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Review Article

Three-dimensional follicular assessment: a review of technique and indications

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ABSTRACT

Follicular maturity assessment and tracking are employed to ascertain mature oocytes' presence and assess the response to ovarian stimulation. This step is crucial to ensure successful outcomes during assisted reproductive techniques. Currently, two-dimensional (2-D) transvaginal ultrasound is performed to monitor follicle growth and determine the optimal time for administration of human chorionic gonadotropin (hCG) hormone. However, the accurate follicle size, count, and multi-follicle maturity assessment require significant expertise and negligible inter-observer variations to maintain the reliability and validity of the measurements. 2-D ultrasound allows only an approximation of the actual follicular volume; therefore, it cannot be used to define standards for follicular maturity assessment. With the improvement of ultrasonography imaging technologies and the supporting software, it is now possible to acquire three-dimensional data sets and perform precise estimation of absolute dimensions, volumes, and mean dimensions of even complex structured follicles. This paper aimed to provide an in-depth review of the use of 3D ultrasound (3D-US) in reproductive medicine and combines an overview of the technique of performing a 3D-US for a fast, valid, objective, and reliable follicular assessment.

Keywords: Assisted reproductive techniques, Three-dimensional ultrasound, Follicular maturity assessment, Endometrial receptivity

INTRODUCTION

The success of assisted reproductive techniques (ART) depends on the number of mature oocytes available at the time of pickup. Therefore, follicular maturation and retrieval are timed to maximize the mature oocyte yield, which can in turn ensure the best possible outcomes for in-vitro fertilization (IVF).[1] Ultrasound is crucial for follicular maturity assessment, follicular tracking, endometrial receptivity assessment, and even in embryo transfer. Following successful ART, regular ultrasound is required to monitor the pregnancy and fetal growth.

In a natural cycle, few follicles in bilateral ovaries compete for dominance. Ultimately, by the end of the follicular phase, only one follicle (dominant follicle) starts maturing, and one mature oocyte develops, while the rest degenerate. Without hormonal stimulation, this dominant follicle is tracked through regular ultrasound performed on every alternate day, till the mature oocyte is released, and the graafian follicle is left behind. This is also marked by minimal free fluid in the pouch of Douglas (PoD), at the end of follicular phase. However, in controlled ovarian hyperstimulation (COH), there is multi-follicular growth, and require complex monitoring techniques for tracking follicular growth and maturation. COH is based on the administration of glycoprotein-based gonadotropins, including follicle stimulating hormone (FSH), luteinizing hormone (LH), and chorionic gonadotropin (hCG).

Different protocols (mainly three) of stimulation are available with major or minor differences.[1]

High doses of FSH triggers the stimulation of ovaries, resulting in follicular growth, and allowing retrieval of multiple mature oocytes.[2] Mature oocytes at the time of ovum pickup are fundamental to ART. Retrieval of good-quality oocytes increases the chances of a high fertilization rate and adequate number of high-quality embryos.[1] However, before initiating ovarian stimulation, ovarian reserve is assessed by antral follicle count, which then dictates the planning of the IVF therapy.

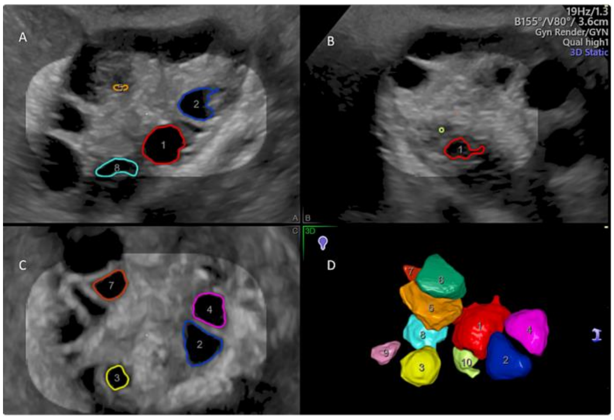

Figure 1: Technique of performing a 3-D Follicular study; after the volume box is switched.

(A) the entire follicle is covered with a 5-7 mm margin. The software calculates the volume based on anechoic intra-follicular areas (B and central) and assigns colours in order of size, with red being the largest. Individual follicles can be counted by studying individual colours (D); no two follicles are assigned the same colour.

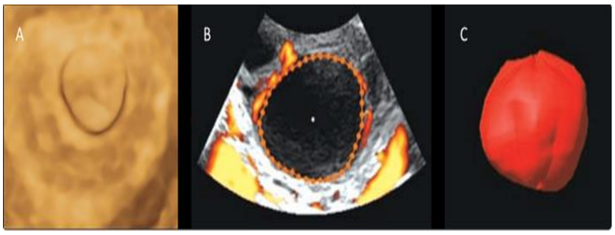

Figure 2: Assessment of Follicular maturity; 3-D evaluation of follicular maturity involves assessing presence of cumulus oophorus.

(A) 3-D power doppler is done by selecting the follicular area, and studying the leash of perifollicular vessels to assess functional maturity (B) It shows the follicular surface covered by vascular supply (C) Higher the vascular coverage, better the follicular maturity.

Follicular maturity assessment and ovarian reserve is, therefore, required before ovulation induction and at the time of the hCG administration, to provide the best chance of in-vitro fertilization.[2] Various biochemical and vascular changes affect the maturation of the follicle, luteinization, ovulation, and endometrial receptivity. Therefore, spectral Doppler evaluation is imperative for the objective assessment of functional follicular maturity, and endometrial blood flow.[3]

Endometrial parameters including thickness, morphology, perfusion, and lack of pathologies are important to ensure successful implantation after embryo transfer.[3] The former are subject to hormonal alterations and show changes over the menstrual cycle. Certain morphological and biochemical conditions, including increased thickness and perfusion, are required for embryo implantation and is is known as the "window of implantation".[1] Follicular maturation has to be in sync with satisfactory endometrial receptivity to achieve and sustain pregnancy, a task negatively affected by the administration of supra-physiological hormones.[1] Currently, transvaginal ultrasound is the preferred method for the assessment of follicles and endometrium.[4]

Ultrasound is non-invasive and painless. Due to lack of harmful radiation, regular ultrasound check-ups are not hazardous to the follicles, or to the patients, and do not pose a risk to the growing embryo.[5] 3D-US allows quick volume calculation of examined objects, its greatest fortitude in contrast to 2D imaging. Recent studies show that volume estimates have better predictive values for pregnancy outcomes. 3D-US is accurate and reproducible, making it widely popular for monitoring follicular development during controlled ovarian hyperstimulation cycles, and for assessing endometrial morphology for receptivity assessment before implantation.[6] While the technique has been in existence for some time, technical advancement was needed to widen the diagnostic applicability of 3D US and make it available for clinical use at affordable prices.[7]

3D power Doppler is, currently, the only technique available that can evaluate vascularity and real-time blood flow in an analyzed sample volume. It provides a unique method for assessing follicular and endometrial global vascularity; better parameters for assessing follicular maturity, endometrial receptivity at the time of hCG administration.[6]

Techniques

A transvaginal high-frequency probe 12-15 MHz is used for scanning. Speckle reduction is used for better resolution when needed.

On 2-D US

Folliculometry is usually performed on alternate days between 11 and 20 days of the menstrual cycle. A single vertical diameter is sufficient for round follicles. For polygonal follicles, the average of three dimensions, measured in perpendicular planes, are required.

Endometrial thickness and morphology are assessed on standard longitudinal images at approximately 2.0 cm from the fundus. Trilaminar endometrial morphology, characterized by central hypoechoic and outer hyperechoic walls, is associated with better results, as compared to non-trilaminar morphology.

Color Doppler is performed to assess the circumference of the follicles that are covered by blood vessels. If blood vessels are scanty, power Doppler is used to assess the vascularity more effectively. The wall filter is set at the lowest value. Gains are adjusted to fill the perifollicular vessels, but not spill color outside the vessels.[3] Angle correction is required.

Uterine artery flow parameters and sub-endometrial blood flow branch typing and parameters are estimated to calculate endometrial blood flow.

On 3D US

Parameter setting: 19 MHz probe setting

For 3D US, the volume box is selected and switched on. It is adjusted to include the entire follicle with a 5-7 mm margin4. The volume should be large enough to cover the follicle from edge to edge. The acquired volume is then evaluated in three perpendicular planes, and its circumference is traced on the screen at every 15° angle.

Post-processing of acquired data in 3D US, allows easy volume calculation, surface rendering, determination of mean diameters accurately, and presentation of data in the inversion mode.[8] The software calculates the volume of the follicle, based on the anechoic intra-follicular areas. Individual colors can count the number of antral follicles (Figure 1).

Inversion mode can highlight subtle differences in density of tissues and structures by assessing the differences in reflection and scattering of ultrasound waves, and aid in better visualization of anechoic structures like cysts and follicles. It can also be used for better determination of tissue boundaries. Captured objects are pictured in different colours allowing clear overview and differentiation. This is particularly helpful in assessing ovarian reserve, and follicle counts.[1] Post-processing of rendered images can be done using surface smooth or light gradient mode to visualize cumulus.

For 3D PD, an ROI including the outside shell, with a wall thickness of approximately 2 mm, is selected for assessment of perifollicular vessels and the surface area covered by a leash of vessels. Following parameters are assessed.

Vascularity index

The number of colour vessels in a given volume i.e., abundance of flow in the selected volume.

Flow index

The average intensity of color in the selected volume i.e., the intensity of flow in a given volume)

Vascular flow index

The ratio of abundance of flow to the intensity of flow in the selected volume i.e., measurement of perfusion of the given volume.

3D US acquire a series of 2D images by manual or automatic systems, through manual movement within the region of interest (ROI), or automatic sweeping within the ROI, respectively. While, the quality and spatial resolution of the 3D acquisitions depend on the quality of 2D US, the latter is associated with higher intra and inter-observer variability. 3D US on the other hand, has less observer variability, and is more standardized and accurate.

Indications

Follicular maturation assessment and growth tracking

Oocyte maturity is the key factor that ensures high-quality embryos, which in turn increases the chances of a viable pregnancy. An oocyte in the metaphase II is considered mature.[1] GnRH analogues and hCG trigger ovulation process. A minimum diameter of 16 mm is needed for gonadotropin stimulation, and of 18 mm for clomiphene citrate stimulation.[3] Follicular volume of 3-7 ml is associated with better results after gonadotropin administration and intrauterine insemination.[3]

FSH stimulation leads to multiple follicular growth and maturation and requires determination of the best time for hCG administration for final oocyte maturation.[1] Accurate follicular tracking is, therefore, crucial for guiding treatment with ovulation-inducing drugs.

3D US measures volume by calculating the anechoic area. This overcomes the lack of third dimension measurement in 2D US. Rough estimates based on single-plane and two-plane diameters lead to inaccurate assessment with undesirable side effects.[9] Moreover, multi-follicular growth tracking requires significant expertise on 2D US to avoid intra and inter-observer variability. For example, in OHSS, there can be over 30 follicles developing in the ovary. It is important that all the follicles are well accounted for and counted properly.[1] 3D US can provide accurate, less variable, standardized volumes. Follicular vascularity evaluation using 3D-PD indices gives an objective assessment of functional maturity (Figure 2).

Assessment of polycystic ovarian disease

Polycystic ovarian syndrome (PCOS) is among the commonest endocrine disorders affecting female fertility, with high incidence among women undergoing IVF therapy10. Ultrasound criteria for the diagnosis of PCOS include visualization of bulky ovaries with multiple, small, peripherally arranged follicles around a dense stroma (Figure 3).[11] Patients usually have larger ovarian volumes (>10 cm^3) with an increased number of antral follicles.[12] PCOS needs to treated in women undergoing IVF, due to an elevate risk of ovarian hyperstimulation (OHSS).[1] 3D US can therefore, through comprehensive analysis, help detect and differentiate between complications like OHSS, bulky ovaries, and anovulatory cycles.

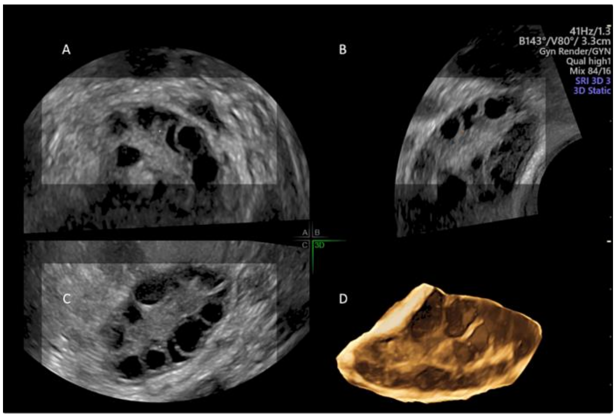

Figure 3: Ovarian volume assessment; 2D ultrasound shows multiple, small, follicles arranged peripherally.
(A) Ovarian volume is assessed in three perpendicular planes. The software calculates (B and C) 3D ovarian structure, placement of follicles, and total ovarian volume (D) for accurate evaluation and follow-up.

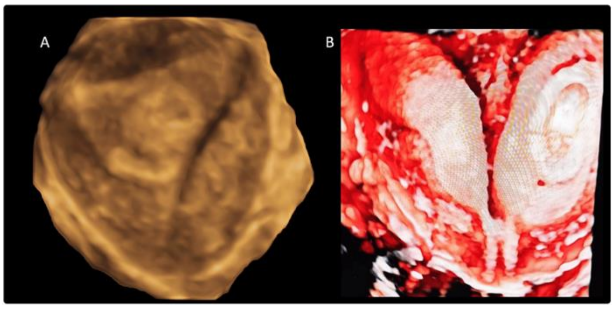

Figure 4: Uterine pathologies; 3D ultrasound is more sensitive for diagnosing submucosal fibroids and polyps.

(A) and uterine anomalies including differentiation between septate and partial septate uterus; (B) it can also show the site of implantation in bicornuate uterus (B).

3D PD indices, including vascularity index, flow index, and vascular-flow index, are used in the management of infertility and for assessment in patients of polycystic ovarian disease. A strong correlation was found between 2D and 3D volumes and 3D PD indices.[13]

Oocyte donors

During a COH cycle, follicles of different sizes respond differently to FSH.[1] Thus, during tracking and monitoring, follicles of different sizes are present. Generally, follicles with larger diameters or volume are considered mature. However, as mentioned above, cytoplasmic and nuclear maturity in oocytes is described as the end of first meiotic division and rest in metaphase II. 3D ultrasound allows the detection of smaller follicles that have matured, through earlier visualization of cumulus.

This leads to shorter lengths of controlled ovarian hyperstimulation cycles, detection of a larger number of follicles, recovery of a higher number of mature oocytes, and hence a more accurate timing of triggering, especially in a multi-operator setting. This ensures increased ovarian stimulation efficiency in oocyte donors.[14]

Endometrial receptivity

Endometrium is the innermost uterine layer and the location of embryo implantation. 3D US is highly sensitive for diagnosing uterine pathologies like adenomyosis, polyps and submucosal fibroids that have a detrimental effect on the patient's fertility and impede embryo implantation, and identifying the type of uterine anomalies (Figure 4).[1] Some of these are easily treatable. The correct diagnosis is crucial in dictating surgical intervention (polypectomy, hysteroscopy) and medical management. Patients with uterine malformations require closer monitoring and pregnancy surveillance due to higher risk of pregnancy loss, premature birth, and other complications.[15,16] 2D US of pelvis does not have such a high degree of accuracy and precision in diagnosing pelvic pathologies. 3D US also allows better discrimination between benign and malignant endometrial pathologies in post-menopausal women.[17]

Before COH is started, the pituitary is downregulated by using GnRH agonists in supra-physiological doses. This creates a state of hypoestrogenism in the body, which is confirmed using either blood estradiol levels or endometrial thickness. The latter has a high predictive value. Endometrial thickness of less than 5 mm is highly suggestive of successful downregulation.[18] Endometrial volumes are now used for a more accurate measurement of pituitary downregulation and determine implantation success or risk of spontaneous abortion. Apart from the thickness and endometrial volume, the endometrial morphology examination is also important to determine if the embryos can be transferred in a fresh cycle, or they need to be cryo-preserved for transfer in subsequent cryo-cycle.[1]

ER is the ability of the endometrium to permit embryo positioning, followed by successful adhesion, implantation, growth, and immune regulation during the window of implantation.[19] This shows shifts during the menstrual cycle and is influenced by multiple regulatory factors. Failure of implantation remains the foremost reason why IVF treatments fail to result in pregnancy.[3] Endometrial receptivity is assessed using parameters like endometrial thickness, endometrial blood flow branches, and endometrial blood flow classification, which are influential factors in pregnancy outcomes. Endometrial volume is now considered more standard, as compared to endometrial thickness. A thickness of <5-7 mm is associated with poor pregnancy outcomes.[19] A good blood supply is also an essential requirement for successful implantation.[3]

3D ultrasound assesses the endometrial volume (as opposed to measuring just the thickness), and number of endometrial blood flow branches with precision using parameters like sub-endometrial vascularity index, flow index, and vascular-flow index (Figure 5), increasing its predictive value in assessing endometrial receptivity (Figure 5).[20]

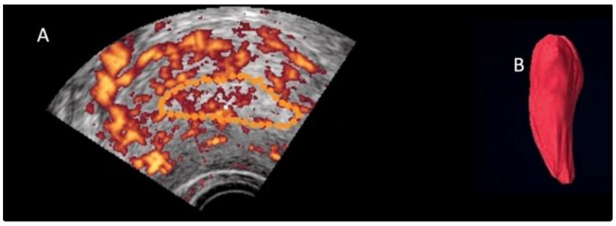

Figure 5: (A) Assessment of endometrial receptivity; 3D volume box is placed along the endometrial cavity; (B) 3D ultrasound and 3D power doppler can calculate endometrial volume, and endometrial and sub-endometrial vascularity, respectively (B).

Correlation with antral follicle count and AMH

Before initiating COH, ovarian reserve and ovarian response to hormonal stimulation are estimated through ultrasound. Ovarian reserve decides the specific IVF treatment, to avoid OHSS with higher doses and failure of IVF with low doses. As a result, it can predict the chances of a successful outcome. This also facilitates pre-treatment counseling of couples and the formulation of an individualized treatment plan.

Ovaries contain different kinds of follicles: primordial, primary, secondary, pre-antral, and antral or Graafian (>2 mm). Only a few of these respond to FSH21, while others atrophy or degenerate. The antral follicles are useful in IVF and the ovarian antral follicle count (AFC) is used to estimate ovarian reserve.[22] This allows segregation and classification of patients from low to high-responding, and calculating risk for developing OHSS and PCOS during gonadotropin stimulation.[23]

3D sonography-based automated volume calculation (SonoAVC) allows antral follicle count using different colors for individual follicles, allowing precise, reliable, and faster estimation of antral follicle count compared to 2D ultrasound. It is specifically developed for follicle observation and improves the inter-observer reliability of antral follicle counts.[24] In contrast to 2D, 3D ultrasounds provide a variety of different image displays facilitating different measurement techniques.[25]

A positive correlation to serum anti Mullerian hormone (AMH) levels was also found.[26] Total antral follicle count (TAFC) includes follicles more than 6 mm, whereas the total ovarian reserve includes all follicles. With increasing number of follicles, the accuracy of 2D US reduces, due to losing track of counted follicles or multiple counting of the same follicle.[27] Multi-follicular growth tracking requires increased expertise and can be performed easily using 3D US with lower observer variability (Figure 1).

DISCUSSION

Optimal oocyte recovery, fertilization, and cleavage are associated with a main follicular diameter of 12 to 24 mm.[6] These correspond to volumes between 3-7 ml.[3] Volumes can only be assessed on 3D-US reliably.

Follicles with cumulus present have higher rates of pregnancy, and those without visualization of cumulus in all three planes, most likely do not contain mature oocytes. 3D ultrasound can confirm the presence of cumulus in smaller follicles, as compared to 2D ultrasound, thus increasing the number of mature oocytes available at the time of ovum pickup.[28]

IVF outcomes are similar in 2D and 3D COHORTs, however, 3D ultrasound is faster, with lesser time required to perform a scan, analyze the data, and record the acquired data. That makes it more efficient.[29] Several studies have proven that 3D ultrasound confers an edge over 2D ultrasound for the accurate assessment of antral follicular counts.

Endometrial volume estimation has shown better reliability in assessing pregnancy outcomes, compared to measurement of 2D endometrial thickness.[18] 3D-US evaluation also enables detection of endometrial polyps and submucosal fibroids, endometrial pathologies, uterine anomalies, obviating the need for the more expensive and less accessible MRI. Uterine anomalies can be demonstrated on 3D-US with extremely high sensitivity and superior spatial resolution. This helps guide surgical/medical management prior to starting IVF treatment.

While follicular flow can be assessed and established by using 2D PSV and perifollicular color maps, 3D PD indices give the most objective and precise information about global follicular vascularization and follicular blood flow.[30,31]

CONCLUSION

3D-US is fast, reliable, and reproducible, making it ideal when different operators are involved. Follicular volume, endometrial volume, and 3D-PD assess follicular morphological and functional maturity, beyond size, and endometrial receptivity objectively. When synced successfully during IVF treatment, these parameters are associated with better pregnancy outcome prognosis.

Higher resolution and better visualization proffered by 3D US, are associated with higher number of mature oocytes available for pickup, smaller COH cycles, and higher efficiency of oocyte donors. 3D-US and 3D-PD of endometrium calculates endometrial volume and endometrial/sub-endometrial vascularity. These can predict endometrial receptivity and successful implantation better than endometrial thickness. Uterine anomalies and pelvic pathologies can be diagnosed with higher sensitivity and specificity, guiding treatment prior to IVF therapy.

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