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ORIGINAL ARTICLE



2-D and 3-D ultrasonographic characteristics of the ovary in women with PCOS and multifollicular ovaries

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ABSTRACT

In this prospective study, seventy-six patients (PCOS group; $n = 36$, multifollicular ovary group; $n = 40$) were evaluated by 2-D and 3-D ultrasonography. VOCAL programme, echogenicity, number of follicles and blood flow parameters were evaluated. The patients with PCOS had a higher total ovarian volume, mean stromal volume and stromal echogenicity (18.6 ± 4.75 to 10.2 ± 3.4 $p < .01$; 12.23 ± 2.53 to 5.02 ± 2.44 $p = .02$; score 1:28 to 10 $p < .01$, respectively). There was no statistically significant difference in terms of mean RI and PI values between the groups ($p > .05$). 3D power Doppler parameters included VI, FI, and VFI values of the patients with PCOS were higher when compared to those of the patients with multifollicular ovary (3.82 ± 2.65 to 1.78 ± 1.2 , $p < .01$; 50.76 ± 4.45 to 40.6 ± 3.64 , $p = .03$; and 2.34 ± 1.02 to 1.12 ± 0.65 , $p = .02$, respectively). Our results revealed that total ovarian volume, stromal volume and echogenicity; VFI, VI, and FI could be useful for differential diagnosis in women with PCOS and multifollicular ovaries.

IMPACT STATEMENT

- **What is already known on this subject?** Ultrasonography is considered the new diagnostic tool for PCOS. Enlarged ovaries with multiple small follicles peripherally located around increased ovarian stroma with increased stromal echogenicity are the sonographic features of polycystic ovaries.
- **What do the results of this study add?** 3-D Doppler ultrasonography may be more specific in the determination of multifollicular and polycystic ovaries when compared to RI and PI in 2-D Doppler ultrasonography. Moreover, 3-D power Doppler ultrasonography could be useful for differential diagnosis in women with PCOS and multifollicular ovaries.
- **What are the implications of these findings for clinical practice and/or further research?** In clinical practice, the differentiation of multifollicular ovaries and polycystic ovaries (PCO) is difficult with the use of 2-D sonography alone. Therefore, 3-D ultrasound and power Doppler may also be used in addition to 2-D ultrasound for the differentiation of multifollicular ovaries and PCO.

KEYWORDS

Polycystic ovarian syndrome; multifollicular ovary; stromal blood flow; 3D power doppler

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age; its prevalence varies between 5 and 10% (Franks 1995). The Rotterdam criteria are commonly used for the diagnosis of PCOS. These criteria consist of oligoovulation and/or anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovaries (observed in ultrasound examination). Two of the three criteria are mandatory to diagnose PCOS. There are four different phenotypes of PCOS: phenotype 1 (oligo-anovulation + hyperandrogenism + polycystic ovaries), phenotype 2 (oligo-anovulation + hyperandrogenism), phenotype 3 (hyperandrogenism + polycystic ovaries), and phenotype 4 (oligo-anovulation + polycystic ovaries) (Giampaolino et al. 2018; Della et al. 2018).

According to the Rotterdam criteria, polycystic ovaries appear in ultrasound examination as twelve or more follicles with a diameter of 2–9 mm in either ovary and an increased ovarian volume ($>10 \text{ cm}^3$) (Balen et al. 2003). These ultrasonographic characteristics of PCOS were also defined by ESHRE and ASRM in 2004 (ESHRE/ASRM 2004). The most specific sonographic sign of PCOS is enlarged ovaries with multiple small follicles peripherally located around increased ovarian stroma with increased stromal echogenicity (Rumack and Levine 2017). Ultrasonography is considered the new diagnostic tool for PCOS (ESHRE/ASRM 2004). Adams et al. also defined multifollicular ovaries; they found that multifollicular ovaries should be considered a normal ovarian variant, and they result from a response to a pulsatile GnRH. They defined multifollicular ovaries as multiple (>6) follicles with a

diameter of 4–10 mm with normal stromal echogenicity and normal in size or slightly enlarged. Multifollicular ovaries are an isolate ultrasonographic sign, without any systemic clinical features in contrast PCOS is metabolic and reproductive disorder which is characterised by sonographic, clinical and biochemical changes (Adams et al. 1985; Zhu et al. 2016).

There are limitations to the use of 2-D sonographic criteria in the diagnosis of PCOS (Lam and Raine-Fenning 2006). The 3-D ultrasound characteristics may offer a better diagnostic tool for PCOS. The correct diagnosis of PCO is necessary to achieve the selection of appropriate intervention (Della et al. 2018). The evaluation of some objective quantitative characteristics, such as AFC, ovarian blood flow, and volume calculation using 3-D may be superior to 2-D ultrasound (Allemand et al. 2006; Raine-Fenning et al. 2003). In clinical practice, the differentiation of multifollicular ovaries and PCO is difficult with the use of 2-D alone. Therefore, 3-D ultrasound and power Doppler may also be used in addition to 2-D for the differentiation of multifollicular ovaries and PCO. The aim of the study was to evaluate 2-D and 3-D ultrasonographic characteristics of ovaries in women with PCOS and multifollicular ovaries.

Material and methods

Participants

A total of seventy-six patients attending the Department of Obstetrics and Gynaecology at Universal Malatya Hospital between November 2012 and February 2013 were included in the study. The study protocol was approved by the ethical committee of the hospital. Written informed consent was obtained from all patients.

Women fulfilling our eligibility criteria were divided into two groups: Group A (the PCOS group, based on the Rotterdam criteria) and Group B (the multifollicular ovary group, based on Adams *et al.*) (ESHRE/ASRM 2004; Adams et al. 1985).

The inclusion criteria were patients with 17 and 38 years of age and a body mass index (BMI) between 20 and 25. The exclusion criteria were patients had any ovarian follicles ≥ 22 mm in diameter, any kind of ovarian cyst on a baseline scan, a previous history of ovarian surgery, a history of taking exogenous hormonal preparation or drug treatment (such as spironolactone and metformin) within the last three months, chronic illness (hypertension, diabetes, etc.), smoking, and regular exercise.

The data collected included age; BMI; serum FSH, LH, E2, testosterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS), and sex hormone binding globulin (SHBG); total ovarian volume; AFC; stromal echogenicity; total stromal volume; RI; PI; VI; FI and VFI.

3-D Ultrasonographic assessments

All ultrasonographic assessments were performed by a single physician, who was blinded to the diagnostic allocation on the second or third day of the menstrual period using a 7.5-MHz endovaginal probe for 2-D and 3-D ultrasounds

(Voluson 730 Pro; GE Healthcare Kretztechnik, Zipf, Austria). The morphological features of both ovaries and the pelvic structures were assessed by B-mode (Figure 1). Power Doppler was concurrently performed with standard settings (colour gain 4 cm/sn, pulse repetition frequency 0.6 MHz, frame rate 4–6, wall motion filter 120 Hz).

Measurement of antral follicles by 2-D ultrasound

AFC was taken for both ovaries using a 2-D ultrasound. Ovaries were evaluated in two planes, longitudinal and transverse, for the calculation of AFC. Antral follicle size was calculated by taking the mean of two perpendicular diameters, one of which represented the largest dimension of each follicle. The scoring system is based on antral follicles: ≤ 10 antral follicles = "0", ≥ 10 antral follicles in only one ovary = "1", and ≥ 10 antral follicles in both ovaries = "2" (Dolz et al. 1999).

Assessment of stromal echogenicity of ovaries

Myometrium echogenicity was used in the assessment of stromal echogenicity of the ovaries. The fundal anterior portion of the uterus and central stromal portion of the ovaries, in which follicles were not observed, were compared for the assessment of stromal echogenicity. Echogenicity was scored as follows: "0" if it was equal to or less than the myometrium or "1" if one ovary had a higher echogenicity than the myometrium (Dolz et al. 1999). If there was no significant difference in stromal echogenicity between both ovaries of the same patient, an average value was taken for both ovaries.

Measurement of resistive index (RI) and pulsatility index (PI) by 2-D doppler

RI and PI were measured using a 2-D Doppler of the stromal arteries, which are located on the central part of the ovaries, where follicles and coding flow are not observed. Mean RI and PI values were calculated by obtaining three successive waveforms for both ovaries. If there was no significant difference in RI and PI values for the ovaries of the same patient, mean RI and PI values were calculated for each patient.

Measurement of total ovarian volume by 3-D ultrasound

The 3-D static surface mode was activated, and an appropriate volume box was placed on the ovary. The 3-D volume scanning produced three simultaneous perpendicular planes. The largest central organ slice was selected as the reference plane, and the ovarian volumes were calculated using the virtual organ computer aided analysis (VOCAL) software programme. Six slices of the ovary at rotational intervals around the main axis of the ovary were created using VOCAL programme. Ovarian contours were manually traced on each slice. The software automatically calculated the volume of the ovary (Figures 1 and 2).

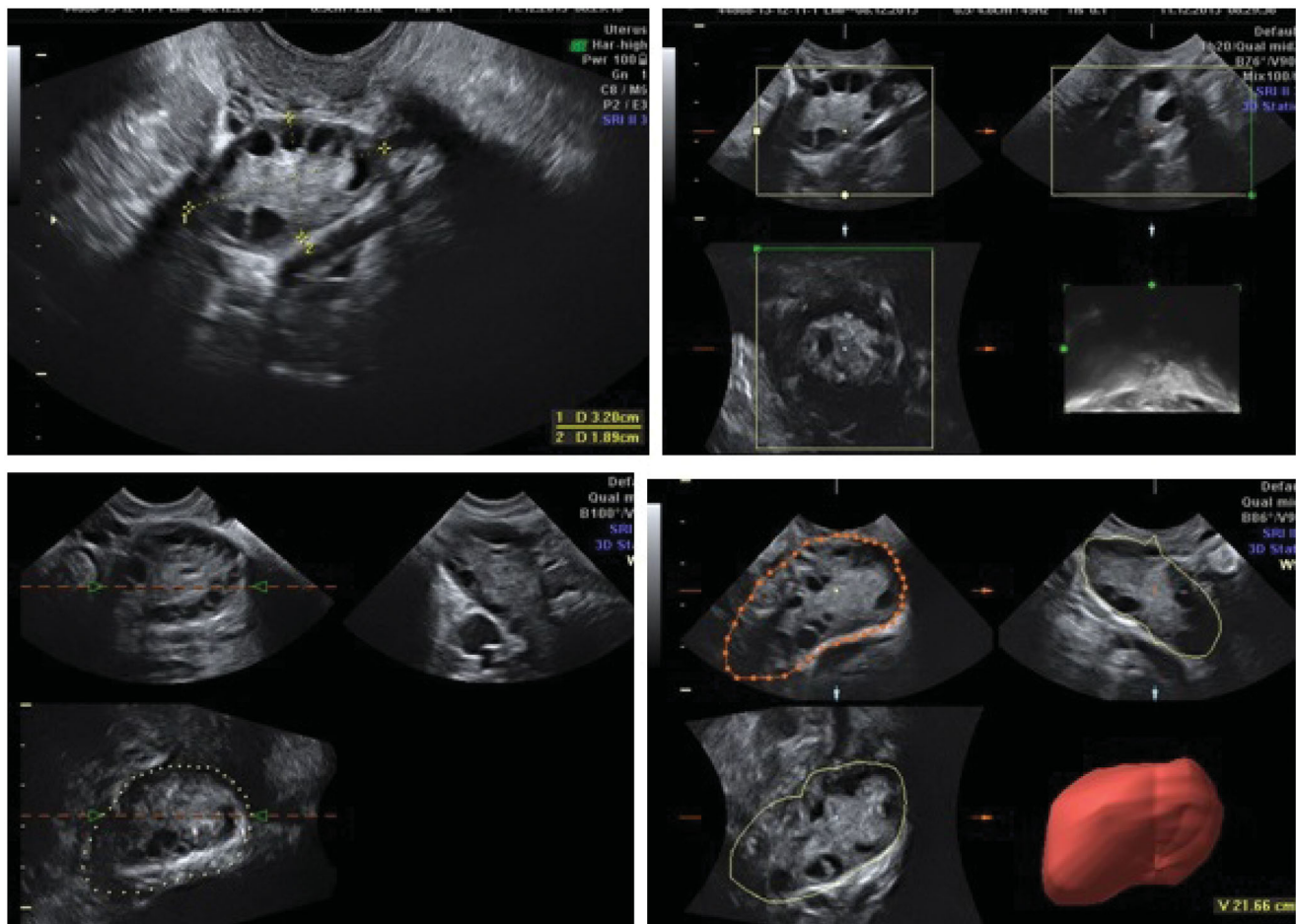


Figure 1. The VOCAL software programme automatically calculated the volume of polycystic ovaries.

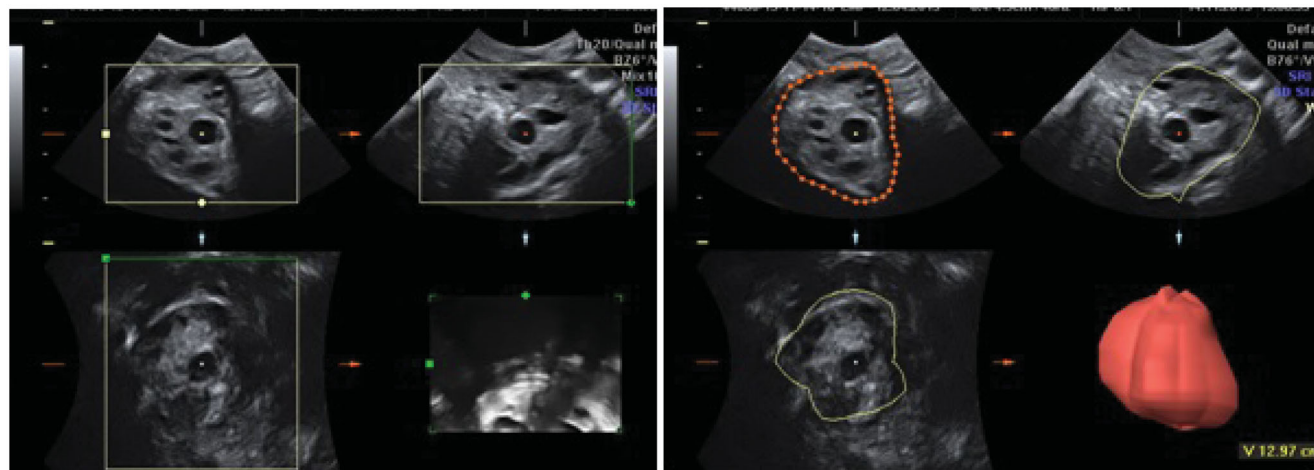


Figure 2. The VOCAL software programme automatically calculated the volume of multifollicular ovaries.

Measurement of total stromal volume by 3-D ultrasound

The stromal volume was calculated using VOCAL software programme. The stromal area was evaluated by outlining the peripheral profile of the stroma with the callipers (Identified by a central area that is slightly hyperechoic with respect to the other ovarian area). Stromal contours were manually traced on each slice. The software automatically calculated the volume of stroma.

Measurement of vascularisation index (VI), flow index (FI), and vascularisation flow index (VFI) by 3-D doppler examination

3-D power Doppler was performed with standard settings (colour gain 4 cm/sn, pulse repetition frequency 0.6 MHz, frame rate 4–6, wall motion filter 120 Hz). The region of interest (ROI) was determined to cover the whole ovary volume. The largest central vascular structure slice was selected as

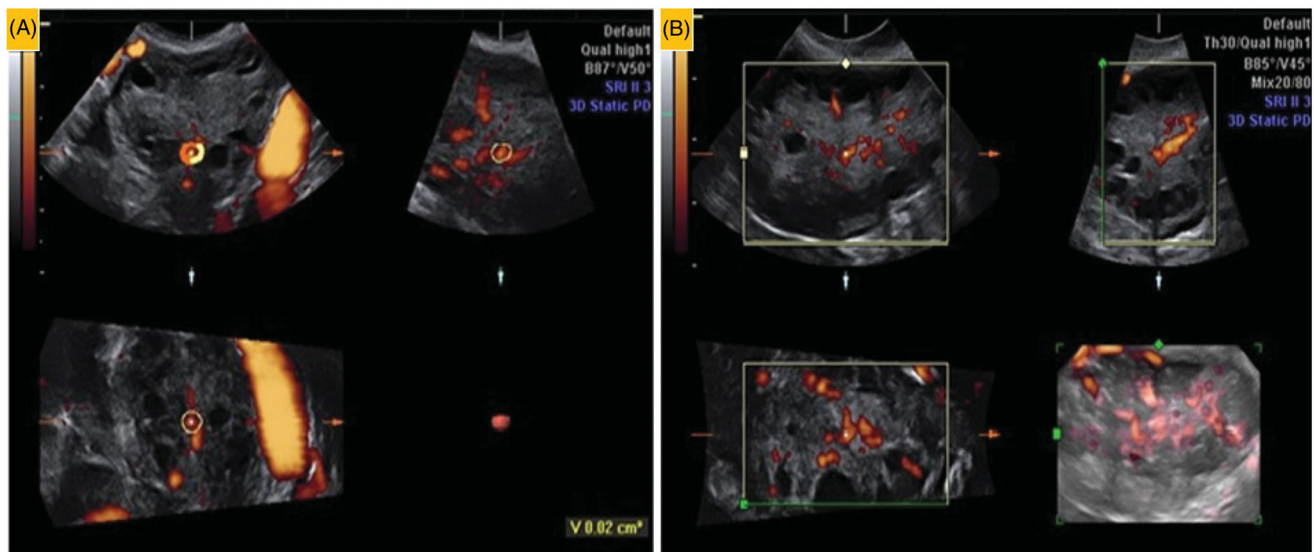


Figure 3. (A) The VOCAL programme showed vascular structure of the polycystic ovaries, (B) 3 D power Doppler examination showed the vascular structures of the polycystic ovaries.

the reference plane, and the VOCAL programme for 3-D power Doppler analysis was used to measure the ovarian volume, the indices of vascularisation, and blood flow. Six slices of the vascular structure with a rotational interval around the main axis of the vascular structure were created via the VOCAL programme (Medison-Kretz) (Figure 3; Pairleitner et al. 1999). The following three indices were calculated: VI, FI, and VFI. VI denotes the ratio of colour voxels to all voxels within the region of interest, and it is expressed as a percentage of the ovarian volume. FI represents the mean flow intensity of blood flow by indicating mean value of colour voxels. VFI is a combination of vascularisation and flow information relating the mean values of colour voxels within ROI, and it is derived by multiplying FI value by VI value (Pairleitner et al. 1999).

Statistical analysis

A sample size and power calculation determined that sufficient statistical power required a total of 36 patients per group (power of 0.80, α of 0.05, and β = 0.20). The power calculation was based on the total ovarian volume. At least 36 patients were included in each group (Ng et al. 2005). Statistical analysis was performed using the Statistical Package for Social Science (SPSS, version 17). The data were reported as the mean $\pm$ standard deviation, number, or percentage. The ultrasonographic and flow data of both ovaries were analysed using the Student's t-test for paired samples. If no significant differences were found between left and right side values, the average of both ovaries was used as a single parameter. The comparison of two groups was performed using an analysis of variance. An independent simple t-test was used to compare the variables. Non-parametric variables were analysed using Mann-Whitney *U* tests. *p*-values of $< .05$ were considered statistically significant.

Results

Thirty-six patients in the PCOS group and 40 patients in multifollicular ovary group were evaluated in our study. Demographic characteristics and biochemical parameters of patients were summarised in Table 1. There was a significant difference between groups in terms of serum LH, testosterone, DHEA-S, SHBG, and LH/FSH ratio. Patients with PCOS had significantly higher serum LH (11.23 ± 4.35 versus 6.23 ± 0.45 , $p < .01$), testosterone (0.92 ± 0.44 versus 0.51 ± 0.17 , $p = .03$), DHEA-S (1.90 ± 0.92 versus 1.06 ± 0.8 , $p < .01^*$), and LH/FSH ratio (2.25 ± 0.56 versus 1.01 ± 0.38 , $p = .02^*$) and a significantly lower SHBG level (246 ± 127 versus 468 ± 305 , $p = .03$) (Table 1).

Total ovarian volumes, AFC, stromal echogenicity, and stromal volumes of ovaries were shown in Table 2. A significant difference was found in ovarian volume between groups. Patients with PCOS had higher ovarian volume (18.6 ± 4.75 versus 10.2 ± 3.4 , $p < .01$). Patients with PCOS also had higher AFC, although this difference is not statistically significant (28 ± 5 versus 26 ± 8 , $p = .09$). There was a statistically significant difference in the measurements of mean stromal volume and stromal echogenicity between the groups. In PCOS group mean stromal volume and stromal echogenicity was significantly higher ($p = .02$ and $p < .01$, respectively). The stromal echogenicity rate of the patients with multifollicular ovary was 25%, while that of patients with PCOS was 77.7%.

3-D power Doppler parameters were presented in Table 3. There was no statistically significant difference in terms of mean RI and PI values between groups ($p > .05$) but patients with PCOS had lower mean RI and PI values. There was a statistically significant difference in terms of VI, FI, and VFI values between groups. VI, FI, and VFI values of the patients with PCOS were higher (3.82 ± 2.65 versus 1.78 ± 1.2 , $p < .01$, 50.76 ± 4.45 versus 40.6 ± 3.64 , $p = .03$, and 2.34 ± 1.02 versus 1.12 ± 0.65 , $p = .02$, respectively). VI, FI, and VFI values of

Table 1. Demographic characteristics and biochemical parameters of the patients.

Variables	Multifollicular ovary (n = 40)	PCOS (n = 36)	p Value
Age	26.3 ± 4.2	28.5 ± 3.7	.08
BMI (kg/m ²)	21.2 ± 2.6	22.3 ± 2.4	.09
Estradiol (pg/mL)	34.2 ± 17.5	42.6 ± 12.1	.07
LH (mIU/mL)	6.23 ± 0.45	11.23 ± 4.35	<.01*
FSH (mIU/mL)	5.22 ± 1.71	5.67 ± 1.43	.81
Serum Testosterone (ng/mL)	0.51 ± 0.17	0.92 ± 0.44	.03*
LH/FSH	1.01 ± 0.38	2.25 ± 0.56	.02*
Serum Androstenedione (nmol/L)	3.68 ± 2.45	3.83 ± 2.36	.18
Serum DHEAS (μmol/L)	1.06 ± 0.8	1.90 ± 0.92	<.01*
Serum SHBG (nmol/L)	468 ± 305	246 ± 127	.03*

All values are expressed as mean ± SD.

**p* < .05 significant difference (Group differences were compared by independent-sample t test).

Table 2. Sonographic characteristics of the patients.

Variables	Multifollicular ovary (n = 40)	PCOS (n = 36)	p Value
Total ovarian volume (mL)	10.2 ± 3.4	18.6 ± 4.75	<.01*
Total number of follicles	26 ± 8	28 ± 5	
Score 0	n = 6	n = 4	.09
Score 1	n = 12	n = 10	
Score 2	n = 22	n = 22	
Stromal echogenicity			
Score 0	n = 30 (75%)	n = 8 (22.2%)	<.01*
Score 1	n = 10 (25%)	n = 28 (77.7%)	
Stromal volume (mL)	5.02 ± 2.44	12.23 ± 2.53	.02*

All values are expressed as a mean ± SD and number (percentages).

**p* < .05 significant difference (Group differences were compared by independent-sample t test).

Score for number of follicle:

Score 0: Number of follicles ≤10;

Score 1: With number of follicles ≥10 in only one ovary;

Score 2: Number of follicles ≥10 in both ovaries.

Score for stromal echogenicity.

Score 0: Ovaries' echogenicity equal or less than myometrium;

Score 1: Ovaries' echogenicity higher than myometrium echogenicity.

patients with PCOS and higher stromal volume were also significantly higher than those with lower stromal volume. But no correlation was found between these values and stromal volume.

Discussion

Our study demonstrated that the total ovarian volume, stromal volume, and echogenicity in women with PCOS are significantly higher when measured by 3-D. RI and PI in 2-D Doppler were lower in women with PCOS, although this measurement is not statistically significant. However, VFI, VI, and FI in 3-D power Doppler were significantly higher in women with PCOS.

PCOS is a heterogeneous endocrine disorder, and a consensus was established for the diagnosis of PCOS by ESHRE and ASRM in 2003. Sonographically, multifollicular ovaries may typically be seen in puberty and in women with hypothalamic amenorrhoea as a result of follicular growth without consistent recruitment of a dominant follicle (Stanhope et al. 1985; Porcu et al. 1994). In concordance with our results, women with PCOS have significantly higher serum LH, DHEAS, testosterone, and LH/FSH ratio and lower SHBG when compared to women with multifollicular ovaries (Teede et al. 2010). These parameters may be helpful for the differential diagnosis between the two clinical entities (ESHRE/ASRM 2004). But the diagnosis sometimes cannot be made via

these biochemical parameters. Therefore, ultrasonographic findings could also be useful in the differential diagnosis of these clinical entities. According to our results patients with PCOS had higher AFC, although this difference is not statistically significant.

Meanwhile, a case control study recently evaluated the effect of AFC for women with PCOS. They recommended to be further evaluated the newly suggested AFC cut-off in patients with PCO (Kim et al. 2020).

Several studies suggested that blood flow and the vascular pattern of tissues are directly related to its morphology and function. In clinical practice, blood flow and vascular patterns of tissues can be determined by colour and power Doppler. Power Doppler is more sensitive to low flow, and it thus overcomes the angle dependence and aliasing of standard colour Doppler. 3-D power Doppler has 3-D and total flow area in the region of interest. The 3-D application could give more detailed information about tissues by displaying 3-D images of the structures on the screen. During the assessment of ovaries, the real stromal volume and blood flow could be reflected more clearly by demonstrating the whole ovarian stromal blood flow with 3-D reconstructive figures with 3-D power Doppler when compared with 2-D (Pan et al. 2002; Pan et al. 2002). With the gradual development of ultrasonography, stromal features, including stromal volume and echogenicity, become more important and may be assessed more subjectively in the diagnosis of PCOS (Pache

Table 3. The stromal volume and 3D Power Doppler signal parameters of groups.

Variables	Multifollicular ovary (n = 40)	PCOS (n = 36)	p Value
RI	0.71 ± 0.35	0.62 ± 0.21	.09
PI	1.02 ± 0.34	0.95 ± 0.23	.09
VFI	1.12 ± 0.65	2.34 ± 1.02	.02*
FI	40.6 ± 3.64	50.76 ± 4.45	.03*
VI	1.78 ± 1.2	3.82 ± 2.65	<.01*

All values are expressed as mean ± SD.

*p < .05 significant difference (Group differences were compared by independent-sample t test).

et al. 1992; Dewailly et al. 1994). In a retrospective study, Giampaolino *et al.* assessed the effect of ovarian volume on the ovulation induction with clomiphene citrate in patient with phenotype 2 PCO. Their results did not demonstrated any correlation between ovarian volume and the success of ovulation and the predictive value of ovarian volume (Giampaolino et al. 2018).

In women with PCOS, elevated LH level increases stromal vascularisation by stimulating neoangiogenesis, catecholaminergic stimulation, and leukocyte and cytokine activation (Panchal and Nagori 2012). A study evaluating blood flow variations using Doppler analysis on the third day of the menstrual cycle in women with PCOS demonstrated increased vascularisation and elevated PI with a typical low RI of stromal ovary vascularisation (Battaglia et al. 1995). Battaglia *et al.* evaluated ovarian and uterine blood flow in women with the peripheral and general forms of PCO and also investigated the correlation between the forms and different hormonal parameters (Battaglia et al. 1998). They found greater stromal echodensity with significantly lower PI in the peripheral cystic group, and an intraovarian flow was not determined in 22% of women with the general form of PCO. According to the differences in blood flow demonstrated by Doppler analysis, they concluded that PCOS does not predetermine a single intraovarian blood flow pattern.

3-D Doppler may be more specific in the determination of multifollicular and PCO when compared to RI and PI in 2-D Doppler, although 3-D is expensive and not widely available. In concordance with our results, Lam *et al.* investigated the ultrasound characteristics of ovaries in women with PCOS, including total ovarian and stromal volume, echogenicity, AFC and ovarian blood flow using both 3-D power Doppler and 2-D Doppler (Lam et al. 2007). They found lower RI and PI in 2-D Doppler and higher VFI and VI in 3-D power Doppler in women with PCOS when compared to the control group. In this study, obese women with PCOS had lower VI and VFI in 3-D power Doppler ultrasonography when compared to hirsute and normal weight women with PCOS. They concluded that women with PCOS have an increased stromal volume and vascularity. The study indicated that VI, FI, and VFI in 3-D power Doppler in patients with PCOS were significantly higher than those of the control group, resulting from a high response to gonadotropins (Pan et al. 2002). They also suggested that 3-D Doppler indices may be more sensitive than 2-D Doppler indices. However, Järvelä *et al.* found that there was no significant difference in VI, FI, and VFI values in women with PCOS and a control group (Järvelä et al. 2002). Another study comparing the 3-D power Doppler parameters

in women with PCOS and polycystic ovaries showed that there was no significant difference in VI, FI, and VFI in 3-D power Doppler between groups (Ng et al. 2006).

This study has some limitations that need to be addressed. First, the study had two case groups, but no control group. Another limitation was the relatively small sample size.

In conclusion, our results revealed that total ovarian volume, stromal volume, and echogenicity in 3-D and VFI, VI, and FI in 3-D power Doppler could be useful for differential diagnosis in women with PCOS and multifollicular ovaries. According to our results, there was no significant difference in terms of RI and PI in 2-D Doppler. Therefore, VFI, VI, and FI measurements are superior in 3-D power Doppler than in 2-D Doppler. Further well-designed trials are required to confirm our results.

Disclosure statement

The authors report no conflict of interest.

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