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Original Research

Value of Three-Dimensional Power Doppler Ultrasound in Quantitative Assessment of Early Diminished Ovarian Reserve During Perimenopause

Yunfei Ma[1], Yiyun Wu[1,*]

[1]Department of Ultrasound, Jiangsu Province Hospital of Chinese Medicine, 210029 Nanjing, Jiangsu, China
*Correspondence: 63483258@qq.com; yfy0043@njucm.edu.cn (Yiyun Wu)
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Abstract

Background: To evaluate the value of transvaginal three-dimensional power Doppler ultrasound (3D-PD-US) in quantitative assessment of early diminished ovarian reserve (DOR) among perimenopausal women. **Methods**: A total of 166 perimenopausal women with DOR were selected from February 2019 to December 2022, including 63 in the early stage and 103 in the mid-to-late stage. Ovarian reserve was assessed by biochemical testing and 3D-PD-US imaging. Biochemical indicators included antimüllerian hormone (AMH), basal serum follicle stimulating hormone (FSH) and estradiol (E_2). 3D-PD-US indicators involved ovarian volume (OV), antral follicle number (AFC), vascularization index (VI), blood flow index (FI), and vascularization flow index (VFI). The accuracies of two methods were compared. **Results**: There were significant differences in OV, AFC, VI, FI, and VFI between the early DOR group and the mid-to-late DOR group (all $p < 0.05$). FSH had significant negative correlations with OV, AFC, VI, FI and VFI, with correlation coefficients of –0.342, –0.381, –0.179, –0.123, and –0.175, respectively (all $p < 0.05$). **Conclusions**: 3D-PD-US may serve as a quantitative method for early detection of DOR in perimenopausal women.

Keywords: three-dimensional power Doppler ultrasound; diminished ovarian reserve; perimenopause; age

1. Introduction

Among all female organs, the ovaries are the earliest to undergo functional decline or even failure [1]. The time point for the ovaries to diminish their reserve varies significantly among individuals [2–4]. Diminished ovarian reserve (DOR) is insidious and progressive [3]. With unknown etiology, DOR is influenced by such factors as age, genetic factors, and iatrogenic factors such as surgery, radiotherapy, and chemotherapy [5–7]. Currently, no treatments can effectively restore ovarian function [4,6,8]. Therefore, early detection of DOR is of great significance for introducing safe and non-invasive measures to delay DOR, thus improving quality of life of perimenopausal women [4,6,7,9].

As recommended by the Practice Committee of the American Society for Reproductive Medicine, ovarian reserve can be assessed by both biochemical tests and ultrasound imaging [10]. Their usefulness has been verified, but ideal markers of ovarian reserve have not been explored [7,10,11]. Biochemical indicators of ovarian reserve include antimüllerian hormone (AMH), follicle stimulating hormone (FSH) and estradiol (E_2) that can measure oocytes or follicular pools directly or indirectly [10]. AMH can inhibit the recruitment of primitive follicles and accurately reflect the size of the antral follicular pool. AMH level fluctuates minorly during different periods of menstruation and can be detected at any time, making it one of the most reliable indicators reflecting ovarian reserve function [11]. The decrease in inhibin B secretion by preantral follicles represses the central negative feedback, leading to an increase in pituitary FSH, as well as late luteal and early follicular FSH concentrations. An earlier onset of new follicular growth and an increase in E_2 concentration follow earlier increase in FSH level. Used as indirect indicators for DOR, FSH and E_2 show a high specificity and but a low sensitivity, and are affected by the Menstrual cycle and have hysteresis. In general, a level of b-FSH less than 10–12 mIU/mL suggests "normal" ovarian reserve, and a higher level suggests DOR [11–13]. Ultrasound imaging can be performed to measure ovarian sinus follicles, volume and blood flow [10,14]. However, the blood flow on ultrasound images decreases in DOR compared to that in the normal ovarian [14]. Therefore, it is difficult to quantitatively measure blood flow using conventional ultrasound. In this light, three-dimensional power Doppler ultrasound (3D-PD-US) has emerged to allow for DOR quantitation through measuring sinus follicles, volume, and blood flow [14–17].

The purpose of the study was to evaluate the value of 3D-PD-US in quantitative assessment of early-stage DOR of perimenopausal women whose FSH is "normal" ($\leq$10 mIU/mL).

2. Materials and Methods

This study is a retrospective cross-sectional observational study. This study was approved by the local Ethics Committee of the hospital. Informed consensus was ob-

tained from every subject for the anonymous use of clinical data. A total of 166 cases of perimenopausal women diagnosed with DOR were collected in our hospital from February 2019 to December 2022. Inclusion criteria were as follows: (1) 3D-PD-US and serum AMH, FSH and E_2 were detected between day 2 and day 4 of the menstrual cycle or during the follicular phase of women without menstruation; (2) the patients still had a normal fertility, or conceived at least once. Exclusion criteria were as follows: (1) a history of hormone therapy in the past three months, such as estrogen, progestogen, oral contraceptives or traditional Chinese medicine; (2) a history of radiotherapy, chemotherapy, or ovarian surgery; (3) endocrine disease, such as Polycystic ovary syndrome (PCOS), diabetes, hyperthyroidism/hypothyroidism or breast diseases; (4) autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Crohn's disease (CD) or Ankylosing spondylitis (AS); (5) ovarian cyst or other ovarian tumor, unclear unilateral or bilateral ovarian, failure to obtain ovarian volume or blood flow by 3D-PD-US. According to FSH, the subjects with DOR were divided into the early stage group (FSH $\leq$10 U/L) and the mid-to-late stage group (FSH $>$10 U/L). Basal AMH, FSH and E_2, the 3D-PD-US indicators (ovarian volume (OV), antral follicle (AFC), vascularization index (VI), flow index (FI), and vascularization flow index (VFI)) were measured in all the subjects between day 2 and day 4 of menstrual cycle or during the follicular phase of women without menstruation.

2.1 3D-PD-US Data Collection and Post-Processing

All ultrasound scans were performed using a Voluson E8 (GE Healthcare Austria GmbH & Co OG, Tiefenbach, Zipf, Austria) and a transvaginal RIC5-9-D volume probe (5–9 MHz) that has 3D power Doppler ultrasound scanning modes. Following the procedures previously described by other authors, follicles ranging from 2 mm to 10 mm were labeled and counted. Using 3D-PD-US OV, AFC, VI, FI, and VFI were measured with Sonography-based Virtual Organ Computer Aided Analysis Imaging Program (GE Healthcare Austria GmbH & Co OG, Tiefenbach, Zipf, Austria). 3D-PD-US was as set at the following parameters: frequency low, smooth 5/5, ensemble 12, line density 6, power Doppler map 5, sweep angle 30°, quality middle 2, wall motion filter low 2, and velocity range 0.9 kHz.

The vocal was manually controlled to cover the whole 3D volume of the ovary with a 30° rotation step; accordingly, 6 contour planes covering 180° were analyzed for each ovary.

Vascularization index (VI) was measured to show the number of blood vessels in the ovary (color voxels) and was expressed as a percentage (%) of the ovarian volume. Flow index (FI) was measured to represent the average intensity of flow inside the ovary. Vascularization flow index (VFI) was calculated by multiplying VI and FI to show vascularization and flow.

2.2 Hormonal Determination

For each subject, 3–5 mL of blood was collected from the forearm elbow vein blood on an empty stomach. The levels of serum AMH, FSH and E_2 were measured by COBAS auto-analyzer (Roche Diagnostics GmbH, Mannheim, Germany) and original reagent kit (Roche Diagnostics GmbH, Mannheim, Baden-Württemberg, Germany).

2.3 Statistical Analysis

Statistical analyses were run on SPSS v26.0 (IBM, Armonk, NY, USA). For categorical data, the χ^2 test or Fisher's exact test was used. For continuous data, the Student's *t*-test was employed. $p < 0.05$ was considered statistically significant. The relationship between 3D-PD-US parameters and FSH was illustrated using Spearman's test.

3. Results

A total of 308 perimenopausal women with DOR were screened from February 2019 to December 2022. According to the exclusion and inclusion criteria, 142 patients were excluded, including 49 disapproving the anonymous use of clinical data; 27 having a history of hormone therapy within previous 3 months; 4 having a history of radiotherapy; 3 having a history of ovarian surgery; 15 having endocrine diseases (7 of PCOS, 8 of diabetes); 6 having autoimmune diseases (1 of SLE, 3 of RA, 1 of CD, 1 of AS); 9 being treated with endocrine therapy after breast cancer surgery; 12 showing ovarian cysts; 4 having other ovarian tumor besides cyst; 13 patients were unclearly showed and failure to obtain ovarian volume or blood flow (11 with unilateral ovary, 2 with bilateral ovaries). Finally, 166 subjects were enrolled (63 patients in the early stage group, 103 patients in the mid-to-late stage group).

Table 1 summarizes both groups' data about age, body mass index (BMI), marital status, menstrual history, menstrual blood volume, dysmenorrhea, AMH, E_2 and FSH. Age, AMH and FSH showed significant differences between the two groups (all $p < 0.05$). The coefficients of Spearman's test showed statistical associations of FSH with OV (–0.342), AFC (–0.381), VI (–0.179), FI (–0.123) and VFI (–0.175), respectively (all $p < 0.05$; Table 2). Table 3 summarizes the 3D-PD-US indicators in both groups. Significant differences in OV, AFC, VI, FI and VFI were found between the two groups (all $p < 0.05$).

4. Discussion

The incidence of cardiovascular and metabolic diseases increases among perimenopausal women, with the decline of ovarian reserve function [2,9]. Early diagnosis is essential for delay DOR and improve the quality of life among perimenopausal women [2,9,18]. DOR is first manifested by a decrease in AMH, followed by a feedback to the increase in FSH [5]. A higher FSH indicates a larger decrease in reserve function [10–13]. Clinical diagnosis of

Table 1. Comparison of general data and biochemical indexes between the two groups (mean ± SD, n [%]).

	Early stage group	Mid-to-late stage group	t/χ^2/F	p
n	63	103		
Age (years)	42.02 ± 2.00	43.18 ± 2.61	4.301[a]	0.040[d]
N, >45 years	4 (6.3%)	22 (21.4%)	5.579[b]	0.018[d]
BMI (kg/m^2)	22.11 ± 3.13	23.33 ± 3.15	1.132[a]	0.289
Marital status				
Married	62 (98.41%)	100 (97.09%)	0.292[b]	0.589
Unmarried	1 (1.59%)	3 (2.91%)		
Menstrual history				
Menstruation menarche (years)	13.37 ± 1.04	13.29 ± 1.03	0.099[a]	0.754
Menstrual cycle (days)	28.16 ± 1.53	28.37 ± 1.36	0.095[a]	0.758
Menstrual period (days)	4.24 ± 0.69	4.18 ± 0.57	1.257[a]	0.264
Menstrual blood volume				
Less than normal	1 (1.59%)	0 (0%)	3.182[c]	0.364
Normal	4 (6.35%)	3 (2.91%)		
More than normal	47 (74.60%)	77 (74.76%)		
Menopause	11 (17.46%)	23 (22.33%)		
Dysmenorrhea				
Yes	11 (17.46%)	19 (%)	0.026[b]	0.873
No	52 (82.54%)	84 (%)		
Biochemical indexes				
AMH (ng/mL)	1.09 ± 0.76	0.38 ± 0.48	23.547[a]	0.000[d]
E_2 (ng/L)	63.49 ± 40.77	59.28 ± 71.97	1.721[a]	0.191
FSH (mIU/mL)	7.90 ± 1.40	31.53 ± 27.20	78.601[a]	0.000[d]

[a] Student's t-test. [b] χ^2 test. [c] Fisher's exact test. [d] $p < 0.05$. BMI, body mass index; AMH, antimüllerian hormone; E_2, estradiol; FSH, follicle stimulating hormone; SD, standard deviation.

Table 2. Correlations between FSH and 3D-PD-US indicators (r).

Item	OV (cm^3)	AFC (Units)	VI	FI	VFI
r	–0.342	–0.381	–0.179	–0.123	–0.175
p	0.000[a]	0.000[a]	0.001[a]	0.025[a]	0.001[a]

[a] $p < 0.05$. 3D-PD-US, three-dimensional power Doppler ultrasound; OV, ovarian volume; AFC, antral follicle number; VI, vascularization index; FI, flow index; VFI, vascularization flow index.

DOR mainly relies on AMH, FSH, ultrasound, and symptoms [2,9,10]. But DOR is insidious in its early stage, and some medical institutions do not provide measurement of AMH level in China. So, delayed diagnosis may lead to loss of an optimal treatment time. Therefore, based on FSH, we defined those with a normal FSH as the early stage DOR group, and those with elevated FSH as the mid-to-late stage DOR group. For the first time, we here clarified that 3D-PD-US could increase the accuracy rate of early assessment for DOR.

Our study found significant differences in AMH between the two groups. The AMH in the mid-to-late stage group was lower than that in the early stage group, which is consistent with previous studies [10,11,19]. AMH reflects the size of the antral follicular pool. Older ovaries exhibit more pronounced decreases in both the quantity and quality of oocytes [19]. Previous studies have confirmed that age is an independent risk factor for DOR [1,3], and negatively correlated with the ovarian reserve function [2,3]. In our study, we found significant between-group differences (all $p < 0.05$; Table 1) in age and the proportion of the subjects above 45 years old (>45 years). The average age in the mid-to-late stage group was older than that in the early stage group, while the proportion of subjects (>45 years) in the mid-to-late stage group was significantly larger. These findings are consistent with previous research findings [2,3,20]. Therefore, for perimenopausal women aged ≤45 years, early detection of DOR is also necessary. However, as women with early-stage DOR present varying ages, the time point for ovarian reserve function examination cannot be determined solely by age.

3D-PD-US can display the 3D shape of the ovaries more intuitively, evaluate the blood flow of the ovaries quantitatively, monitor changes in ovarian morphology, functionality and hemodynamics dynamically and comprehensively. It is recommended by the Practice Committee of the American Society for Reproductive Medicine for evaluating ovarian reserve function. Previous studies have shown that the OV, AFC, VI, FI, and VFI of 3D-PD-US

Table 3. Comparison of 3D-PD-US indicators between the two groups (mean ± SD).

Group	OV (cm^3)	AFC (Units)	VI	FI	VFI
Early stage group	4.56 ± 2.25	4.71 ± 2.49	6.66 ± 6.15	30.29 ± 6.37	2.18 ± 2.35
Mid-to-late stage group	3.15 ± 1.83	2.59 ± 1.96	4.60 ± 4.86	28.40 ± 6.39	1.48 ± 1.72
t	5.921	8.14	3.197	2.626	2.91
p	0.000[a]	0.000[a]	0.002[a]	0.009[a]	0.004[a]

[a] $p < 0.05$.

are effective parameters for predicting ovarian reserve function, with high sensitivity and specificity [6–9]. In this study, the coefficients of Spearman's test showed statistical associations of FSH with OV (–0.342), AFC (–0.381), VI (–0.179), FI (–0.123) and VFI (–0.175), respectively (all $p < 0.05$; Table 2). With the increase of FSH, the 3D-PD-US indicators OV, AFC, VI, FI, and VFI fell significantly in DOR patients. AFC has shown a stronger correlation with DOR than OV, which is consistent with previous research findings [11,21]. Therefore, 3D-PD-US, with its advantages of non-invasiveness, safety, ionizing radiation free and convenience, may be recommended to evaluate the ovarian reserve function status of perimenopausal women.

Furthermore, all 3D-PD-US indicators showed significant differences between the early stage group and the mid-to-late stage group (all $p < 0.05$), suggesting that 3D-PD-US may be employed to monitor ovarian reserve status and early detect DOR in perimenopausal women. 3D-PD-US compensates conventional ultrasound for the difficulty in diagnosing early reserve function decline. Nevertheless, this study is limited by a small sample size and its observational nature. The value of 3D-PD-US should be further validated in larger-size and retrospective analysis.

5. Conclusions

3D-PD-US has a high diagnostic value for ovarian reserve function in perimenopausal women and may be replicated in clinical early detection of DOR.

Availability of Data and Materials

The data for this study is publicly available. You can request data from the author (Yunfei Ma) through email (439925904@qq.com).

Author Contributions

YM and YW designed the research study. YM and YW performed the research. YM analyzed the data. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

According to the regulation of National Health Commission- "Ethical Review of Life Sciences and Medical Research Involving Human Beings", the study using anonymized data is exempt from ethical review. This study is a retrospective cross-sectional Observational study. Although our research can be exempted from ethical review, we fully respect the wishes of the research subjects by phone. 49 patients were excluded who didn't approve the anonymous use of clinical data.

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Conflict of Interest

The authors declare no conflict of interest.

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