REVIEW ARTICLE

The New Three-dimensional Ultrasound Modes allow a Better Polycystic Ovary Syndrome Ultrasound Diagnosis beyond the Rotterdam Criteria

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex endocrine condition affecting reproductive-aged women. The exact etiology of this condition remains elusive and the definition of the syndrome has undergone several revisions in which ovarian morphology plays a central role. This review describes several and modern three-dimensional (3D) ultrasound modes including: HDlive™, virtual organ computer-aided analysis (VOCAL), inversion mode, Radiance System Architecture™, for the study of PCOS-related ovarian morphology. This state-of-the-art technology should be employed—when available, in the evaluation of PCOS-related ovarian morphology.

Keywords: Ovary, PCOS diagnosis, Reproductive endocrinology, Rotterdam criteria, Three-dimensional/HDlive/ radiance system architecture ultrasonography.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive-aged women, affecting 12 to 21%,[1] and the second most common cause of women infertility after aging.

Polycystic ovary syndrome is a complex endocrine condition due to its heterogeneity and uncertainty about its etiology. Several research groups have suggested that the origin of PCOS lays within genetics (polygenic) and/or environmental androgen effects in fetal life, and involves the fetal (re)programming of metabolic/

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endocrine axes, especially carbohydrate metabolism and adrenal secretion.[2]

Due to the fact that diagnostic criteria rely on clinical, biological and morphological criteria, the definition has undergone several revisions.[3]

At the National Institutes of Health (NIH) consensus conference held in 1990[4], PCOS was defined as:
- Chronic anovulation with
- Clinical and/or biochemical hyperandrogenism after excluding other mimicking etiologies, such as thyroid or adrenal dysfunction.

In 2003, the Rotterdam consensus of the European Society for Human Reproduction/American Society of Reproductive Medicine (ESHRE/ASRM)[5] proposed that the diagnosis should include two out of three of the following criteria:[5,6]
- Oligo and/or anovulation
- Clinical and/or biochemical hyperandrogenism
- Polycystic ovaries on ultrasound
  Other etiologies must be excluded.

Most recently, in 2009, the Androgen Excess and PCOS (AE-PCOS) Society[7,8] published a task force report emphasizing that PCOS was primarily a hyperandrogenic disorder and should, therefore, include hyperandrogenism (hirsutism and/or hyperandrogenemia) and ovarian dysfunction (oligoanovulation and/or polycystic ovaries) in the definition; thereby encompassing the Rotterdam ultrasound criteria but requiring hyperandrogenism for the diagnosis.

In 2011, the Amsterdam ESHRE/ASMR-sponsored 3rd PCOS consensus workshop group identified different phenotypes and separated the most classic phenotype, characterized by ovarian dysfunction and polycystic morphology.[9]

Finally, some progress has been made toward reaching unity about the diagnostic criteria with the recommendations of the expert panel following the NIH evidence-based methodology on PCOS in 2012.[10,11]

CLINICAL CRITERIA

Oligo/anovulation is usually described in women with menstrual cycles greater than 35 days apart. It is important to remember that even women with regular

cycles may be anovulatory. Irregular or absent menstrual cycles may lead to subfertility or infertility.

Hyperandrogenism, acne, hypertricosis or hirsutism are characteristic PCOS features. Hirsutism is difficult to assess as most women treat it, so it might not be obvious on examination. Hyperandrogenemia is measured with free testosterone; free androgen index (FAI) or bioavailable testosterone. This hyperandrogenemia, insulin resistance and obesity are pathophysiological features of PCOS.

In 2009, a task force report of the AE-PCOS society for the PCOS,[8] by using combinations of these criteria, identified four different PCOS phenotypes:
- Hyperandrogenism (clinical or biochemical) and chronic anovulation (H-CA)
- Hyperandrogenism and polycystic ovaries on US but with ovulatory cycles (H-PCO US)
- Chronic anovulation and polycystic ovaries hyperandrogenism (CA-PCO US)
- Hyperandrogenism, chronic anovulation and polycystic ovaries (H-CA-PCO US)

The identification and individualization of specific phenotypes in women having PCOS seems to be justified from the metabolic point of view.

BIOCHEMICAL CRITERIA

The biochemical recommended testing in adolescents presenting with PCOS-like symptoms are:
- Thyroid stimulating hormone (TSH), FSH, LH, prolactin and 17β-estradiol (in amenorrheic adolescents).
- Anti-müllerian hormone (AMH) which shows an equal or better performance than antral follicular count (AFC).[12,13]
- Testosterone—total serum concentration as first-line recommendation.
- Free testosterone, also as first-line recommendation
- Free androgen index (T/SHBG × 100)
- Dehydroepiandrosterone sulfate (DHEAS) and androstenedione levels are optional.
- 17-OH progesterone

Once PCOS has been confirmed, fasting and 2-hour glucose tolerance test, fasting insulin and lipid panel should be determined.

ULTRASOUND CRITERIA INCLUDING STATE-OF-THE-ART 3D VISUALIZATION MODES

Transvaginal Two-dimensional Ultrasound

For the study of PCOS-associated ovarian morphology (PCOM), the international societies (ESHRE, the European Society of Endocrinology and the AE-PCOS society)[5,11] recommend the following:
- The use of transvaginal ultrasound including scan frequencies of 7.5 to 8 MHz or more.

- An early follicular phase scan.
- All follicles ≥ 2 mm should be included in the AFC being measured in the three spatial planes (Fig. 1).

Early comparative studies showed intra and interobserver variations in the two-dimensional (2D) transvaginal AFC in the general population and a 12 to 13% rate of underestimation in the exact number of antral follicles present within the ovary,[11] however, recent studies, using new three-dimensional (3D) US modes, increase this underestimation rate up to 20 to 30%.[12-17,22]

Besides this limitation, and in order to maintain standard criteria for research purposes, 2D transvaginal scan remains as the recommended method for antral follicle count in the general population including PCOS patients, and the count of at least 12 follicles remains the limit to categorize a polycystic appearance ovary.[6]

Herein, we describe several and modern 3D ultrasound modes for the study of PCOS-related ovarian morphology; these new methods are scarcely mentioned on the medical literature since the clinical experience is limited; further, they are sometimes described as 'future techniques'.

Tomographic Ultrasound Image (TUI)

This software performs millimetric sections within the acquired volumetric image of the ovary in the three spatial planes, and allows the visualization and quantification of all the antral follicles present (Fig. 2).
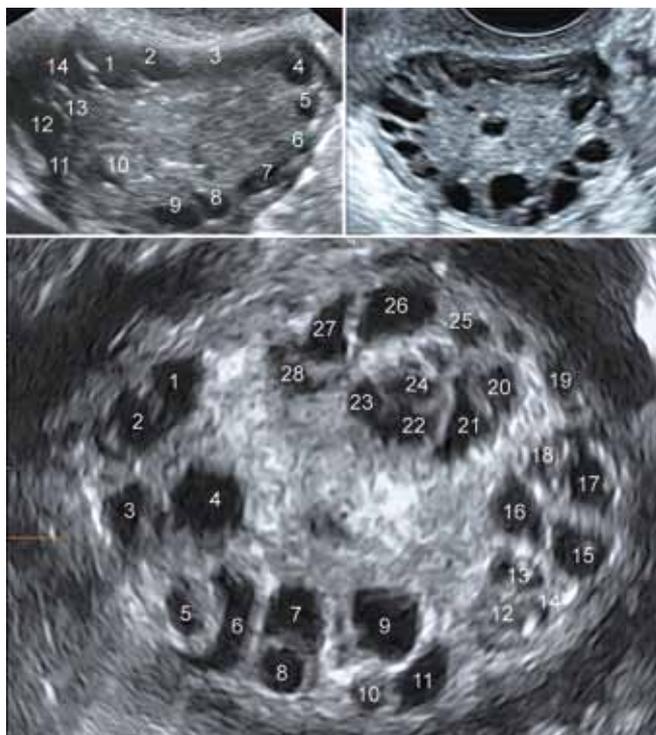

Fig. 1: Transvaginal 2D scan using a 7.5 MHz probe in a PCOS patient: antral follicles are measured and numbered in this image in two different spatial planes
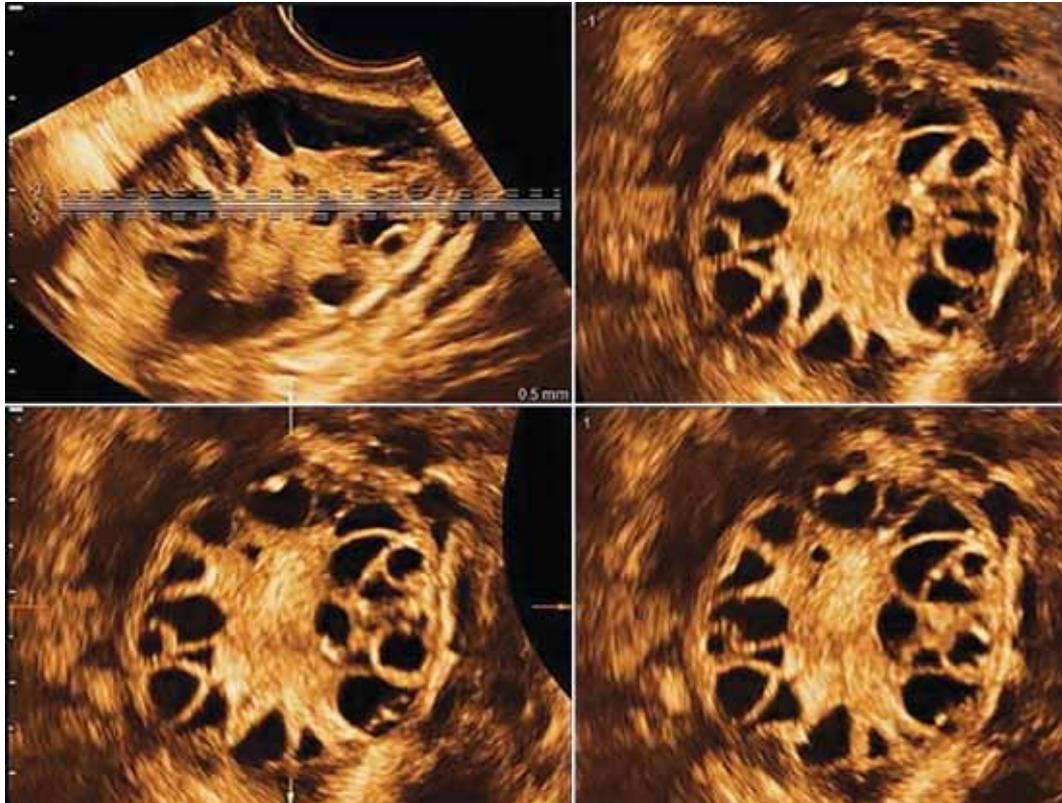
Fig. 2: Tomographic ultrasound image (TUI) in a transversal plane of a polycystic ovary (Voluson E8 GE®). The AF can be clearly seen in separated sections. The amplitude of the sections can be selected at convenience

Inversion Mode

This 3D US software converts econegative structures into ecopositive and *vice versa*, somehow similar to the old movie films used in the early days of cinema which used black and white (positive) or inverted (negative) images. In this ecographic mode, we can see the ovaries in 'negative'. Since the antrum space within the follicle is full of fluid—econegative—this software turns them into ecopositive. Moreover, the software eliminates the surrounding ovarian tissue, hence, the antral follicles appear in white color [or sepia in the current case (Fig. 3)] thus making AF counting easier.

This new rendering technique requires good image quality to identify the follicular margin,[18,19,21] however, cannot accurately assess AF of less than 5 to 6 mm[20] (Fig. 3).

It is quite simple to obtain this type of images, it only requires a 3D sweep on the region of interest and then selecting the inversion mode within the software panel, due to its simplicity this software is strongly recommended for clinical practice.[21]

Virtual Organ Computer-aided Analysis (VOCAL)

The sonographer performs several sections of the ovary, then the software integrates these sections and automatically calculates the volume of the region under study. This 3D US mode is ideal for ovarian volumetric calculation[21] (Figs 4 and 5).

Automatic Volume Calculation (AVC)

The software of this 3D US mode delineates and designates in different colors each AF facilitating enormously its counting (Figs 6 and 7). At the same time, evaluates three diameters and the total volume of each follicle, showing those which are more developed.[18-21]

Three-dimensional Digital Doppler Angiography

This 3D US mode shows the whole ovarian or follicular vasculature depicted in 3D US Doppler color or energy. The quality of the image is superior to those obtained with 2D transvaginal Doppler (Fig. 8).

Three-dimensional HDlive and Radiance System Architecture

Three-dimensional ultrasound has been used during the past 20 years by many investigators due to its excellence in showing ovarian images.[11,14-25] Three-dimensional ultrasound imaging (Figs 9 and 10) has potential advantages over standard 2D US:

- Reduced intra and interobserver variability[16,18-21,24,25]
- Shorter time for US procedures[18,24,25]
- Post-processing imaging analysis[20]
- The use of fully automated 3D technology is an exciting prospect, with much better results in counting AF than 2D.
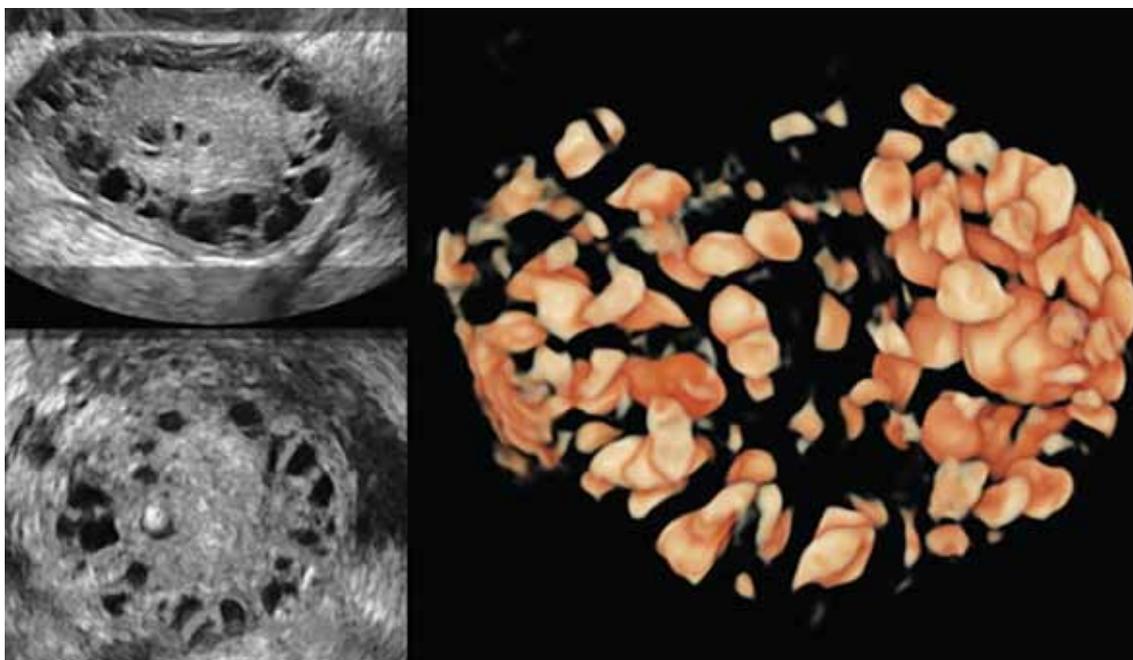
Fig. 3: Comparative imaging of an ovary displaying PCOM: Left—2D ultrasound scan, right—3D ultrasound scan using the inversion mode (Voluson E8 GE®)
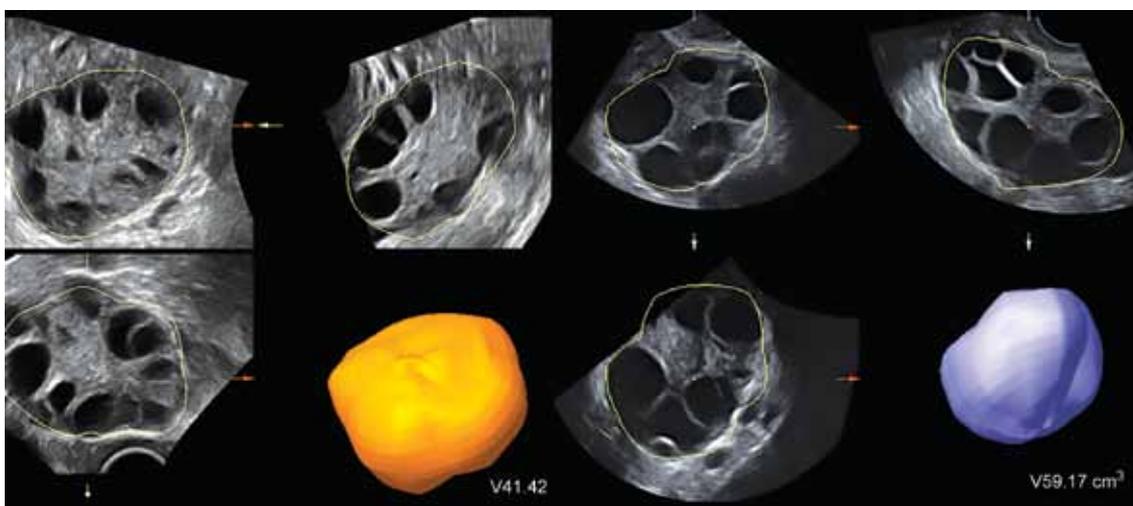

Fig. 4: Virtual organ computer-aided analysis (Voluson E8 GE®) measurement of the ovarian volume in two cases of PCOS. The software automatically calculates the ovarian volume
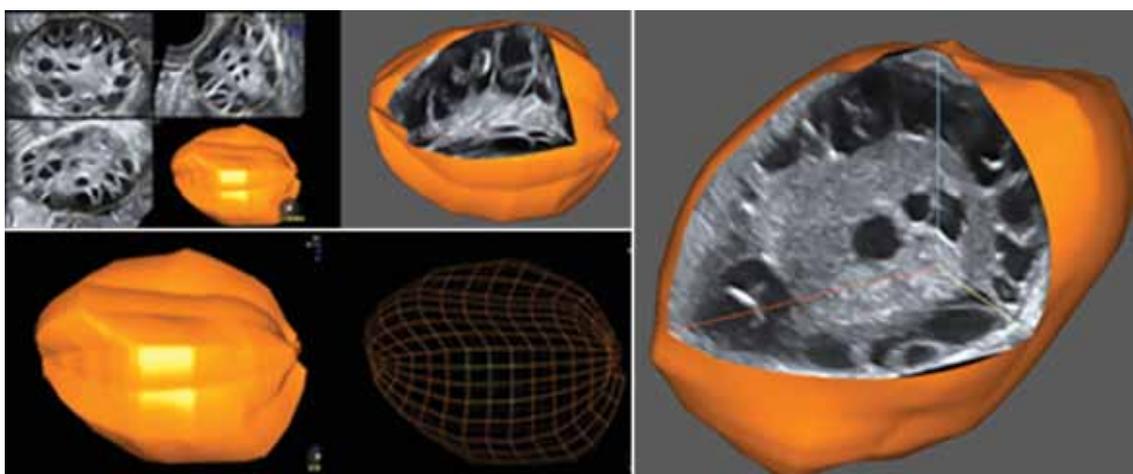

Fig. 5: Volume calculation of the ovary using VOCAL and NICHE mode (Voluson E8 GE®). This mode shows the ovarian surface in orange, the medulla in gray and numerous sorrounding AF, of similar size, on the periphery
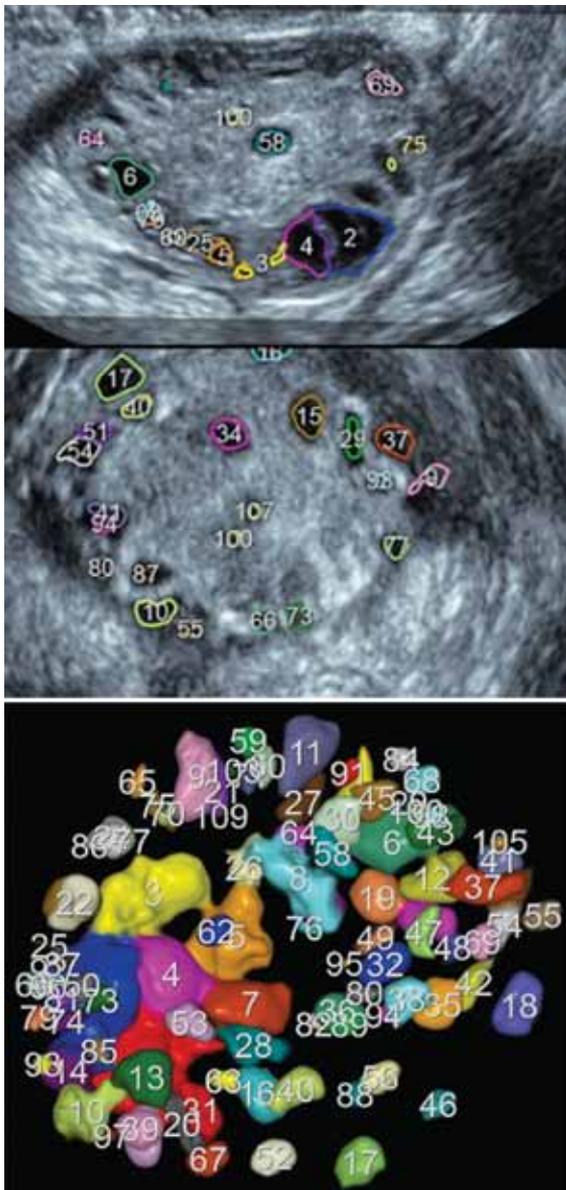
Fig. 6: Automatic volume calculation (Voluson E8 GE®) in a PCOM. The software shows each follicle in a different color and calculates the three diameters and volume automatically

Radiance System Architecture (Voluson E10 GE®), a new software to depict AF and ovarian cortex, increases much more the image quality (Figs 11 to 13).

However, 3D US and all these new modes cannot be recommended for routine clinical practice until:

• Additional data on reliability are available.
• The high cost of 3D imaging equipments may be reduced.
• Nevertheless, manual follicle counts from stored 3D data currently provide the optimal method of AFC assessment and would minimize many of the practical pitfalls of conventional 2D US evaluation.
• Nonetheless, it was agreed that the use of real-time 2D US imaging is adequate for measurement and counting of AF in routine clinical practice.[26,27]

POLYCYSTIC OVARIES AND ULTRASONOGRAPHIC EVALUATION

With the advent of ultrasonography, follicle excess has become the main aspect of polycystic ovarian morphology (PCOM)[1] as well as the main aspect of PCOS diagnosis.

Since 2003 Rotterdam criteria,[5] the following transvaginal US criteria were recommended[6] and most clinician have been using:

• A threshold of 12 follicles, measuring 2 to 10 mm in diameter per whole ovary
• And an ovarian volume (OV) of ≥ 10 ml, But that now seems obsolete.[28]

STATE-OF-THE-ART: NEW CRITERIA AND US MODES

We remark newly 3D US criteria that should be used and the modern 3D US modes introduced that—whenever possible—should be employed.
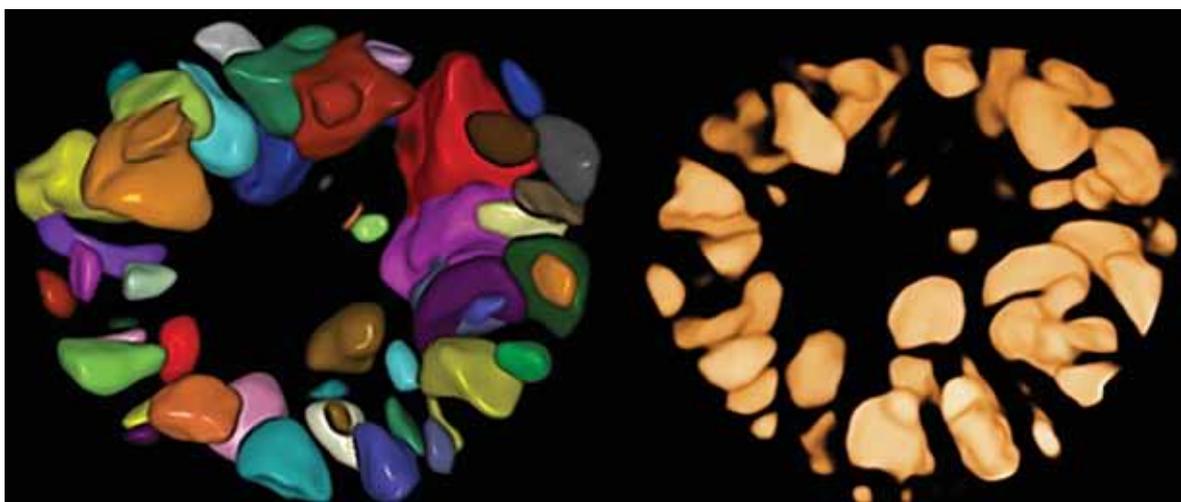

Fig. 7: Comparison between AVC (left) and inversion mode (right) for AF count
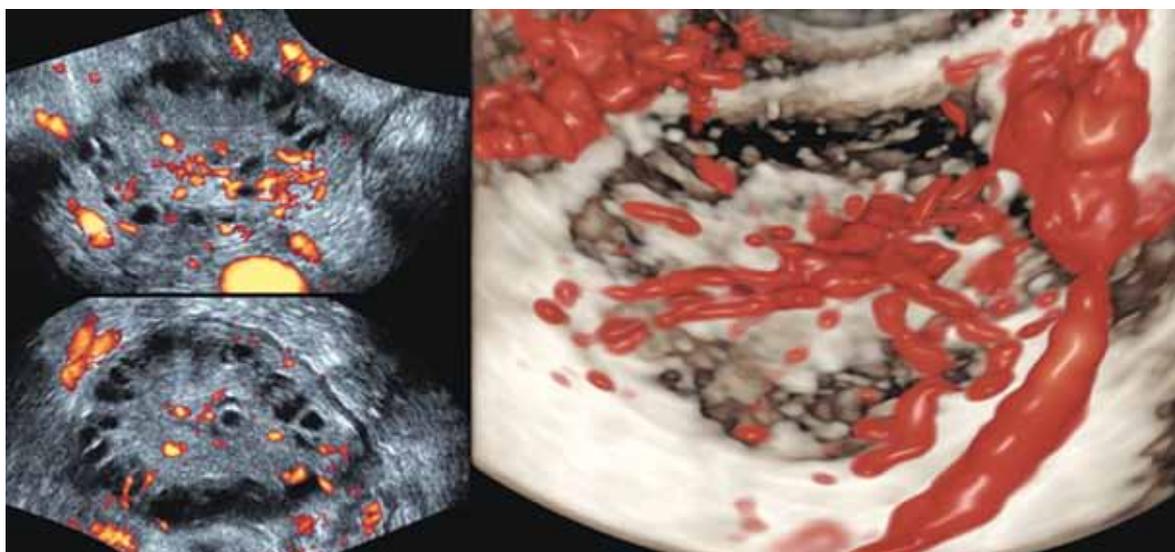
Fig. 8: Three-dimensional US color Doppler angiography of the ovary in a case of PCOS (Voluson E10 GE®). Compare the quality of the images depicted within 3D angiography (right) to 2D transvaginal Doppler energy (left)
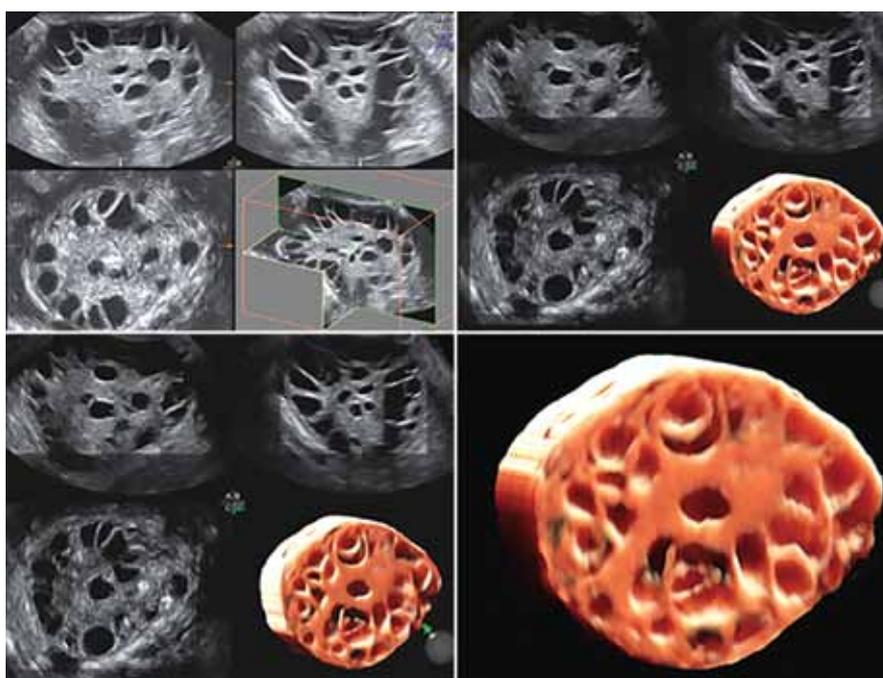

Fig. 9: Polycystic ovarian morphology 3D US HDlive is an excellent mode to increase the image quality

POLYCYSTIC OVARIAN MORPHOLOGY

Polycystic ovarian morphology designates the morphologic appearance of the polycystic ovaries under transvaginal sonographic view. Herein, we have described specific morphologic appearance in PCOS using advanced ultrasonographic software, this morphologic descriptions strictly correlates to current diagnostic criteria,[30] and moreover, is—in our opinion—more specific than the description given in Rotterdam criteria:[5,29]

- Polycystic ovaries always show an ovarian surface—the albuginea—which is very thin and highly bright (Figs 11 to 13), unfortunately little diagnostic value is given to this feature.

- The follicular distribution is located, almost in all cases, surrounding the ovarian cortex (Figs 1, 2, 4 to 7, 10, 13 and 16).

Although this peripheral distribution is a typical feature of PCOM (in our opinion of outstanding interest for diagnosis), some previous considerations should remain:

- It is not always present in both ovaries, can be unilateral.
- Can be diffuse occupying the whole ovary.
- The size of the AF ranges between 2 and 9 mm, and is frequently similar in both ovaries (Figs 5 to 11).
- All these morphologic characteristics are usually present in both ovaries providing that the etiologic

Fig. 10: Comparison of conventional 3D US (above) *vs* 3D HDlive (below) images of PCOS. HDlive increases the quality imaging view of 3D US

factors act over both ovaries. However, it is accepted that the presence of these morphologic features only in one ovary is enough for diagnosis. Unilateral polycystic ovary is an infrequent but still clinically significant finding.[5]

- Although chronologic age is the most important predictor of both the qualitative and quantitative ovarian reserve, there is considerable variability in the timing of the female reproductive aging process.[26,27,30-34]

Taken together, assessment of ovarian follicle number has become the main item of PCOM. Establishing the normal values for follicle number per ovary (FNPO) as well as for ovarian volume (OV), and especially the setting of accurate thresholds for distinguishing normal ovaries from PCOM is still a great controversy.[30]

The total number of AF present is highly variable, but in order to reach a correct diagnosis, it is still enough to find over 12 follicles at least in one ovary.[5]

However, it seems that this cut-off limit is not accurate; in fact, when new ultrasonographic technologies are applied to AFC, this cut-off limit seems to be underestimated.
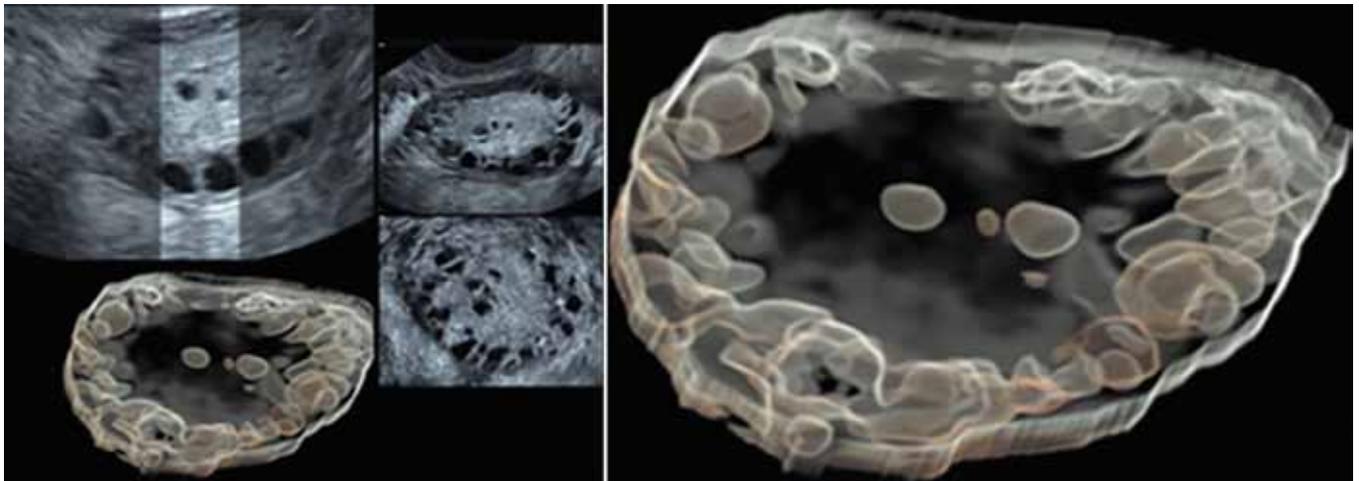

Fig. 11: Radiance system architecture (Voluson E10 GE®) of an ovary with PCOS (right), compared to 2D US (left). This is the most advanced 3D US mode which allows a wonderful view of the isolated antral follicles and the ovarian tunica albuginea
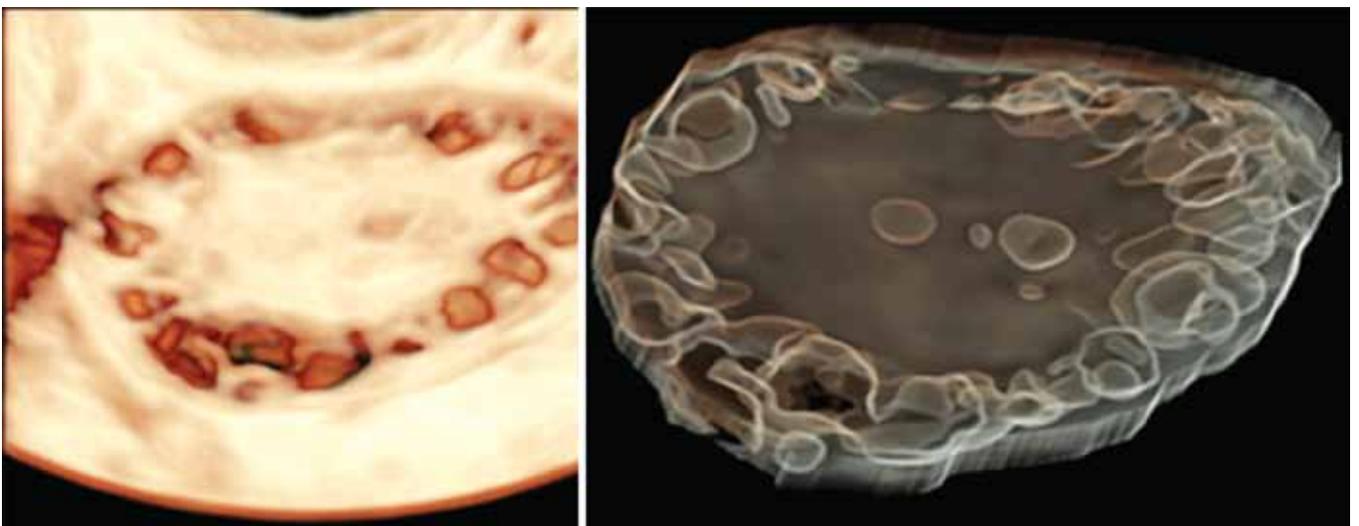

Fig. 12: A PCOM depicted using 3D HDlive (left) *vs* Radiance System Architecture (right)
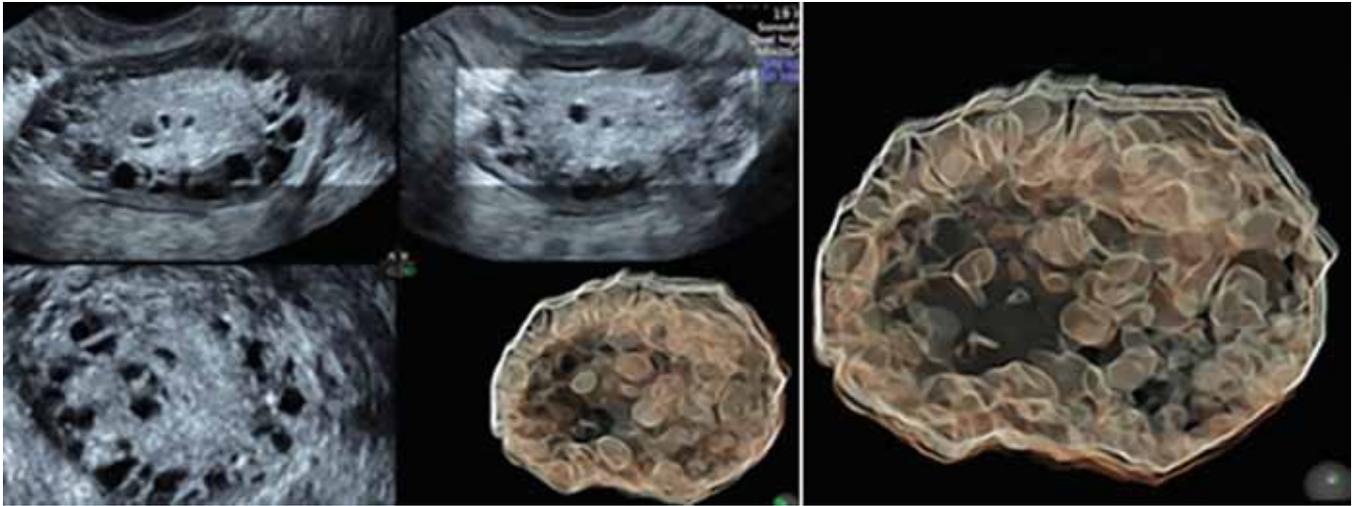
440

Fig. 13: Polycystic ovary syndrome-related ovarian morphology image using Radiance System Architecture. Each AF is clearly depicted with much better resolution than with standard 2D US

Several studies in the last two decades have addressed comparatively 2D *vs* 3D scan accuracy, and the results clearly showed than 2D underestimate in 12 to 13% the exact number of AF available,[11,21] moreover, recent studies report even higher differences of up to 25 to 30% of underestimation.[14-16] Modern 3D ultrasonographic modes like AVC and inverse mode seem to be more accurate when measuring AFC;[18] likewise, VOCAL appear to be very important for volumetry.[16,17,21,22]

Recent publications focused on PCOS patients showed a mean number of AFC of 19 to 26 follicles.[12,13,28] This situation has arisen from the marked improvements in the level of spatial resolution afforded by newer ultrasound scanners.

Ovarian volume or area (OA) were also included as mandatory diagnostic criteria by ESHRE.

Ovarian volume appears to be a good surrogate marker of PCOM although, when compared with FNPO, it has less sensibility for discriminating between patients with PCOM and controls in all the studies comparing both parameters. As the AFC declines progressively over time,[32-34] it provides a more useful clinical marker of ovarian responsiveness than OV.

Large-scale studies over the three last decades have shown that OV is inversely correlated with age (climacteric and menopause)[10] intake of anovulatory pills, GnRH-agonist and antagonist[21] and diabetes but positively correlated to puberty (beginning of hormonal secretion) and normal ovarian cycles.[24,26,27,31,35,36]

Two-dimensional transvaginal scan measures two planes in order to obtain the ovarian area, and three planes for obtaining the volume of the ovary after the application of the ellipsoid formula ($v = 4/3 \pi LD + APD + TD/2$); however, there is a high probability of interobserver variations.

Based on all the previous considerations, our critical review shows that OV has less diagnostic potential for PCOM compared to AFC. The usefulness of OV compared to follicle excess remains unclear.

European Society for human reproduction and latter studies suggested maintaining the threshold for increased OV $\geq$ 10 ml. By using AVC and VOCAL,[14-17,21] the measurement of the ovarian volumetry can be more exactly obtained (Figs 3 to 6 and 8). If such technology is not available, then OV is recommend rather than FNPO for the diagnosis in routine daily practice but not for research studies that require the precise full characterization of PCOM in each patient.

The Medulla of the Ovary

Surprisingly, Rotterdam criteria do not consider the characteristics of the ovarian medulla as relevant for diagnosis.[5]

Decades ago, specific characteristics of this ovarian zone for PCOS patients were described, including an increased vascularization, thickness and echogenicity. Some authors considered it as an additional feature of PCOS (Figs 14 and 15).[11,21]

This review emphasizes that both ultrasonographic features: thickness of the medulla and antral follicles distribution and count should be included for a correct description of PCOM. However, regarding the thickness of the ovarian medulla, there was a lack of consensus on quantitative measure for standardization.[28] The ratio of ovarian stroma to total ovarian size may be a good criterion for the diagnosis of PCOS, with a cut-off value of 0.32 and has been suggested that its size is associated with more hyperandrogenemia.[37]

Nowadays, a new way to quantify the thickness of the medulla is to use the double VOCAL technique or combining it with the niche mode. The software quantifies at the same time the medullar volume as well as the total OV (Fig. 15).
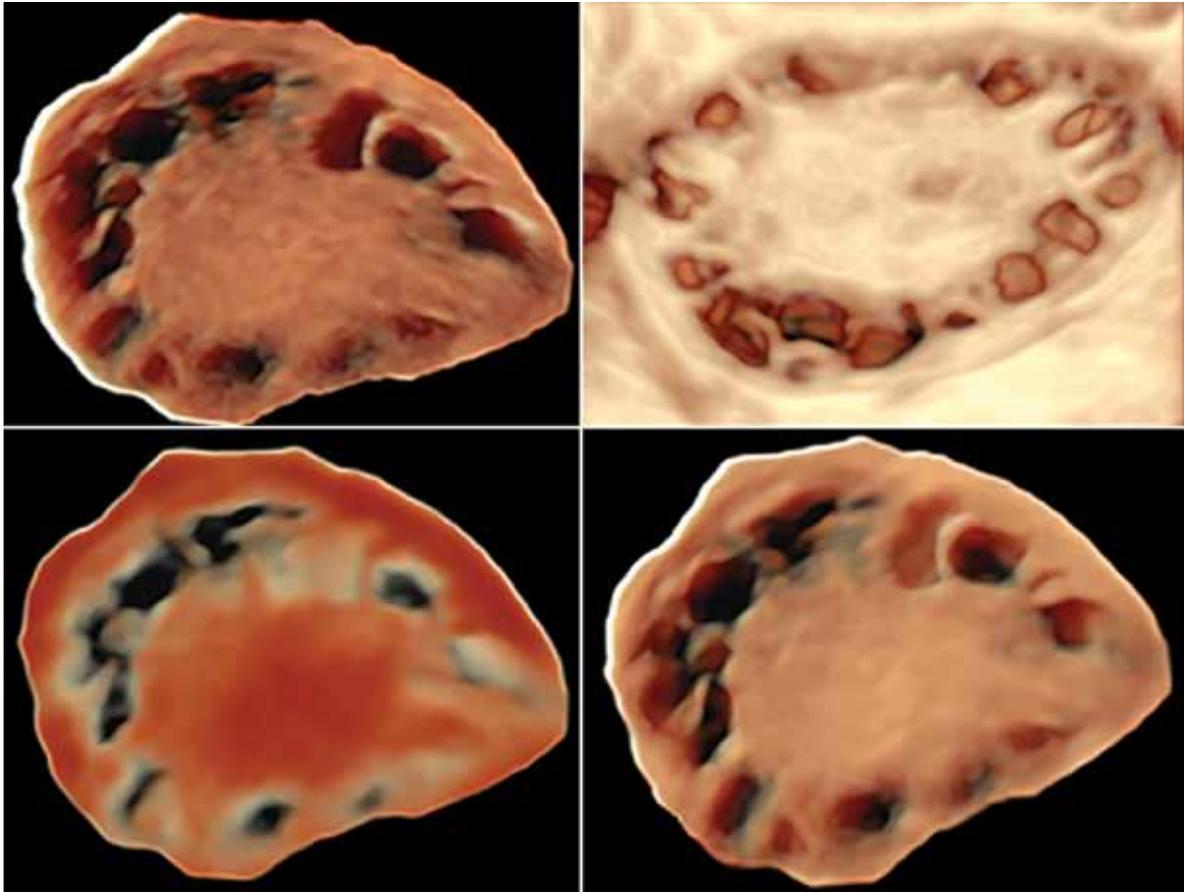
Fig. 14: Left: Polycystic ovarian morphology using HDlive without (upper left) and with (bottom left) maximum transparency. Note the increased thickness of the medulla and the AF located peripherally in the ovarian cortex

Fig. 15: Double VOCAL and NICHE modes showing the medulla of the ovary in red and orange. Around this structure, numerous small AF. The yellow arrows depict total ovary and medullary volumes. The software calculates the volumes automatically

Vascularization of the Medulla

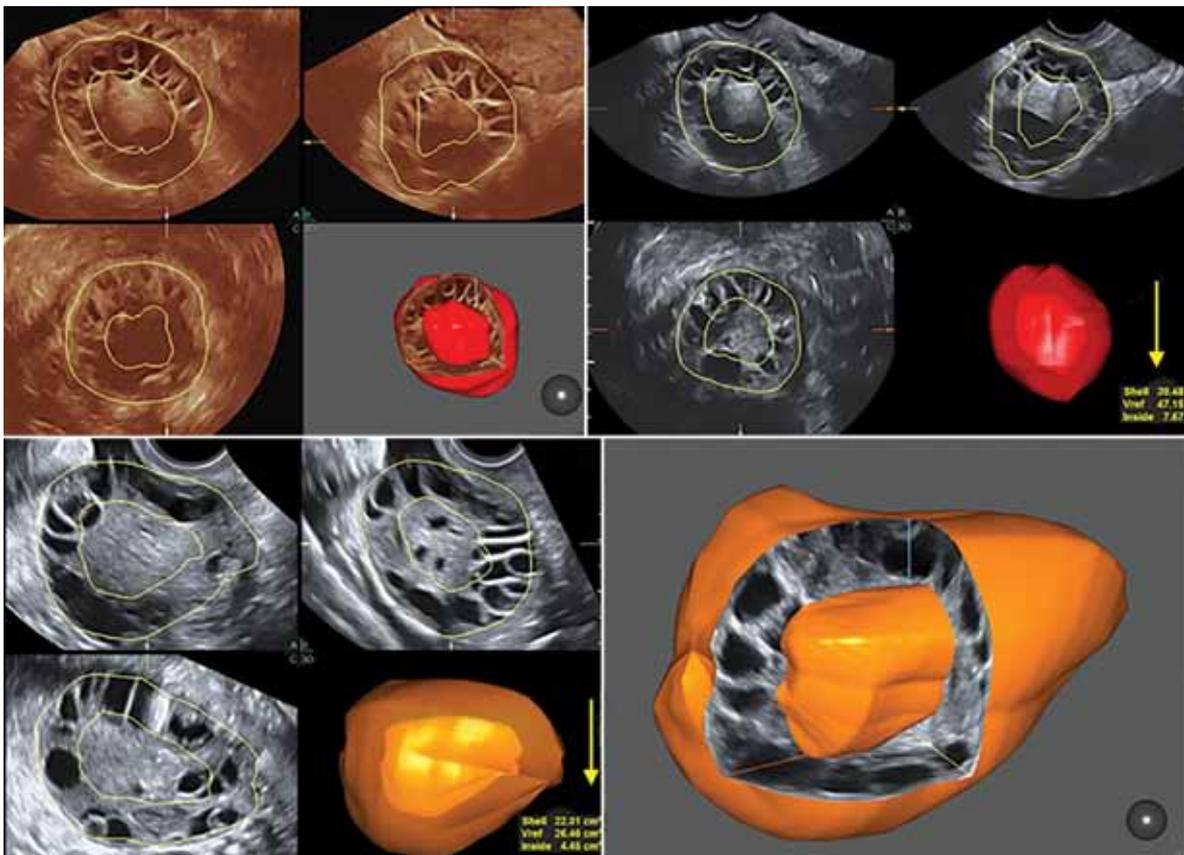Polycystic ovary syndrome patients shows an enhanced vascularization of the ovarian medulla which remains constant during the menstrual cycle, this feature is not seen in patients having normal hormonal periods which show cyclic changes.[38]

With the pioneering studies focused on Doppler, it was observed that resistance and pulsatility indices were very low in PCOS patients—related to good vascularization—but without changes throughout the cycle, this feature seems to be characteristic of PCOM,[21,38,39] although it is not internationally accepted.[39] This situation might change with the introduction of 3D digital Doppler angiography.[21,22] This new 3D US mode has allowed observing the following key events in this group of patients:

- A markedly increase in vascularization (Fig. 16).
- Three-dimensional parameters [vascularization (VI), flow (FI), vascularization flow (VFI) indices] significantly higher.
- These features deserve further study to be included as new markers for PCOM.

Several studies have compared these vascular indices between normo and hyperandrogenic women having PCOS.[22,41,42] The results showed statistically differences as well as a direct relationship between increased flow and hyperandrogenemia:

	Normoandrogenic	vs	PCOS patients
VI (0–100)	2.79%		3.85%
FI (0–100)	31.79		33.54
VFI (0–100)	0.85		1.27

Besides its enormous interests, we may underline that other several authors remark the lack of uniform data and absence of cut-off values which would still make vascular parameters by Doppler impractical for discriminating between PCOM and normal ovarian morphology.[28]

In our opinion, 3D digital Doppler angiography should be considered as a valuable and potent diagnostic tool.

CONCLUDING REMARKS

- Recent papers have been published focusing on the redefinition of PCOS, nevertheless, the Rotterdam consensus remains as the most widely accepted across Europe, Asia and Australia and, until a new Consensus, should be still used.[5-7,11,29]
- Initial investigations must exclude other endocrinological abnormalities: thyroid function, prolactin luteinizing (LH) and follicle stimulating hormone (FSH) levels.
- Ovarian US morphology as diagnostic criteria (Rotterdam consensus) plays—in our consideration—a central role, superior to hyperandrogenemia, however, as shown in this review, there is a lack of consensus.
- Indeed, PCOS and control population share a significant overlap in ovarian morphology, and a large proportion (estimates range from 10 to 48%) of adolescents who do not have PCOS may have polycystic-appearing ovaries.[43-45]
- A PCOS should never be considered in young women until 2 years of establishing normal menstruations.[7]
- There are cases with typical PCOM and hyperandrogenemia but with OV less than 10 cc. Ovarian volume
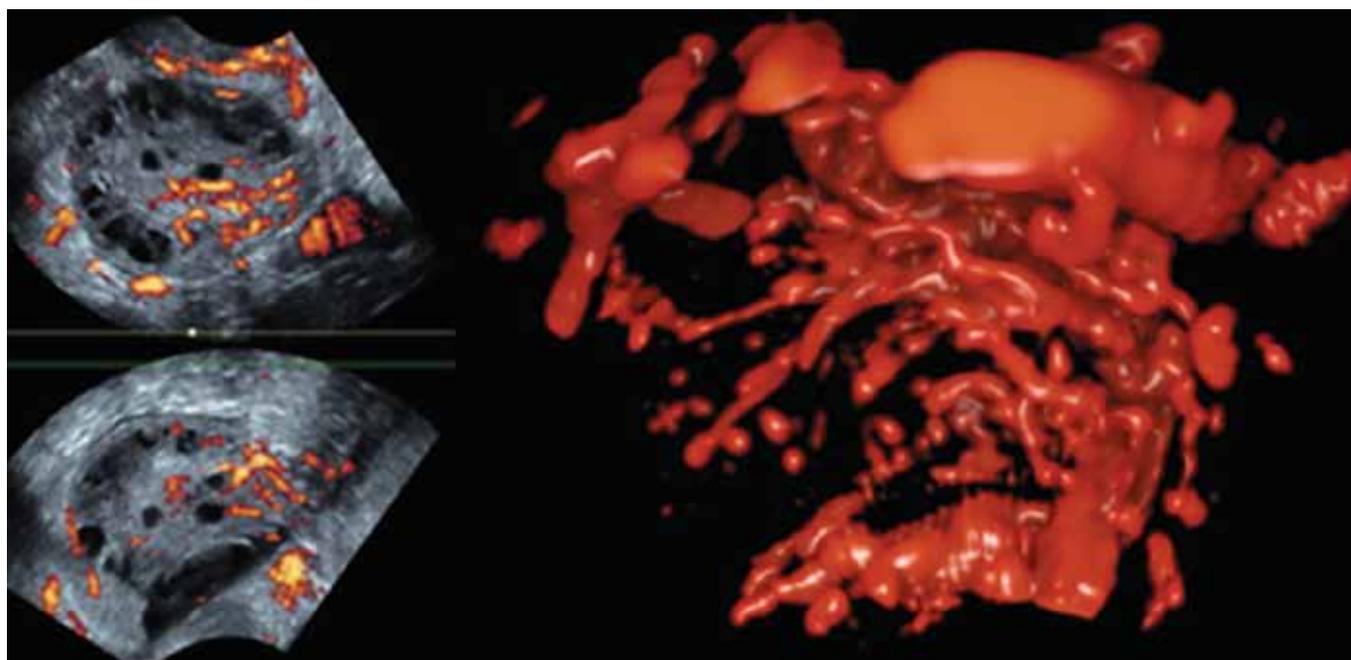

Fig. 16: Vascularization of the medulla in PCOS patients. Note the increased vascular component, gross and long vessels and the absence of changes in flow velocity

and/or area are not as interesting as published by the Rotterdam consensus.

- The measurement of the medulla, especially if combined with 3D angio-Doppler is a good adjunctive tool in diagnosing PCOS.
- Tomographic ultrasound image (TUI) is only of modest interest for PCOM evaluation. It's neither necessary nor recommended.
- Inversion mode and AVC are much more specific, reliable and recommended than the simple transvaginal 2D for AF counting.
- 3D US HDlive™ and Radiance System Architecture™ software improve image quality and should be used if available.
- Anovulatory drugs: This medication may interfere with the ultrasonographic appearance of the AF, presumably due to a reduction in testosterone levels, thus, the hormonal and ultrasonography assay are recommended after 3 months of cessation.
- The finding of PCOM in ovulatory women not showing clinical or biochemical androgen excess may be inconsequential.[28]
- Finally, the new 3D US modalities seem to be superior to the standard 2D transvaginal scan, and should be employed when available.

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