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REVIEW ARTICLE

Baseline Scan and Ultrasound Diagnosis of PCOS

Sonal Panchal, CB Nagori

ABSTRACT

Success of any assisted reproductive technology is dependent on selection of correct stimulation protocol. This is based on prestimulation assessment of female to assess ovarian response and reserve. But, this assessment can also be done by ultrasound scan on 2nd to 3rd day of menstrual cycle, named as 'baseline scan'. This scan is done to categorize ovary into one of the four types: Normal ovaries, low reserve ovaries, poorly responding ovaries and polycystic ovaries. Patients with polycystic ovarian syndrome have variable pictures of ovaries on ultrasound. Understanding the evolution of polycystic ovarian syndrome can explain these variations. Moreover, ultrasound findings can also be correlated with the biochemical and hormonal derangements. This scan also predicts the ovarian reserve and response that can guide to decide the stimulation protocols for ART. This scan includes the use of b mode, Doppler and 3D ultrasound with 3D power Doppler. It consists of assessing ovarian size, antral follicle count (AFC), stromal echogenecity and stromal flow chiefly. Dose calculation is chiefly done based on ovarian volume, AFC and stromal flow.

Keywords: Baseline scan, PCOS, Dose calculation, Hormonal correlation.

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INTRODUCTION

Success of any assisted reproductive technology (ART) is chiefly dependent on two decisions, selection of correct stimulation protocol and correct timing of human chorionic gonadotropin (hCG). Selection of correct stimulation protocol is based on prestimulation assessment of female to assess ovarian response and reserve. This can be done by assessment of baseline hormones, antimullerian hormone (AMH), follicle stimulation hormone (FSH) and estradiol on 2nd and 3rd days of menstrual cycle. But, this assessment can also be done by baseline ultrasound (US) scan before starting stimulation on 2nd and 3rd day of menstrual cycle. This scan can be also named as 'baseline scan'.

Baseline Scan

This scan is done when hormonal levels are at baseline, ovaries are silent and have no active follicle or corpus luteum.

Route of scan has to be transvaginal always. Using transabdominal approach for ovarian assessment may miss at least 42% of the ovarian anatomical details.[1] All the scans are done using B-mode US with color Doppler, pulse Doppler, three-dimensional (3D) US and 3D power Doppler. Using color Doppler in this assessment is mandatory because a large number of biochemical or hormonal changes occur during the menstrual cycle, which reflect as vascular and morphological changes in the ovaries and uterus and vascular changes can be assessed by Doppler. 3D US is especially useful for volume measurements. Volumes when calculated by 3D US using VOCAL (volume calculation) software, are much more reliable than volumes calculated by two-dimensional (2D) US. 3D power Doppler assessment has been found to be highly promising as it gives idea about the global vascularity of ovaries, instead of one or two selected vessels being interrogated on pulse Doppler. Moreover, 3D US has an advantage of storing the volumes and reproducing the images and measurements.

This scan is done to categorize ovary into one of the four types: Normal ovaries, low reserve ovaries, poorly responding ovaries and polycystic ovaries. Or in other words to predict the ovarian reserve and response that can guide to decide the stimulation protocols for ART.

Technique for Baseline Scan of Ovaries

B-mode ultrasound assessment of the ovaries consists of assessment of ovarian diameters and volume and counting of antral follicles as quantitative assessment and qualitative assessment of stromal density.

Once ovary is located the probe is rotated to find out the longest diameter of ovary and is stored as one frame on a dual screen. Then probe is rotated 90° to get a true transverse axis of ovary. Measure the largest longitudinal, transverse and anteroposterior (AP) diameter of the ovary in centimeters (cm), and ovarian volume can be calculated by the formula (x x y x z x 0.523; Fig. 1).

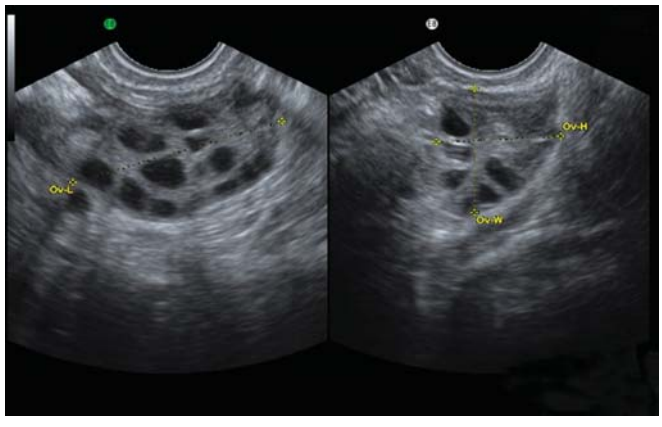

Fig. 1: Longitudinal and transverse sections of ovary

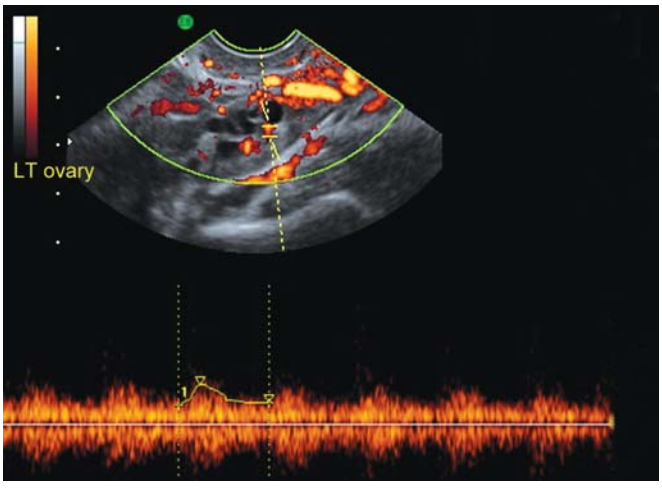

Fig. 2: Measuring ovarian stromal flows

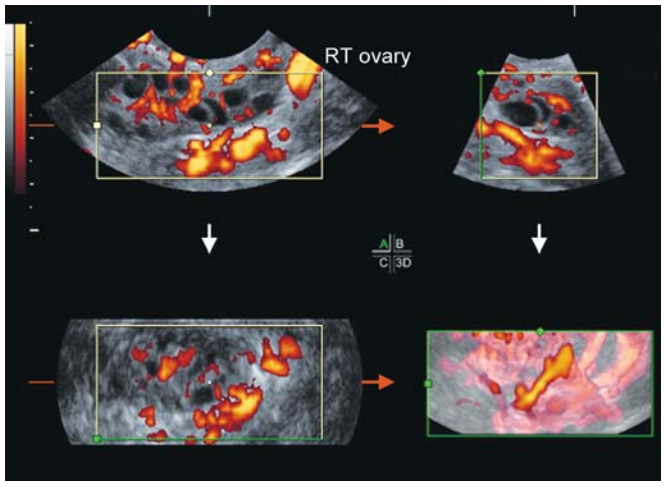

Fig. 3: 3D power Doppler volume of ovary

Number of antral follicles are counted in the longest section of ovary, but are better counted in the whole ovary by taking a 2D sweep across whole ovary. This method is very feasible and reliable when number of antral follicles is approximately 10 to 15. But when number of follicles is much more as in polycystic ovaries, the calculation using B-mode scroll may be inaccurate.

Stromal echogenecity is assessed against echogenecity of myometrium, especially if ovary and uterus are at almost same depth from probe. Normally ovarian stroma is hypoechoic or isoechoic to myometrium.

Color or power Doppler box is now placed on the ovary, so that whole ovary in included in color box. Doppler is used to see presence of vessels in ovarian stroma (Fig. 2). The vessel that is close to any of the follicles is not a stromal vessel. If vascularity is present, pulse Doppler is used for quantitative assessment of the flows—intraovarian resistance index (RI) and peak systolic velocity (PSV). For color Doppler pulse repetition frequency (PRF) is set at 0.3, wall filters lowest with optimum gains and balance settings. For pulse Doppler also lowest PRF and wall filters are used as stromal flows at base line scan are low velocity flows. The vessel selected for interrogation is a vessel that is shows brightest color on color Doppler.

After having completed the Doppler study, volume studies are initiated. 3D ultrasound provides a new method for objective quantitative assessment of follicle count, ovarian volume, stromal volume and blood flow in the ovary.[2] Power Doppler box is set to include whole ovary and then 3D volume of ovary is acquired (Fig. 3). This volume of ovary is used to calculate ovarian volume, stromal volume and to count number of antral follicles. Global vascular indices VI (vascularity index), FI (flow index) and VFI (vascularity flow index) may be calculated from the same ovarian volume.

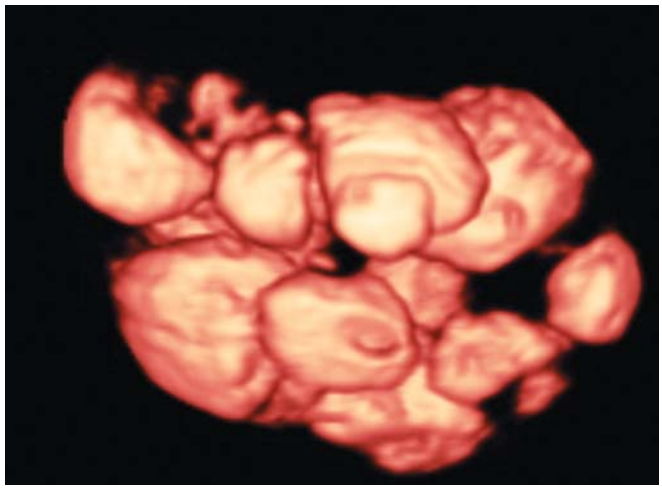

Fig. 4: Follicles as seen on inversion mode rendering

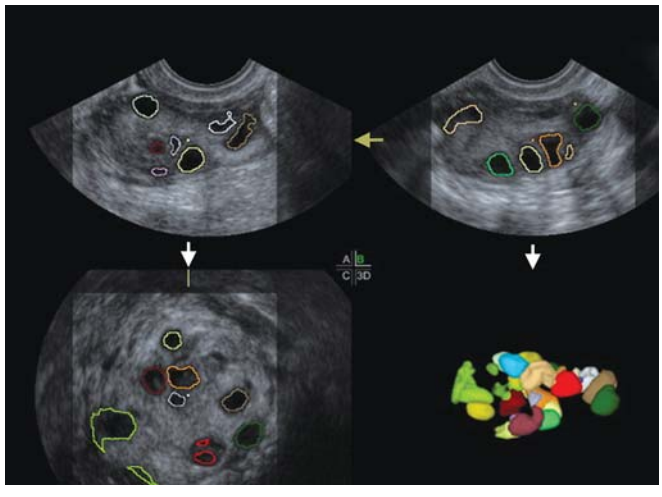

Fig. 5: Antral follicles as seen on Sono AVC

Antral follicles can be counted by using inversion mode rendering (Fig. 4) or using a software called Sono AVC (automated volume calculation; Fig. 5). Region of interest (ROI) is selected to include the whole ovary in all


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Date of Exam: 22.04.2010 Page 3 / 4
Exam Type:

Name NAYANA G 730 Pat. ID D10878-10-03-04-5

Ovary: Left Total#: 12							Ovary: Right Total#: 10						
Nr.	d(V) mm	dx mm	dy mm	dz mm	mn. d mm	V cm³	Nr.	d(V) mm	dx mm	dy mm	dz mm	mn. d mm	V cm³
1	9.3	17.0	9.6	5.8	10.8	0.42	1	13.1	20.7	15.6	7.9	14.7	1.18
2	7.6	10.7	8.2	5.2	8.0	0.23	2	9.7	13.6	11.7	6.5	10.6	0.48
3	7.4	11.0	7.5	5.7	8.1	0.21	3	8.6	10.7	9.0	7.0	8.9	0.33
4	5.8	6.9	6.4	4.7	6.0	0.10	4	7.2	10.1	7.2	5.5	7.6	0.20
5	5.7	13.5	7.7	4.2	8.5	0.10	5	7.0	9.6	6.6	5.8	7.3	0.18
6	5.7	6.6	6.2	4.7	5.8	0.10	6	5.1	7.2	5.7	3.4	5.4	0.07
7	5.4	6.8	6.6	3.9	5.8	0.08	7	4.7	6.2	5.2	3.4	4.9	0.05
8	5.0	7.6	5.4	3.7	5.6	0.07	8	4.2	5.7	4.6	3.1	4.4	0.04
9	4.6	5.6	4.5	4.1	4.7	0.05	9	3.2	3.6	3.4	2.8	3.3	0.02
10	3.0	5.4	3.0	1.8	3.4	0.01	10	3.1	3.7	3.0	2.7	3.1	0.02
11	2.2	3.3	2.1	1.6	2.4	<0.01							
12	2.1	3.2	2.3	1.7	2.4	<0.01							

Endo Thickness 4.89 mm

Pelvic Floor

funneling yes no

urethral kinking yes no

Fig. 6: Result sheet of Sono AVC

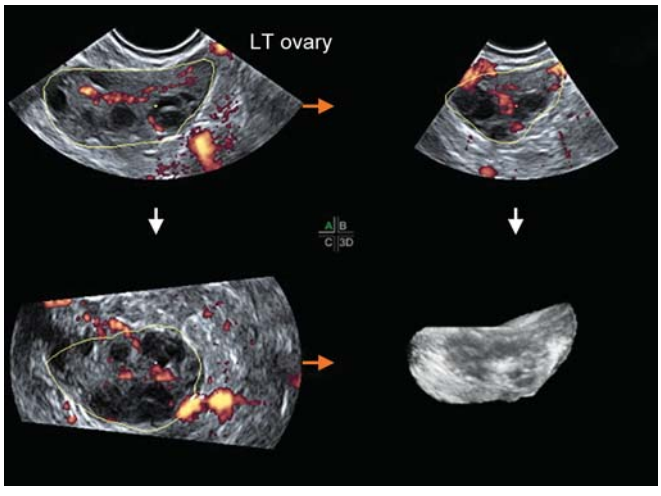

Fig. 7: Ovarian volume calculated by VOCAL

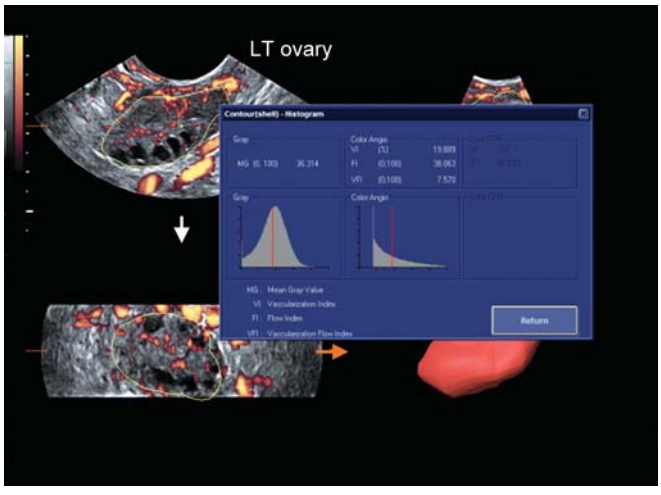

Fig. 8: 3D power Doppler volume histogram of ovary

three orthogonal planes on acquired ovarian volume. Sono AVC is based on inversion mode rendering, but further color codes each follicle and also shows x, y and z diameters, mean diameter and volume of each follicle on result sheet (Fig. 6).

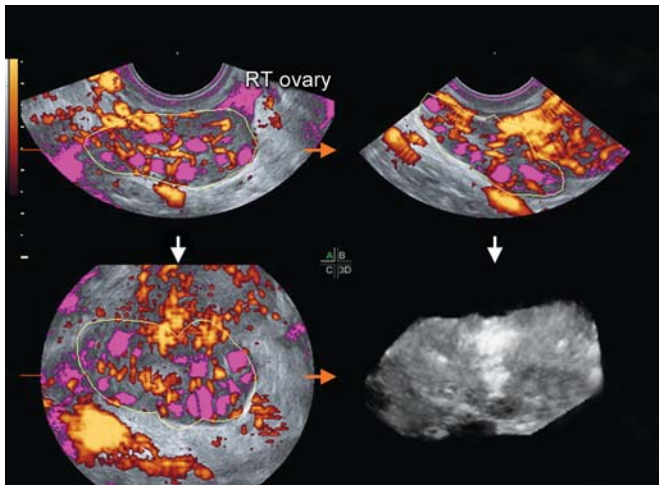

Fig. 9: Stromal volume calculated using threshold volume after VOCAL of ovary

A software called VOCAL (volume calculation by computer) is used to define ovarian volume (Fig. 7). VOCAL calculates volume of any structure by rotating it 180°. A rotating angle of 6° to 30° can be selected. A circumference is drawn around the structure of interest at every rotation and at the end of 180° total volume is calculated. On this calculated ovarian volume with power Doppler, applying volume histogram gives values of 3D power Doppler indices, vascularity index (VI), flow index (FI) and vascularity flow index (VFI) (Fig. 8). VI is an index for abundance of flow in the selected volume, FI is an index for average intensity of flow in a selected volume and VFI is a perfusion index. Applying threshold volume on the same VOCAL calculated volume will define stromal volume when threshold is set to differentiate follicles from rest of the ovarian tissue (Fig. 9).

Based on the above described assessment of the ovaries, they are categorized into normal, low reserve, poorly responding or polycystic ovaries. This helps to decide the stimulation protocol and also to decide any additional or supportive therapy required for a particular patient.

Normal Ovaries

Normal ovaries are the ones that have a largest diameter of 2 to 3 cm, ovarian volume of 3 to 6.6 cc, antral follicle count (AFC) per ovary of 5 to 12, isoechoic stroma (Fig. 10), stromal RI of 0.6 to 0.7, stromal PSV 5 to 10 cm/sec, stromal FI 11 to 14. Ovaries with these features are categorized as normal ovaries because they respond to standard stimulation protocols and produce adequate follicles for the concerned assisted reproduction technology. These standard protocols are 75 IU for intrauterine insemination (IUI) cycles and 150 to 225 IU for *in vitro* fertilization (IVF) cycles.

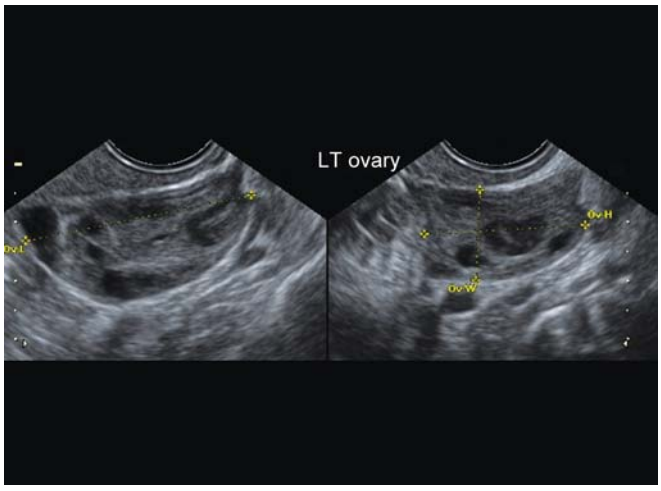

Fig. 10: Normal ovaries

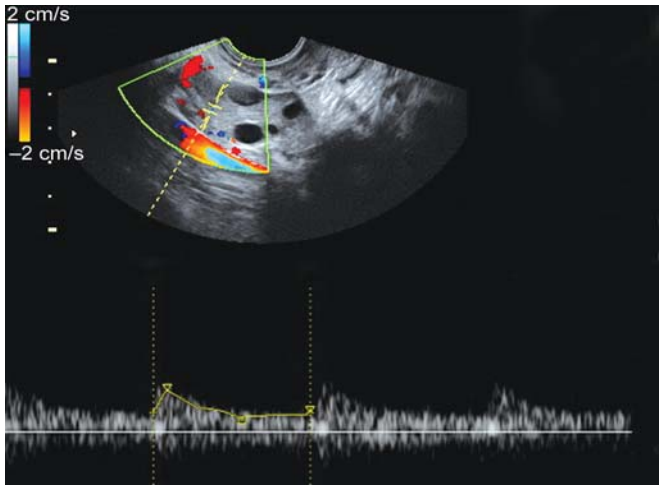

Fig. 12: Poor responding ovary: With little stromal flow

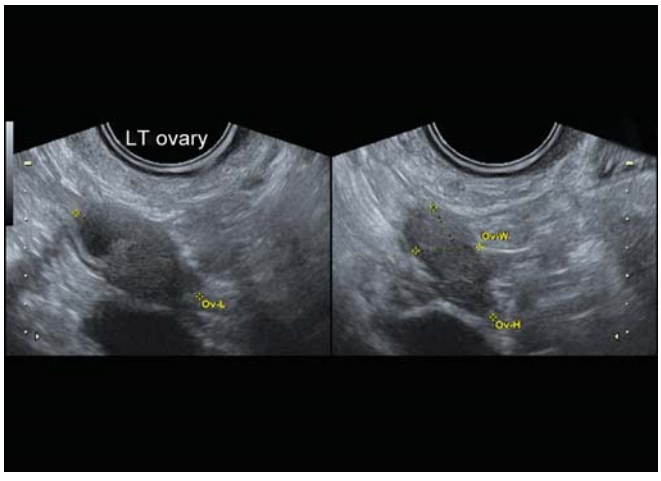

Fig. 11: Low reserve ovaries: Small size and <3 antral follicles

Low Reserve Ovaries

Low reserve ovaries are the ones, that have low reserve, and reserve in ovaries means number of reserve follicles in ovary. Therefore, these are the ovaries that have less number of antral follicles, less than 5 per ovary. As they have less number of antral follicles they are also small in size (Fig. 11). They have a largest diameter of less than 2 cm and volume less than 3 cc. As the reserve or AFC is low, these ovaries produce less number of follicles at the end of stimulation.

Poorly Responding Ovaries

Poorly responding ovaries are the ones that respond poorly to any stimulation. This means that these ovaries require larger doses of gonadotropins for stimulation. This poor response can be attributed to the poor blood flow to these ovaries. Measurement of ovarian stromal flow in early follicular phase is related to subsequent ovarian response in IVF treatment.[3] Ovarian stromal PSV after pituitary suppression is predictive of ovarian responsiveness and outcome of IVF treatment.[4] These ovaries show high resistance (RI > 0.7), low velocity (PSV < 5 cm/sec) flow (Fig. 12).

Low reserve and poorly responding ovaries are often used as synonyms, but these are different entities. This is so because reserve relates to antral follicle count or ultimate yield of follicles/ova at the end of stimulation, whereas response relates to sensitivity of ovary to ovulation stimulating agents to produce those follicles. This means that any ovary would be a permutation combination of one of the characteristics from each of these two groups. One group has normal response, poor response and hyperresponse and the other group has normal reserve, low reserve and high reserve or polycystic ovaries. It is, therefore, a combination of findings like AFC and stromal flow: RI, PSV and FI that would ultimately decide the optimum stimulation protocol.

Though before we do that let us discuss ultrasound for diagnosis and management of polycystic ovarian syndrome.

Polycystic Ovaries

Diagnosis of polycystic ovarian syndrome (PCOS) has been a controversial and debatable issue always. Earliest description of polycystic ovaries appears to date from 1845 as 'sclerocystic ovaries'.[5] Other names suggested are polyfollicular syndrome or ovarian dysmetabolic syndrome. Three major ways to diagnosis of PCOS:

1. Clinical findings
2. Laboratory testing
3. US findings

   Approximately 20 to 30% of women in reproductive age have polycystic ovaries and about half of these have signs and symptoms of PCOS. According to ESHRE/ASRM consensus 2003 the diagnosis of polycystic

ovarian syndrome consists of at least two of the three following criteria:

1. Oligo and/or anovulation
2. Hyperandrogenism: Biochemical or clinical
3. Polycystic ovaries on ultrasound: This means an ovary that is 10 cc in volume and/or has more than 12 antral follicles.

And enlarged spherical ovaries >10 cc, has shown good correlation between US and diagnosis of polycystic morphology and histopathological criteria for polycystic ovaries.[6] But there are controversies regarding the ovarian enlargement in PCO. Concerning the ovarian volume setting the threshold at 7 cc offered the best compromise between specificity (91.2%) and sensitivity (67.5%). In comparision, specificity and sensitivity were 98.2 and 45% respectively with threshold at 10 cc.[7] Ovarian volume 6.6 cc has shown 91% sensitivity and 91% specificity for polycystic ovarian syndrome.[8] Moreover, according to S Kupesic, ovaries that are normal in volume can be polycystic as demonstrated by histological and biochemical studies (in 20%). Polycystic ovarian morphology has, therefore, been found to be a better discriminator than ovarian volume between polycystic ovarian syndrome and control women.[9] This discussion indicates that ovarian volume alone cannot be used as a parameter for diagnosis of polycystic ovaries. Therefore, morphological features of polycystic ovaries needs consideration.

Morphological characteristics of polycystic ovaries consist of number and arrangement of antral follicles, stromal echogenecity and vascularity.

Antral Follicle Count

Antral follicle count (AFC) of 12/more (2-9 mm) has been used as a characteristic for polycystic ovaries according to Rotterdam criteria. Setting the threshold at 12 for 2 to 9 mm FNPO offered the best compromise between specificity (99%) and sensitivity (75%).[10] Though polycystic histology and morphology has been found in ovaries having AFC between 5 and 15. AFC also thus cannot be used as the characteristic of polycystic ovaries.

At this stage a short understanding of pathophysiology and hormonal correlation of ultrasound findings may be helpful.

Pathophysiology

Polycystic ovaries are a result of chronic anovulation. Mildly raised androgen levels, in early follicular phase in PCOS patients, leads to recruitment of several follicles. It is believed that androgen leads to early follicular development but further progression is not normal due to hyperinsulinemia and/or other metabolic influence linked to obesity.[10] All these follicles do not become dominant. This is so because there is partial conversion of androgen to estrogen and there is also cumulative effect of minimal estradiol production by multiple follicles leading to negative feedback for FSH and positive feedback for luteinizing hormone (LH). These factors lead to maturation arrest of these follicles and premature luteinization leading to atresia. These luteinized atretic follicles ultimately contribute to the stroma leading to stromal abundance.

AFC in PCO and its Hormonal Implications

Antimullerian hormone (AMH) is a biomarker that predicts the number of antral follicles and is involved in follicular arrest for women with PCOS. AFC and ovarian volume showed significant correlation with AMH, total testosterone and free androgen index but not with age, body mass index (BMI) or homeostasis model assessment of insulin resistance (HOMA-IR). AMH and total testosterone were main determinants for ovarian volume in stepwise regression model. AMH, obesity, IR and high androgen levels relates to large size of antral follicle pool and ovarian volume on PCOS. Obesity and IR may enhance follicular excess by dysregulation of AMH through pathway of hyperandrogenemia.[11]

The mean follicular number per ovary (FNPO) of follicles 2 to 5 mm in size was significantly higher in polycystic ovaries than in controls, while it was similar within 6 to 9 mm range. Within 2 to 5 mm range, significant relationship was found between FNPO and androgens but FNPO in the range of 6 to 9 mm was significantly and negatively related to BMI and fasting serum insulin level.

This indicates that antral follicle count and size of antral follicles can derive a lot of information about the biochemical status of the patient.

Arrangement of Follicles

The antral and atretic follicles get arranged peripherally or are dispersed in the stroma and thus may categorize polycystic ovary as peripheral and general cystic pattern. In peripheral cystic pattern there is typical garland like arrangement of follicles and in generalized cystic pattern, the follicles can be seen throughout the ovary[12] (Fig. 13). Though one school of thoughts believe that peripheral cystic pattern polycystic ovaries and generalized cystic pattern polycystic ovaries have different histopathological and endocrine bases,[13] another theory is different. According to this the ovary is multifollicular in adolescence. Because of the pathophysiology explained earlier, follicles that are <9 mm are exposed to LH and undergo atresia and make stroma denser giving rise to generalized cystic polycystic

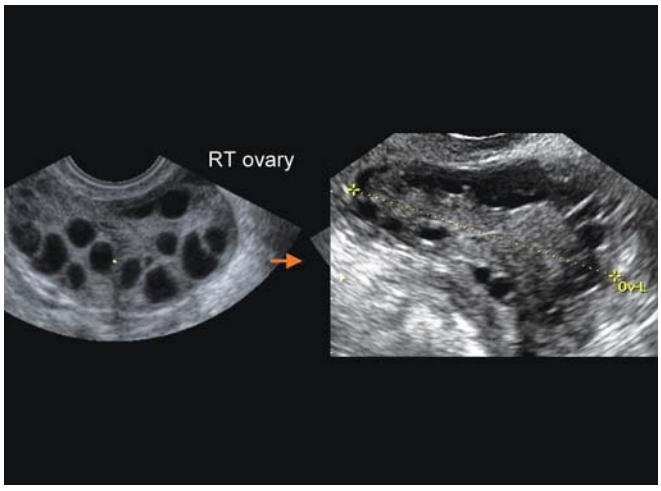

Fig. 13: Generalized polycystic pattern and peripheral polycystic pattern: PCO

ovary. If at this stage the condition is left untreated, gradually the follicles in the central part of the ovary, in an effort and process of recruitment reach the periphery or are pushed out to periphery by expanding stroma and undergo atresia ultimately leading to peripheral cystic polycystic ovary.[14,15] So multicystic ovary to generalized cystic PCO, to peripheral cystic PCO is a process of evolution of the disease. This indicates that the patients who have more severe form of disease or a long standing disease have a peripheral cystic pattern and evidently will have worse hormonal milieu as compared to those who have generalized polycystic pattern.

Stromal Abundance

Hyperdense stroma and stromal abundance have been described with polycystic ovaries since the first definition of the syndrome by Stein-Levanthal.

Stromal abundance can present as increased echogenecity because stroma is densely packed and increased stromal area or increased stroma volume in large ovary. Patients having long standing PCOS and long standing anovulation have more dense stroma. Most severe form of stromal abundance, hyperthecosis, presents large ovaries with almost absence of cystic lesions: Solid looking ovaries (Fig. 14).

Assessment of Stromal Abundance

Polycystic ovaries show a hyperechoic stroma but assessment of this hyperechogenecity is subjective not only to the operator but also to equipment settings.[16,17] Though ovarian stroma can be stamped as hyperecoic when its more echogenic than myometrium. This hyperechogenecity is especially useful for its differentiation from multicystic ovaries, that are normally seen in adolescence and have multiple follicles, of variable sizes and nonhyperechogenic stroma. Increased stromal echogenecity for diagnosis of PCO has a sensitivity of 94% and specificity of 90%.[18] (Fig. 15).

But recent studies have shown that mean stromal echogenecity or total ovarian echogenecity as measured by histogram are not different in controls and PCOS. But, stromal index (stromal echogenecity/total ovarian echogenecity) was significantly higher in PCOS than controls.[19]

Not only the echogenecity but total stromal volume is also increased in polycystic ovaries. This can be measured on ultrasound as stromal area in the most longitudinal section of ovary on 2D US. As for echogenecity this also has been found to be sensitive for diagnosis of polycystic ovarian disease. Stromal area: 4.6 cm^2 has 91% sensitivity and 86% specificity for diagnosis of PCOS. The ovarian area can also be measured in this same section. Ovarian area of 5.3 cm^2 has 93% sensitivity and 91% specificity for diagnosis of PCOS.[8]

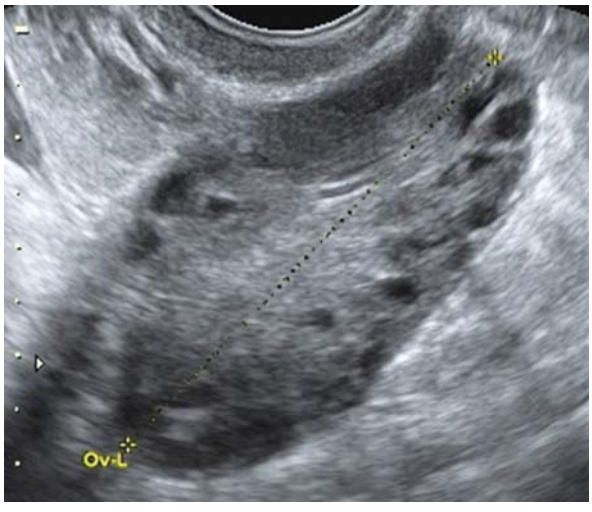

Fig. 14: Solid looking PCO

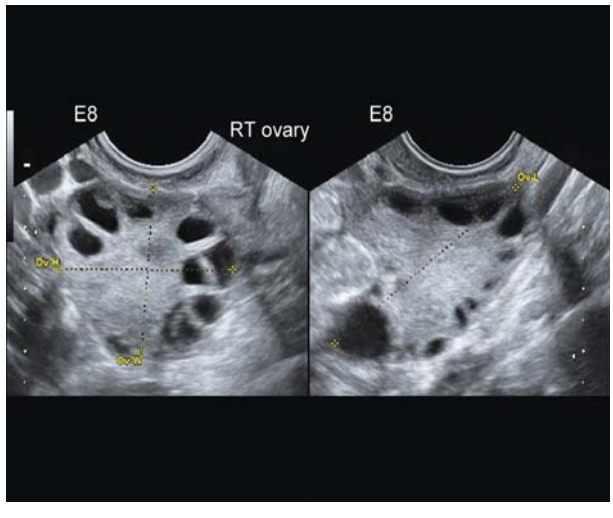

Fig. 15: Dense hyperechoic stroma of PCO

But, the ratio of stromal area to ovarian area has been found to be more reliable. S/A ratio also has a strongest correlation with S androgens especially testosterone and androstenedione and insulin.[20] Stromal area/ovarian area ratio of >0.34 is diagnostic of PCOS and can be correlated with S androstenedione.

Sensitivity for diagnosis of PCOS:

- Ovarian volume 13.21 cc 21%
- Ovarian area 7 cm^2 4%
- Stromal area 1.95 cm^2 62%
- Stromal/total area 0.34 100%

Mean stromal area/mean ovarian area ratio of 0.34 and above also has a specificity of 100% in the same study.[21]

Stromal Abundance in PCO its Hormonal Implications

The proportion revealed between the stroma and the ovary surface in the median section (S/A ratio) had been indicated as a reliable marker for hyperandrogenism. Hyperandrogenic subjects showed higher values of stromal area and S/A ratio, with no difference in ovarian volume and ovarian area.[22] S/A has also been found to be the best significant predictor of elevated androgen and testosterone levels. This parameter may be used in routine clinical practice for improving US diagnosis of PCOS.[21] Stromal to ovarian ratio is lower in normal females. Stromal abundance may be better assessed by stromal volume than with stromal area. Stromal volume can be assessed by using threshold volume on vocal calculated ovarian volume.

3D US provides a new method for objective quantitative assessment of follicle count, ovarian volume, stromal volume and blood flow in the ovary.[14] Ovarian volume calculation by 3D US has been found to be useful over 2D evaluation of ovarian long diameter or volume by 2D US.

Ov. volume	*Right*	*Left*
Normal	5.3 ± 2.0 cc	5.7 ± 1.6 cc
PCOD	12.2 ± 4.7 cc	10.5 ± 3.6 cc[19]

Right ovary is larger in PCOS patients, whereas left ovary is larger in normal patients.

Theca cells of PCOS women hyperrespond to gonadotropins (LH) and produce excess androgens. This is due to an escape of their normal downregulation to gonadotropins. This dysregulation is linked to excess of insulin and insulin-like growth factor (IGF)-1. Hyperinsulinemia is a key factor to the pathogenesis of PCOS. Insulin augments LH-stimulated androgen production by stromal cells. Androgen in turn causes proliferation of stromal and theca cells. This leads to increased stroma in the PCO. Stromal volume was positively correlated with serum androstenedione concentrations in patients with polycystic ovarian syndrome.[23] Increased androstenedione secretion as shown earlier is due to hyperinsulinemia.[24]

A prospective study of 50 polycystic ovarian syndrome patients with 50 non-PCOS patients was done over a period 6 months with clinical examination, baseline ultrasound scan with 2D and 3D US and fasting and postprandial insulin levels. Group A: 50 patients with normal ovulation, no hirsutism, normal menstrual cycle and normal ovarian size and group B: 50 patients with PCOS, according to Rotterdam criteria. Age range of patients for both groups was between 25 and 35 with mean age for group A was 30.4 years, mean age for group B was 29.7 years. Mean BMI in both the groups was 28, range from 25 to 32 $kg/m.^2$

Patients with BMI < 25 kg/m^2, proved diabetes mellitus, any other endocrinological derangement (thyroid, adrenal, etc.), follicles larger than 9 mm or residual corpus lutea on day 3 and ovarian mass lesions (cystic/solid) were excluded from the study cohort. Ovarian volume and stromal volume were calculated by applying threshold volume to the VOCAL calculated volume. The threshold is set to differentiate follicles from stroma. Fasting and postprandial insulin levels were checked for all on the same day. Insulin estimation was done by chemiluminescence method. For postprandial insulin measurement patient was given 75 gm of glucose after fasting blood sample and then blood sample for PP insulin was taken after 2 hours. Values of ovarian and stromal volume and AFC were all averaged for both ovaries in each patient. Two tailed Pearson correlation was checked for ovarian volume, stromal volume and stromal volume to ovarian volume ratio with fasting insulin and postprandial insulin level each. Ovarian and stromal volumes were compared and correlated with both fasting and postprandial insulin levels.

Positive correlation was seen between ovarian and stromal volumes and fasting and postprandial insulin levels. With Pearson correlation significance level of 0.01 (2-tailed) the correlation for:

- Ovarian volume to fasting insulin is 0.651
- Ovarian volume to PP insulin is 0.409
- Stromal volume to fasting insulin is 0.736
- Stromal volume to PP insulin is 0.428

Stromal and ovarian volumes and AFC correlated significantly well with the fasting insulin levels, more than with postprandial insulin levels in obese PCOS patients. It is the stromal volume that can be best correlated with fasting insulin levels followed by ovarian volumes and AFC.[25]

A similar study has also been done earlier. Study by Pache et al has shown that the degree of insulin resistance can be correlated with ovarian volume and stromal echogenecity.[24] A retrospective observational study done

with 50 PCOS patients showing correlation between ovarian and stromal volumes with fasting and postprandial insulin levels. But in this study neither BMI, nor age group were defined. In PCOS patients a strong and similar correlation was seen between ovarian and stromal volumes to fasting and postprandial insulin levels.

Stromal Vascularity

Based on 3D US, women with PCOS have an increased stromal volume and vascularity. Even with same echogenecity, PCOS has more stromal flow. In polycystic ovaries even on 3rd day of the cycle intraovarian stromal flow is seen and they have moderate to low resistance flow with RI of 0.50 to 0.58.[26]

Elevated LH levels may be responsible for increased stromal vascularization due to neoangiogenesis, catecholaminergic stimulation and leukocyte and cytokine activation. This vascularity is inversely related to LH/FSH ratio. Tonic secretion of LH in early follicular phase in PCOS is also associated with theca and stromal cell hyperplasia and consequent androgen production. This androgen hypersecretion is responsible for not only increased follicular recruitment but also for vasoconstrictive effect on the uterine arteries. This effect is thought to be due to activation of specific receptors in arterial walls and collagen and elastin deposition in smooth muscle cells. Uterine artery pulsatility index (PI) is >3 and sometimes the diastolic flow is absolutely absent. Even in later phases of the cycle this effect continues. This leads to inadequate perfusion of the endometrium and is thought to be responsible for blastocyst implantation failure and high abortion rate in PCOS.

Stromal Vascularity in PCO and its Hormonal Implications

Looking to the hormonal correlation with the Doppler findings, it is evident that in patients in whom the hormonal milieu is worse the Doppler findings are more prominent. As discussed earlier the peripheral cystic pattern of PCO is an advanced stage then generalized cystic pattern and so the intraovarian vascularity and uterine artery resistance are more in peripheral polycystic ovaries than in generalized polycystic ovaries. In 22% of GCP, PCO intraovarian vessels are not recognized.[27] Stromal vascularity is significantly higher in women with PCOS who are hyperandrogenic and lean rather than normoandrogenic and obese.[28] Fertile controls and PCOS women had similar total ovarian 3D power Doppler flow indices. Normal weight PCOS women had significantly higher total ovarian 3D power Doppler flow indices than their overweight counterparts.[29] Higher age, obesity and amenorrhea as compared to young age, normal weight and oligomenorrhea show higher uterine artery resistance and increased ovarian stromal flow. These are the patients who also have higher LH and higher androstenedione levels and higher ovarian volumes. Uterine artery resistance has also been found to be higher in obese than in lean patients and is also associated with hyperinsulinemia, high triglycerides, low high density lipids and higher hematocrit values. Oligoanovulatory patients with PCO but without hyperandrogenism have mild endocrine and metabolic features of PCOS.[30]

But the results were different when 3D and 3D power Doppler were used. Women with PCOS had higher AFC (median 16.3 *vs* 5.5 per ovary), ovarian volume (12.56 *vs* 5.6 ml), stromal volume (10.79 *vs* 4.69 ml) and stromal vascularization (VI 3.85 *vs* 2.79%, VFI 1.27 *vs* 0.85). Though 2D power Doppler indices were not higher in PCOS than in controls. Ovarian stromal FI is higher (33.94 *vs* 29.30) in hirsutes than in normoandrogenic PCOS women. But in PCOS women with obesity the vascularity was lower than in normal weight women (VI 3.25 *vs* 4.51%, VFI 1.22 *vs* 1.56%).[31] Comparing ovarian stromal blood flow and serum vascular endothelial growth factor between fertile women with normal ovaries and infertile women with PCOS, it was found that both the groups had similar total ovarian VI, FI, and VFI values after controlling for the age. Though VI, FI and VFI were significantly higher in normal weight PCOS than in obese PCOS women.[29] The vascularization indices VI, FI and VFI are significantly higher in PCOS than in normal females which explains excessive response to gonadotropins in PCOS females. 3D vascularization quantification has been found to be more sensitive than 2D vascularization quantification.[32]

Though a study from Finland shows no difference in VI, FI and VFI values in PCO and normal ovaries. But in normal ovaries, FI was found to be higher in left ovary.[33] Total ovarian VI and VFI were significantly lower in women aged 41 or more. AFC has the best correlation with the age, followed by S FSH and ovarian 3D power Doppler indices. The rate of decline of total ovarian VI was 0.18% per year.[34]

Though our understanding of ultrasound findings has substantially increased in the near past, certain controversies still need explanation:

1. *Low flow in PCO*: Many of the PCO patients consume large doses of gonadotropins. These are the patients which on US do show multiple follicles, large ovaries and dense stroma, and on doppler they do have a moderate resistance flow but have a low PSV. They may be grouped clinically as resistant PCO.

2. *Moderate resistance in uterine arteries*: Not all patients with PCOS have high androgen levels which is responsible for high uterine artery resistance, this is especially so in generalized cystic pattern of PCOS. Another cause of moderate resistance in uterine artery instead of high resistance is adenomyosis or fibroid in the fibroid commonly associated with PCOS.

Deciding Stimulation Protocol

Predictors of ovarian response are[35]

- Number of antral follicles
- Ovarian stromal FI
- Total ovarian stromal area
- Total ovarian volume

In that order of importance.

AFC is a better marker than basal FSH for selection of older patients with acceptable pregnancy offer.[12] Antral follicle count and ovarian volume showed significant correlation with AMH, total testosterone and free androgen index.[36]

Precise calculation of antral follicle count, therefore, can help in predicting the ovarian response. This can be done on 2D US or by 3D with inversion mode rendering and Sono AVC as described earlier. This method is more precise as there is least chance of follicles being missed or being counted twice. But postprocessing is required for accurate calculations. It takes longer to perform, because of the need for postprocessing, and obtains values that are lower than those obtained by the 2D and 3D-MPV techniques. However, the AFC obtained by SonoAVC-PP is likely to be lower because this method measures and color codes each follicle preventing recounting. Antral follicle when counted by inversion mode: Likely to get 60% of follicles of the counted antral follicles in IVF cycles.[37] Intraobserver and interobserver reliability of automated AFCs made using 3DUS and SonoAVC a preferred method.[38]

It has been shown by Zaidi et al that stomal blood flow velocity after pituitary suppression was an independent predictor of ovarian response.[3] Measurement of ovarian stromal flow in early follicular phase is related to subsequent ovarian response in IVF treatment.[4] Ovarian stromal PSV after pitutary suppression is predictive of ovarian responsiveness and outcome of IVF treatment.[35] Kupesic has shown correlation in the ovarian stromal flow index and number of mature oocytes retrieved in an IVF cycles and pregnancy rates.[35] Stromal FI (<11 low responder, 11 to 14 good, >15 risk of OHSS).

Based on all these facts and findings we understand that ovaries that have high resistance, low velocity flow require higher doses of gonadotropins for stimulation. Whereas those with low resistance, high velocity flow require lower doses of gonadotropins for stimulation. Number of antral follicles decide the ultimate yield of mature follicles at the end of stimulation. If number of antral follicles is high, it would be preferable to proceed with low doses of gonadotropins, whereas in ovaries that have low AFC, it is preferable to start with high doses of gonadotropins. Patients with higher age and high BMI require higher doses for stimulation whereas those of young age and with low BMI require lower doses for stimulation.

The final dose calculation can be based on following factors and we would prefer to simplify it like this:

	Increase the dose when..	*Decrease the dose when..*
Age	>35 years	<25 years
BMI	>28 kg/m^2	<20 kg/m^2
AFC	<3 (FNPO)	>12 (FNPO)
Stromal RI	>0.7	<0.5
Stromal PSV	<5 cm/sec	>10 cm/sec
Stromal FI	<11	>15
Ovarian volume	<3 cc	>10 cc

To select the correct stimulation protocol, doses are titrated depending on findings present in a particular patient from high dose table or low dose table.

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