same ovary is likely to be 1.12. The interobserver *r* was slightly higher (1.23) than the intraobserver *r*.

Vascularization index

The range for VI values was between 2.17% and 23.76%. The intra-CC was 0.89 and *r* was 3.70, the latter suggesting that two separate VI measurements performed by a single observer are unlikely to be more than 3.7% apart. The inter-CC was 0.73 and *r* was 5.69. In common with ovarian volume measurements, VI measurements were more accurately repeated by only one observer. LOA showing the level of variability between two observers are plotted in Figure 1. As shown, 95% of disagreement between the two observers lay between −4.8283 and 4.9281 for VI.

Flow index

The range for FI was between 39.19 and 58.45. For FI measurements, the intra-CC (0.82) and $r = 3.97$ expressed high repeatability. According to *r*, two FI measurements on the same subject made by one observer are unlikely to be more than 3.97 units apart. The inter-CC was only 0.29 and the interobserver *r* was 7.87. FI measurements were more accurately repeated by a single observer. Interobserver limits of agreement for FI lay between −8.4980 and 8.08404 (Figure 1).

Vascularization flow index

The VFI values varied between 0.90 and 12.08. The intra-CC (0.90) was higher than the inter-CC (0.70) and the intraobserver *r* (1.83) was lower than the interobserver *r* (3.12), indicating again that the intraobserver repeatability of the VFI measurements was better than the interobserver repeatability. Interobserver limits of agreement for VFI lay between −2.6219 and 2.6909 (Figure 1).

Mean grayness

The MG values were between 30.20 and 54.70. Both intra-CC and inter-CC were high (0.91 and 0.81, respectively) and the *r* was reasonable (intraobserver 3.64 and interobserver 5.41), suggesting good reproducibility,

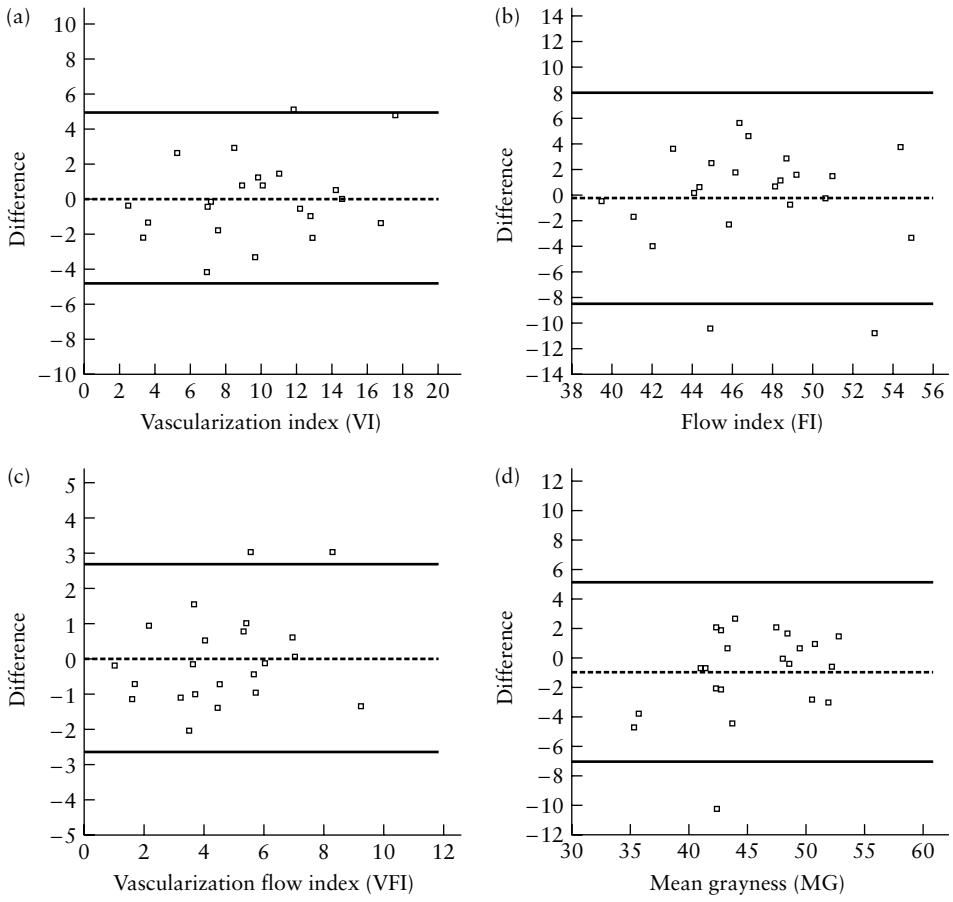

Figure 1 Differences plotted against means for vascularization (VI), flow (FI), vascularization flow index (VFI) and mean grayness (MG). The examinations were performed by two separate observers. (a) VI in the ovaries, mean difference 0.0499, upper limit of agreement (LOA) 4.9281, lower LOA −4.8283. (b) FI in the ovaries, mean difference −0.2070, upper LOA 8.08404, lower LOA −8.4980. (c) VFI in the ovaries, mean difference 0.0345, upper LOA 2.6909, lower LOA −2.6219. (d) MG in the ovaries, mean difference −0.9723, upper LOA 5.0833, lower LOA −7.0279.

no matter whether there was one or two observers. Interobserver LOA for MG lay between −7.0279 and 5.0833 (Figure 1).

DISCUSSION

With this method there are two main sources of intraobserver and interobserver variability: the variability associated with 3D-volume acquisition and the variability associated with contour definition. The aim of this study was to determine the intraobserver and interobserver reproducibilities of 3D-volume acquisition. The contours were all defined manually by only one observer (I.Y.J.) and the intraobserver reproducibility of contour definition was not assessed.

The reproducibility associated with the assessment of indices may be worsened by problems in assessing the volume of the ROI, which must be determined before indices can be calculated. If the assessed volume of the ROI does not exactly correspond to the true volume of the organ, besides affecting the accuracy of the volumes obtained, it affects the accuracy of color-scale or gray-scale indices calculated thereafter and may even multiply the error in them. Therefore it is possible that if the assessment ROI volume is not reproducible during repeated measurements, neither will be the calculation of the indices.

According to earlier studies, the reproducibility of 3D ultrasonography in assessing the volume of the target organ has proved to be accurate[14,15]. In a study by Kyei-Mensah *et al.* three different observers independently measured 20 stored ovarian volumes scanned with 3D ultrasonography. They observed that 3D ultrasonography produces highly reproducible ovarian volume measurements[14]. The same authors have demonstrated that the true volume of ovarian follicles is measured more accurately by a 3D ultrasound system than by 2D sonographic techniques[15]. Our results agree with these studies, since both intraobserver and interobserver reproducibility in volume assessments was good, nevertheless the intraobserver variability was slightly less than the interobserver one.

This is the first study to assess the reproducibility of tissue grayness or brightness measurements by means of the MG index using 3D ultrasonographic equipment. A possible clinical application of this might be in the diagnosis of polycystic ovaries (PCO). Increased stromal echogenicity is often considered typical of PCO[16,17], although it has been acknowledged that its recognition is highly subjective[18]. We found MG assessments to be highly repeatable, both between measurements performed by one observer and those by two separate observers. Two MG measurements on the same subject would differ by no more than 3.64 units if there was only one observer and 5.41 units if there were two separate observers. Thus, with the use of this new technology we are now able to determine objectively the differences in tissue brightness, for example between normal and PCO.

Pairleitner *et al.*[10] have previously assessed the reproducibility of the VI, FI and VFI obtained using the same 3D power Doppler ultrasound equipment but an earlier version of the software. The authors concluded that the FI and VI gave reproducible information whereas VFI was less reproducible. In their software the indices were calculated from a cube which covered most of the target organ but also some of the surrounding tissue, and therefore the accuracy of the earlier version of the software cannot be directly compared to that of the current version.

In our study the intraobserver variability of the color-scale indices (VI, FI, VFI) was equal to the variability in the gray-scale index (MG). The interobserver variability in VI, FI and VFI was larger than the intraobserver one, FI representing the lowest reproducibility. The theoretical scale for VI, FI and VFI is 0...100, nevertheless in this material the range was much narrower for each of them. According to our results the smallest change in the index for which it is possible to differentiate measurements performed by two separate observers was 5.69 for VI, 7.87 for FI and 3.12 for VFI. Particularly for FI, the difference observed is quite large. It is likely that the fluctuations in blood flow cause greater changes in the color voxels than the gray-scale index, which remains nearly constant. The system did not allow any coordination between volume acquisition and the cardiac cycle but, because acquisition lasted between 15 to 25 s, the effect would probably have been insignificant.

The variation in the measurements of color indices maybe partly due to the artifacts produced by power Doppler. Amso *et al.* quantified power Doppler energy images on 2D real-time scanning and observed a significant image-to-image variation[6]. Nelson *et al.* divided the type of power Doppler artifacts into gain and motion-induced ones[19]. In this study we had similar settings for each scan, which should have excluded any effects caused by the gain. As for motion artifacts, motion flash is caused by external movements, which causes color in places where there is no flow, showing streaks and pseudo-vessels in the image. Since the ovaries lie close to the iliac vessels, movements in the iliac artery affect the ovaries, especially when they are enlarged. In addition, despite the fact that the probe was kept steady during the scans, we did not ask the patient to stop breathing, which may have caused some ovarian movement as well. The ovarian motion caused by arterial and breathing movements may have led to power Doppler artifacts and variation in the VI, FI and VFI measurements observed in our study. Because the interobserver reproducibility in these indices was poorer than the intraobserver reproducibility, there might be a factor or factors which are dependent on the observer and not on the patient or on the ultrasound equipment used. Possibly individual style of scanning may induce variation in the artifacts caused by the motion of the ovary and surrounding organs.

Despite being the latest technical modality, 3D power Doppler imaging is still subject to some of the limitations of the conventional technique[20]. The assessment of reproducibility of the indices from 3D volumes defined

the limits beyond which the true change in the parameters cannot be observed. In clinical practice and research it is essential to estimate the magnitude of the change in the parameter (index) which is considered clinically significant and then define whether the measuring equipment is capable of detecting it or not. Because interobserver variability was larger than intraobserver variability, the expected magnitude of the change in the parameter affects the decision whether it is possible to use more observers instead of only one.

We have assessed the reproducibility of gray-scale and color-scale indices in volumes obtained using 3D power Doppler ultrasonography. According to our results it seems that in general the measurement of these indices is reproducible thus allowing their use in clinical practice and research, bearing in mind the limitations involved in this technique as described above.

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