Ultrasound in Infertility

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ABSTRACT

Evaluation of the complete cycle instead of only pre hCG scan is an essential for follicular monitoring. Using color Doppler in this assessment is mandatory because it allows to assess the functional status of follicle and endometrium. 3D ultrasound is useful for volume measurements, and 3D PD for assessment of global vascularity.

Baseline scan is done to predict the ovarian reserve and response and decide the stimulation protocols for ARTs. Uterus is assessed for receptivity. But baseline scan also diagnoses PCOS. This is by counting antral follicles, stromal flows and stromal and ovarian volume. Ultrasound features of ovary on baseline scan can also be correlated closely with the baseline hormonal status of ovaries—LH, FSH and Androgen. Ultrasound is a key tool to decide follicular maturity and endometrial receptivity and to decide the time of hCG and time of IUI. Doppler plays a major role in correct decision making and 3D and 3D power Doppler add to the details and also improves the success rates of different ARTs. Luteal phase also can be better explained by the use of Doppler. Ultrasound to hormonal correlation in both preovulatory and luteal phase helps plan the ART for positivity.

Keywords: Baseline scan, Preovulatory scan, Endometrial receptivity.

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INTRODUCTION

Fertility is a complex equation of continuous hormonal changes. Assessment of infertility needs a thorough assessment of the hormonal changes occurring during menstrual cycle in a female and, therefore, evaluation of the complete cycle instead of only pre hCG assessment is essential. Hormonal changes can be studied as biochemical changes directly or indirectly by studying the effects of these hormones in the form of morphological and vascular changes in uterus and ovaries. This can be done by ultrasound scans.

Route of scan has to be transvaginal always. All scans are done using B-mode, color Doppler, pulse Doppler, 3D ultrasound and 3D power Doppler. 3D ultrasound is especially useful for volume measurements. 3D power Doppler, has also been proved to be highly promising in several studies, as it gives idea about the global vascularity of the structure examined.

Baseline Scan

This scan is done on 2nd or 3rd day of the cycle. It is known as a baseline scan because at this time of the cycle, the ovaries have no active follicles and estrogen and progesterone are both at lowest levels, endometrium is thin like a single line as it has shed off during menstruation and this is a baseline status of the ovaries (Fig. 1).

This scan is done to classify the ovaries in one of the four categories: Polycystic ovaries (PCO), normal ovaries, low reserve ovaries and poorly responding ovaries or in other words, to predict the ovarian reserve and response which can guide to decide the stimulation protocols for ART. Ovarian reserve assesses the number of follicles likely to develop or mature by ovarian stimulation and assessing response is to calculate the dosage of drugs required for these follicles to grow. Uterus is assessed for the procedure: intrauterine insemination (IUI) or embryotransfer (ET) and for receptivity.

Baseline Scan of Ovaries

2D 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. Color or power Doppler is used to see the presence of vessels in the stroma and pulse Doppler is used for quantitative assessment of the flows— intraovarian resistance index (RI) and peak systolic velocity (PSV). 3D and 3D power Doppler volume of the ovary is acquired for assessment of ovarian volume and stromal volume and counting number of antral follicles. Global vascular indices VI (vascularity index), FI (flow index) and VFI (vascularity flow index) may be calculated by volume histogram. VI indicates abundance of flow in the calculated volume, FI indicates average intensity of flow and VFI indicates perfusion of this volume.
Normal ovaries have (ovaries with normal reserve and response) the following:
- Diameter: 2-3.5 cm
- Volume: 3-6.6 cc
- Stromal RI: 0.6-0.7
- Stromal PSV: 5-10 cm/sec
- Follicle number per ovary: 5-12

**US and Doppler Features for Polycystic Ovaries**

Enlarged ovaries, multiple antral follicles, stromal abundance and atypical endometrium are features of polycystic ovaries as described in literature. But each feature needs to be evaluated independently. According to Rotterdam criteria (ESHRE/ASRM 2003 consensus) polycystic ovarian syndrome is defined as:  

Two of the following three criteria and exclusion of other etiologies:
- Oligo and/or anovulation
- Hyperandrogenism: clinical or biochemical
- Polycystic ovaries on ultrasound.

According to this definition, polycystic ovaries on ultrasound are ovaries that are more than 10 cc in volume and/or has > 12 antral follicles.

**Enlarged Ovaries**

On 2D US an ovary which has a longest diameter of > 3.5 cm is considered enlarged. When scanning the ovary, the probe is rotated and spanned at various angles to find out the most longitudinal section of the ovary. To calculate the volume of the ovary on 2D US, a second section is taken by rotating the probe 90° from this most longitudinal section. On this section, two perpendicular measurements are taken: one perpendicular and one parallel to the ultrasound beam (Fig. 2). Volume is calculated by applying the formula $(x \times y \times z) \times 0.523$. Calculation of ovarian volume by VOCAL (volume calculation by computer: virtual organ computer aided analysis) on 3D US is much more accurate (Fig. 3). For VOCAL usually rotating angle of 30° is used but in case of an ovary which is not perfectly oval, angle of 15° may be preferred.

According to Adam Balen, ovarian volume of 10 cc is a criteria for polycystic ovarian disease. ‘For an ovarian size of 10 cc a good correlation has been shown between ultrasound diagnosis of polycystic morphology and histopathological criteria for polycystic ovaries.’

But reports have also shown that an ovarian size of 6.6 cc has 91% sensitivity and 91% specificity for PCOS. And yet another report says ‘however, ovaries which are normal in volume can be polycystic as demonstrated by histological and biochemical studies (in 20%).’ This means ovarian volume alone cannot be used as the criteria for diagnosis of PCO.

Polycystic ovarian morphology has been found to be a better discriminator than ovarian volume between polycystic ovarian syndrome and control women.
These morphological features are:
• Number of antral follicles
• Arrangement of antral follicles
• Stromal predominance.

Multiple Antral Follicles (2-9 mm)

Counting antral follicles especially in PCO patients is difficult on 2D ultrasound. If 3D volume of the ovary is acquired and Sono AVC (automatic volume calculation) (Fig. 4) is used the antral follicle count can be reliably done. Sono AVC color codes each follicle and on report sheet presents diameter and volume of each follicle (Fig. 5). Using Sono AVC for counting antral follicles requires postprocessing.

Setting the threshold at 12 for 2 to 9 mm FNPO offered the best compromise between specificity (99%) and sensitivity (75%).

Though a study by Dewailly et al in 2011, shows that FNPO > 19 had a sensitivity of 81% and specificity of 92% for PCO. (AMH > 35 pmol/l with sensitivity of 92% and specificity of 97%). An average value of 26 or more follicles per ovary is a reliable threshold for detecting polycystic ovaries in women with frank manifestation of PCOS. Sensitivity and specificity for diagnosis of PCOS for FNPO (26) was 85 and 94% and for OV (10 cc) was 81 and 84%. But the same study has also quoted that the lower follicle threshold may be required to detect milder variants of the syndrome.

Predominant Hyperechoic Stroma

Stromal abundance the most consistent character and key factor for the diagnosis of PCOS. Polycystic ovaries show a hyperechoic stroma but assessment of this hyperechogenicity is subjective not only to the operator but also to equipment settings. Ovarian area of 5.3 cm² on strict longitudinal ovarian section and stromal area of 4.6 cm² has high sensitivity and specificity for PCOS. But stromal area/ovarian area ratio has been found to be even more efficient for diagnosis of PCOS.

Sensitivity for diagnosis of PCOS
• Ovarian volume 13.21 cc 21%
• Ovarian area 7 cm² 4%
• Stromal area 1.95 cm² 62%
• Stromal/total area 0.34 100%

Stromal volume can be calculated by acquiring a 3D volume of the ovary and calculating the volume by VOCAL, and then using threshold volume, adjusting the threshold to exclude the follicles from total ovarian volume. This helps to separately calculate the volume of the structures below threshold (follicular volume) and above threshold (Stromal Volume) (Fig. 6).

Apart from these major features, PCOS patients show atypical endometrium or out of phase endometrium due to the hormonal derangements like high LH and early rise of estrogen due to multiple follicle recruitment and conversion of androgen to estrogen. For these reasons, endometrium in early follicular phase appears multilayered in PCOS patients and becomes echogenic.
just before ovulation. In control patients left ovary is larger than left and in PCOS patients right ovary is larger.3

There are certain Doppler characteristics of the PCO. These ovaries show stromal flow on the baseline scan. On pulse Doppler these vessels show RI: 0.50-0.58.12 This is because of high LH levels. If these blood vessels also show a PSV of > 10 cm/sec, they are prone to hyperstimulation. High androgen levels in these patients also lead to high uterine artery pulsatility index (PI) (Figs 7A and B).

It is interesting to note here that higher uterine artery PI and lower ovarian stromal vessel resistance is seen in PCP (peripheral cystic pattern) ovaries than GCP (generalized cystic pattern) ovaries, higher age, amenorrhic, rather than oligomenorrheic patients and in obese, hyperinsulinemic and hirsuit patients.

FNPO in the range of 6 to 9 mm was significantly and negatively related to body mass index and fasting serum insulin level.6

Third type of ovaries are poorly responding ovaries. They may be of any size but have Intraovarian stromal RI > 0.68, and PSV < 5 cm/sec. These ovaries have low vascularity and therefore respond only to higher doses of stimulation.

Fourth type is low reserve ovaries which are small sized ovaries with < 2 cm in longest diameter or < 3 cc in volume and have < 3 antral follicles. They will produce very few follicles with any stimulation.

Based on this, predictors of ovarian response are:13

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

In that order of importance.

Antral follicle count is a better marker than basal FSH for selection of older patients with acceptable pregnancy offer.14 Antral follicle count and ovarian volume showed significant correlation with AMH, total testosterone and free androgen index.15

Precise calculation of antral follicle count, therefore, can help in predicting the ovarian response. This can be done on 2D ultrasound by scanning across the whole ovary while counting the follicles by eyeballing. A more precise method is using Sono AVC (automatic volume calculation) (Fig. 8). Antral follicle when counted by inversion mode: likely to get 60% of follicles of the counted antral follicles in IVF cycles.16

SonoAVC 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 because this method measures and color codes each follicle preventing recounting. Intraobserver and interobserver reliability of automated antral follicle counts made using three-dimensional ultrasound and SonoAVC a preferred method.17

It has been shown by Zaidi et al that stromal blood flow velocity after pituitary suppression was an independent predictor of ovarian response.18

Kupesic has shown correlation in the ovarian stromal flow index and number of mature oocytes retrieved in an IVF cycles and pregnancy rates.13
Uterus

Base line scan of the uterus is done to assess the uterocervical length by ultrasound. Longitudinal section of the uterus is imaged on the screen where whole of the uterus is seen with endometrial cavity starting from fundus to the internal os and external os. For all ART procedures may it be intrauterine insemination (IUI) or embryo transfer (ET), the uterocervical length is measured from the fundal end of the endometrium to the external os and is known as functional or physiological uterocervical length (Fig. 9). Doppler of the uterine artery is done. Uterine artery RI > 0.79 indicates that high doses of stimulation will be required for endometrial maturation. Subendometrial flow on day 2 is present indicates a low receptive endometrium for implantation. This is so because on day 2 ovaries are silent, estrogen levels are very low and, therefore, no endometrial vascularity is expected normally. If it is present it is either high basal estrogen or inflammation of the endometrium leading to increased vascularity.

Preovulatory Scan

Antral follicles grow to become dominant and mature follicles. A follicle that grows to 12 mm is a dominant follicle, it grows at a rate of 2 to 3 mm/day and ovulation occurs at 18 to 24 mm size usually. Follicular flow can be first detected when follicular size is 10 mm and its resistance starts falling 2 days prior to ovulation.

Features of a Mature Follicle

A rounded 16 to 18 mm sized follicle with thin walls and no internal echogenicity is seen. A sonolucent halo appears surrounding the follicle 24 hours prior to ovulation. Cumulus oophorus, a small projection from wall in the follicular lumen may be seen (Fig. 10A).

On color Doppler vascularity is seen surrounding 3/4 th of the follicular circumference (Fig. 10B). On pulse Doppler these vessels show RI 0.4-0.48, PSV > 10 cm/sec (Fig. 10C). If the follicular vascular indices are not in the defined range, it means that the follicle is not yet physiologically mature and, therefore, stimulation still needs to be continued. The follicle is said to be functionally mature when PSV is 10 cm/sec, that is the time when the LH surge starts and under the effect of that LH, the perifollicular PSV keeps on rising constantly. Follicular blood flow velocity starts increasing approximately 26 to 29 hours before rupture and continues till 72 hours after rupture. Rising PSV with steady low RI suggests that the follicle is close to rupture.20 Steady or decreasing PSV with rising RI suggests that the follicle is proceeding towards LUF.22

We have done a study of 500 IUI cycles, based on this to find out appropriate time of IUI based on pre hCG perifollicular vascularity. Single IUI was done between 36 to 38 hours and double IUI were done, one at 12 to 14 hours and other at 36 to 38 hours in all patients in whom perifollicular PSV > 15 cm/sec. When perifollicular PSV on the day of hCG was more than 20 cm/sec, and perifollicular RI was within normal range, double IUI has given significantly higher pregnancy rates.23 This indicates that in patients with higher perifollicular pre hCG PSV, IUI done at 12 to 14 hours has been fruitful.

Fertilization of a follicle with a PSV of less than 10 cm/sec, has high chances of fertilizing into an embryo with chromosomal abnormality.
Application of 3D US for Follicular Assessment

When there is multifollicular development as in PCO the follicular shapes become polygonal and, therefore, follicular diameter may not be a reliable parameter. Follicular volume by 3D is a more reliable parameter. Follicular volumes of between 3 and 7 cc are optimum for oocyte retrieval (Fig. 11A). The limits of agreement between the volume of the follicular aspirate and 3D volume of the follicle were 0.96 to – 0.43 with 3D and 3.47 to – 2.42 by 2D volume estimation.

Cumulus can be seen in more than 80% of follicles using 3D US rendering. This is possible only in upto 40 to 45% of follicles by 2D US. On the day of hCG, if cumulus like echo is not seen in all three planes in the follicle, it is less likely to be mature fertilizable oocyte (Fig. 11B).

3D power Doppler gives idea about the global vascularity of the follicle (Fig. 11C). The 3D power Doppler indices, VI, FI and VFI give quantitative global assessment of vascularity (Fig. 11D). We have done a large study taking this into consideration. Based on study of > 1000 cycles, follicular volume of 3 to 7.5 cc, VI > 6-20 and FI > 35 if used as additional features for a mature follicle apart from the follicular features mentioned previously, increased the pregnancy rates significantly.

Features of a Mature Endometrium

On 2D ultrasound an endometrium of 6 mm or more in thickness, preferably 8 to 10 mm that is multilayered is thought to be good enough. But its morphological assessment is essential. Because of orderly organization of glandular elements in proliferative phase, endometrium is generally hypoechoic in this phase, though the morphology can be graded as follows.

Endometrial Grading

Grade A endometrium: Multilayered with intervening area at least as echogenic as myometrium.

Grade B endometrium: Multilayered with intervening area hypoechoic to myometrium.

Grade C endometrium: Homogenous hyperechoic endometrium.

Spiral arteries supply the endometrium and these are the vessels that undergo substantial changes during menstrual cycle. Estrogen causes vasodilatation and progesterone antagonizes the effect. Therefore higher E2/P ratio is responsible for higher blood flow through uterine vascular bed.

On color Doppler, the vascularity of the endometrium is classified by Applebaum as follows (Figs 13A to D).

Zone I: Blood vessels reaching endo-myometrial junction surrounding the endometrium.

Zone II: Blood vessels reaching hyperechoic endometrial edge.

Zone III: Blood vessels reaching intervening endometrial hypoechoic zone.
Zone IV: Blood vessels reaching endometrial cavity- the central line.

On pulse Doppler, these blood vessels should show RI 0.49-0.59 and PI 1.1-2.3. These blood vessels should cover 5 mm² area of a particular zone of endometrium.

Absent subendometrial and intraendometrial vascularization on the day of hCG, appears to be a useful predictor of failure of implantation in IVF, irrespective of morphological appearance.29

It has also been observed that when pregnancy is achieved in absence of endometrial and subendometrial flow on the day of embryo transfer, more than half of these pregnancies will finish as spontaneous miscarriage.30

Estrogen receptors are also present in uterine arteries and cause vasodilatation in these vessels also. The normal values of uterine artery indices are RI 0.60 to 0.80 and PI 2.22-3.16. Even if TVCD of follicle is normal, endometrial and the uterine artery indices should be within normal limits for implantation. Embryo transfers in IVF cycles are also cancelled if the uterine artery PI > 3.2.31 Doppler study for uterine receptivity should be done on the day of hCG. hCG administration induces significant increase in the resistance of uterine artery for 48 hours which can affect its evaluation on the day of follicular aspiration/rupture therefore Doppler study for uterine receptivity should be done on the day of hCG.19

Figs 12A to C: Grade A, B and C endometrium

Figs 13A to D: Zone 1, 2, 3, 4 vascularity seen in endometrium on color and power Doppler
Moreover, the Doppler studies are better done at the same time every day as circadian rhythm, is seen in uterine artery flow in periovulatory phase.

As for the follicle, the endometrium also if evaluated by 3D and 3D power Doppler for maturity before hCG, gives better implantation rates. It gives endometrial volume instead of endometrial thickness. Volume is considered to be a better parameter than endometrial thickness. Moreover, 3D power Doppler gives idea about the global vascularity of the endometrium not only in terms of quality but also in terms of quantity, which should be a more useful parameter than 2D Doppler where we can see vessels only in one plane at a time and also interrogate a few vessels not all by pulse Doppler. In our study, 3D power Doppler indices that we have found reliable are endometrial volume > 3 cc, FI > 20 and VFI > 5 (Fig. 15). There are several other people who have also worked on endometrial receptivity assessment by 3D and 3D power Doppler. A study by Luis T Merce et al of 40 IVF cycles has shown endometrial volume of 3 to 7 ml as most favorable for conception. Median values for a favorable endometrium is 4.28 ± 1.9 ml. Endometrial volume of > 2.0 ml has a significantly higher pregnancy rates and no pregnancies were recorded with endometrial volume of < 1 ml.

No pregnancy occurred when endometrial volume < 3 ml and VI < 10. Exceptionally better pregnancy rates are achieved with endometrial volume > 7 ml and subendometrial VI between 10 and 35%. A study by Kupesic et al shows lower resistance index of 0.49 to 0.57 in subendometrial vessels and FI of 11.0-15.4 in conception cycles as compared to 9.5 to 13.3 otherwise.

VFI on the day of hCG is more sensitive than volume, VI and FI for prediction of pregnancy. VFI > 0.24 has
- Sensitivity of 83.3%
- Specificity of 88.9%
- PPV 93.8%
- NPV 72.3%
- For prediction of pregnancy with 33% pregnancy rate.

Secretory Scan

With the rupture of the follicle corpus luteum is formed. Corpus luteum is a cystic structure with thick shaggy walls and echogenicity in the lumen. But, it is known to have variable appearances like ground glass echogenicity in lumen or lace like echogenecities (Fig. 16A). Secretory changes are seen in the endometrium in the form of echogenicity of the endometrium which starts from outside, proceeding to the central line making a ring sign of the endometrium (Fig. 16B). Posterior wall of the uterus also appears more echogenic in this phase due to acoustic enhancement by the endometrium due to fluid accumulation.
Assessment of the corpus luteum, endometrium and uterine artery by Doppler gives information about the luteal phase normalcy and progesterone levels. A clear correlation between RI of corpus luteum and plasma progesterone levels has been seen in natural cycle.

RI of the corpus luteum can, therefore, be used as an adjunct to plasma progesterone assay as an index of luteal function.\(^3\)

A healthy corpus luteum will show a vascular ring surrounding itself on color Doppler and on power Doppler these vessels show RI 0.35-0.50, PI 0.70-0.80 and PSV 10-15 cm/sec\(^2\),\(^3\) (Fig. 17A). This resistance starts increasing on days 23 of the cycle in a nonconception cycle to RI 0.5-0.55 (Fig. 17B). Endometrial Doppler shows branches of spiral arteries piercing the outer echogenic margins of the endometrium and better if it reaches the central line with RI 0.48-0.52. Uterine artery at this time of the cycle has PI of 2.0-2.5 and PSV 15-20 cm/sec. Based on these values abnormal parameters indicate luteal phase problems like Luteinized unruptured follicle or luteal phase defect.

### Luteinized Unruptured Follicle

On 2D US shows persistent follicle with thick walls and progressive loss of cystic appearance which is difficult to differentiate from corpus luteum. Endometrium is thick and echogenic and no fluid is seen in POD. On Doppler perifollicular RI is 0.51-0.59, which is higher than normal and remains almost constant till the end of the cycle. Nondominant ovary also shows similar Doppler indices. Endometrium is echogenic but endometrial flow is absent\(^2\) (Fig. 18).

### Luteal Phase Defect

On Doppler corpus luteum shows high resistance flow with RI: 0.58 + 0.04. Bilateral ovarian RI shows no
difference. Increased resistance is also seen in spiral arteries RI:0.72 ± 0.06. This is the case when luteal phase defect is due to insufficient corpus luteum (Fig. 19). But when luteal phase defect is due to insufficient progesterone receptors in the endometrium, the endometrial flow is normal but endometrium is thin, nonhyperechogenic and shows scanty vascularity (Fig. 20).

Segmental uterine and ovarian artery perfusion demonstrate a significant correlation with histological and hormonal markers of uterine receptivity and may aid assessment of luteal phase defect.

Optimally done ultrasound assessment suffices for cycle assessment and hormonal investigations can be done less frequently.

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