Three-dimensional ultrasound markers of ovarian reserve in fertile and infertile females

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Abstract: Objective: Evaluation of the differences between fertile and infertile females regarding the three-dimensional ultrasound markers of ovarian reserve and between the right and the left ovaries in women in each group. **Participants and method:** After inclusion, One hundred infertile and one hundred fertile women had a 3D power Doppler transvaginal scan in the early follicular phase (between the third and the fifth day of the menstrual cycle). The outcome measures included are: antral follicle count (AFC), small(2-6mm) AFC, larger (7-9mm) AFC, total ovarian volume (OV) and ovarian vascular indices; vascularization index (VI), flow index (FI) and vascularization flow index (VFI). The data from the right and left ovaries were compared. **Results:** The mean AFC, small AFC (2-6mm), mean VI and mean VFI of fertile group was significantly higher than the infertile group. The mean OV of infertile group was significantly higher than the fertile group. The difference between the mean of larger (7-9 mm) AFC and mean FI were statistically insignificant. Regarding the RT and the LT ovaries in the infertile group AFC, FI and VFI show a statistical significant difference; OV and VI show a statistical non-significant difference. In the fertile group; AFC, OV, VI, FI and VFI show a statistical non-significant difference. **Conclusion:** AFC especially small (2-6 mm) follicles are the best ultrasound marker of the ovarian reserve.OV alone should not be considered as a predictor of ovarian reserve. Evaluation of the ovarian stromal vascularity needs further research as a marker of ovarian reserve. Evaluation of the right and the left ovaries separately is recommended but further studies are needed to confirm that.

1- Introduction

Infertility, defined by the failure to achieve a clinical pregnancy after twelve months or more of regular unprotected sexual intercourse(1)**.**

Ovarian reserve (OR) refers to the number and quality of oocytes that, at any given age, are available to produce a dominant follicle late in the follicular phase of the menstrual cycle. By estimating the OR, a prediction of the remaining reproductive lifetime could be assessed as well as the likely success of assisted reproductive techniques (ART) such as *in vitro* fertilization (IVF) (2).

A true ovarian reserve is the number of primordial follicles present in the ovaries that can currently only examined histologically. However, ultrasound and endocrine measures of ovarian reserve appears to correlate to this true reserve and have been widely accepted as markers of ovarian reserve (3)**.**

In clinical practice, both ovaries are considered together as a combined unit during ovarian stimulation. The endocrine markers such as FSH, LH, E2, antiMullerian hormone (AMH) and inhibin B provide information of ovarian reserve of both ovaries as a combined unit. Ultrasound is so far the only method that allows a direct assessment of each ovary as a separate entity (4)**.**

Three- dimensional ultrasound not only permits improved spatial awareness and volumetric and quantitative vascular assessment of the ovaries but also provides a more objective tool to examine stromal echogenicity. Furthermore three-dimensional ultrasound provides a new method for the objective quantitative assessment of follicle count, ovarian volume, stromal volume and blood flow within the ovary as a whole(5).

Three-dimensional (3D) ultrasonography and power Doppler angiography (PDA) allow the evaluation of the ovarian volume, the number of antral follicles, and ovarian blood flow at the same time. All these variables have been linked to the ovarian response and human oocytes development competence. The volume measurement by 3D ultrasonography is more reliable than that obtained by two-dimensional ultrasonography. The ovarian volume calculated from serial multiple slices or, more recently, from a rotational method using the Virtual Organ Computer-Aided Analysis (VOCAL™) imaging program has very good reproducibility. Good reproducibility of the antral follicle count has also been shown by means of this technique(6)**.**

The ovarian volume, AFC, vascularization index, flow index, and vascularization-flow index were

determined by 3-D Power Doppler Angiography (PDA) and all were shown to have excellent intra-observer and inter-observer reproducibility (7)**.**

Further, the ovary functional stage (basal after pituitary suppression or stimulated after gonadotropin treatment) does not modify the reliability of any of these measurements (7)**.**

Thus, 3-D US and PDA offer the advantage of evaluating all parameters in a single US examination, thereby improving the clinical evaluation of ovarian parameters. In addition, ovarian images captured by 3-D sonography can be stored and evaluated later, thereby uncoupling the need to analyze the data during ovarian examination (8)**.**

SonoAVC™ (Automatic Volume Calculation) is a software program that can identify and quantify ovarian hypo echogenic regions within a 3D dataset and provide automatic estimation of their absolute dimensions, mean diameter and volume of fluid-filled areas of the ovary. Each different volume of cyst or follicle is color coded separately; SonoAVC can evaluate follicular development within the ovary(9)**.**

Pre-ART ultrasonographic AFC has been shown to be an excellent predictor of ovarian reserve and response, with significant superiority in relation to other markers. Studies also demonstrated significant correlations between AFC and commonly performed serum ovarian reserve tests and between AFC and AMH. In a recent systematic review, AFC alone was as accurate as different combinations of clinical, biochemical and other ultrasonographic markers in predicting IVF response(10)**.**

Also that AFC at cut off 5 should allow identification of poor response with 89% sensitivity, in spite of low specificity 39%(11)**.**

Based on the available data, it can be concluded that the accuracy of the AFC for predicting poor response in regularly cycling women is adequate at a low threshold level, but because of the very limited numbers of abnormal tests, AFC has hardly any clinical value for pregnancy prediction. Added to the false positive rate of ☐5%; the test will not be suitable as diagnostic test to exclude patients on the basis of the presumed diagnosis of advanced ovarian ageing. It may be used as a screening test for possible poor responders and for directing further diagnostic steps like a first IVF attempt, where the ovarian response to hyper stimulation will provide additional information (12)**.**

AFC with up to 10 mm in mean diameter is 10.1 ± 3.0 for normal responders and 5.7 ± 1.0 antral follicles, respectively, for poor responders(13)**.**

Meta-analyses conducted in a systematic review of studies evaluating the diagnostic accuracy of all the ultrasound based tests of ovarian reserve, including antral follicle count (AFC), ovarian volume and stromal blood flow in predicting fertility outcomes showed that women with AFC less than four were 8.7 times more likely not to get pregnant after IVF than women with AFC four or more. The sensitivity and specificity of AFC to predict cycle cancellation was 66.7% and 94.7%, respectively. Women with an AFC of less than four were 37 times more likely to have their cycle cancelled than women with AFC of four or more(14)**.**

The total antral follicles count includes small antral follicles (2-6mm)that are largely gonadotropin independent but selectable due to their responsiveness to gonadotropins and larger antral follicles (more than 6mm) that are gonadotropin dependent (15).

Special attention has been given to small antral follicles. In an observational study, normal response to stimulus for ART was significantly elevated for women with AFC $\geq$ 5 with mean diameter up to 5 mm(16)**.**

The number of small antral follicles (2-6 mm) is significantly related to age and also, independent of age, to all endocrine ovarian reserve tests, suggesting that the number of small antral follicles represents the functional ovarian reserve(17)**.**

Some studies show concern about the use of OV as a test of ovarian reserve and raised doubts on its reliability being evaluated by two-dimensional ultrasound (18)**.**This leads some authors to recommend assessment by three-dimensional ultrasound in order to minimize interobserver variability (3)**.**

Opinions are divided on considering ovarian volume (OV) as an adequate gonadal reserve test. Ovarian volume measurement, at a cut off value of 3 cm^3, showed specificity of 92%for prediction of cycle cancellation and 93% for non-pregnancy(14)**.**

However, others did not observe OV differences in ART between young women with normal response (means of 4.1 ± 0.66 mL) and poor response (means of 3.36 ± 0.71 mL) (13)**.**

Similar results were reached when evaluating women at high risk for cycle cancellation. There was a trend towards ovarian volume measurement being able to predict a poor response, but this trend did not reach statistical significance. Ovarian volume did not vary significantly between cycles in this group of patients(19)**.**

The review of ten well-designed studies on ovarian volume for that purposes concluded that OV presented little applicability in the prediction of poor response or pregnancy. The AFC performed statistically significantly better than ovarian volume in the prediction of poor response. The overall accuracy for predicting no pregnancy was poor for both tests (20)**.**

Three-dimensional PDA makes possible the quantification of complete blood flow of the region of interest from the analysis of the power Doppler signal. The "histogram facility" of the VOCAL imaging program automatically obtains 3 vascularity indices: vascularization index (VI), flow index (FI), and vascularization-flow index (VFI), which potentially can reflect the vascular density, blood flow, and tissue perfusion respectively(6).

In brief, the vascularization index (VI) indicates the proportion of the volume showing a flow signal in the total volume of the ovary. It does not contain any information on flow signal and intensity. The flow index (FI) is an average of the intensity of flow signal inside the ovary that carries no significance by itself. The vascularization flow index (VFI) is a combination of the information of vessel presence and amount of flow made by multiplying the FI and VI(21).

Ovarian blood flow (OBF) has been extensively assessed in natural and stimulated reproductive cycles. There was a significant negative correlation between age and ovarian perifollicular blood flow (PFBF) in women undergoing IVF which was only observed very late in the follicular phase of ovarian stimulation(22).

Ovarian PFBF of follicles > or =5 mm was subjectively assessed using a modified grading system (grades 0-4). It was found that high grade ovarian PFBF in the early follicular phase during IVF is associated with both high grade PFBF in the late follicular phase and a higher clinical pregnancy rate(23).

Do measurements of ovarian vascularity add anything to validate the use of three-dimensional sonography as a marker of ovarian reserve?

A study had used three-dimensional power Doppler angiography after pituitary 'down-regulation' and during gonadotrophin stimulation to compare ovarian vascularity in 33 women with normal ovarian reserve, as judged by antral follicle counts, to 12 women who had demonstrated a previous poor response. All three indices of vascularity were shown to increase significantly during gonadotrophin stimulation in the group with normal ovarian reserve only but this was related to the antral follicle count, lowering the importance of this marker(ovarian vascularity) as an independent variable (24).

Others claimed that the clinical value of Doppler studies for ovarian stromal blood flow was unclear. This is why ovarian vascular flow may not be used to determine inclusion of infertile couples in ART programs or to infer its results(14).

In evaluating the differences in the three-dimensional (3D) ultrasound markers of ovarian reserve between the ovaries within an individual; a study was carried out submitting two hundred seventy patients undergoing investigation for subfertility. Variations in 3D ultrasound markers of ovarian reserve between the two ovaries within same individual were estimated. Two hundred fifteen subjects were analyzed for ovarian volume and antral follicle count, and 205 subjects for 3D power Doppler indices. Significant differences were noted (median, range) in the AFC especially seen in follicles measuring more than 6.0 mm and in ovarian volume. But significant correlation was noted between the two ovaries in 3D power Doppler indices(6).

However an earlier study was performed to estimate side-to-side variation in antral follicle counts. Forty-one patients between the ages of 20 and 42 years undergoing monitoring for *in vitro* fertilization-embryo transfer (IVF-ET) were evaluated ultrasonographically for antral follicle number. The antral follicle counts were determined for each ovary by experienced ultra-sonographers at the time of suppression check ultrasonography. It stated that there was no significant difference between right and left antral follicle counts (25).

As part of a prospective study evaluating the three-dimensional ultrasound markers of ovarian reserve, we studied the differences in fertile and infertile females and the differences between the right and the left ovaries in women in each group.

2. Patients and Methods
2.1. Study population
From April 2012 to November 2013, patients were recruited at Department of Obstetrics & Gynecology in Benha University hospital. Women enrolled in the study were divided into study & control groups:

Study group:
One hundred women diagnosed as having infertility (more than 12 months of no conception).

Control group:
One hundred normal fertile women; they all had regular spontaneous menstrual cycles with a baseline transvaginal scan showing normal ovaries.

Exclusion criteria of both groups were:
1. Ovarian follicles ≥ 10mm or ovarian cyst on the baseline scan.
2. Persistent corpus luteum.
3. Previous ovarian surgery and evidence of other pelvic pathology.
4. The use of exogenous hormones in the past 3 months.
5. Presence of endometriomas.
6. Male or coital factor of infertility.
7. Tubal or uterine factor of infertility.
8. Age less than 18 years or more than 35 years.

Informed consent was obtained from all participants. The study protocol was reviewed and approved by the

Medical Ethics Committee for Research Projects of faculty of medicine, Benha University.

2.2. Study protocol

After inclusion, between the third and the fifth day of menstrual cycle, All women had a 3D transvaginal pelvic ultrasound scan where all data were acquired using a GE Voluson 730 pro ultrasound system (GE Healthcare, Zipf, Austria) equipped with 7.5 MHz transvaginal transducer. Identical fixed pre-installed power Doppler ultrasound settings were used in all women: frequency 3–9 MHz, pulse repetition frequency 0.6 kHz, gain –4.0 and wall motion filter 'low 1' (40 Hz at pulse repetition frequency 0.6 kHz). The women were examined in the lithotomy position with an empty bladder in the early follicular phase. The 3D ultrasound probe was introduced into the vagina. Once a satisfactory longitudinal view of the ovary had been obtained, the ovary was centralized within the 3D sector on the screen, and the ultrasound machine was switched into the power Doppler mode. Then, the 3D ultrasound mode was switched on. The woman was asked to remain as still as possible, and a 3D power Doppler data set of the ovary was acquired. The resultant multiplanar display was examined to ensure that the whole ovary had been captured in the volume. Volumes of satisfactory quality and with no artifacts were stored on a hard disk for future analysis. The ultrasound outcome measures included are: antral follicle count (AFC), total ovarian volume (OV) and volumetric ovarian vascular indices measured by power Doppler three dimensional ultrasound: vascularization index (VI), flow index (FI) and vascularization flow index (VFI). SonoAVC follicle™ program was used to asses antral follicle count where the number and the size of every follicle in the ovary was calculated and color coded and stratified in a table. Antral follicles were divided into small antral follicles (2- 6 mm) and larger antral follicles (7-9mm). The virtual organ computer- aided analysis (VOCAL™) imaging program was used to calculate the volume and vascularity indices of both ovaries. The acquired volumes yielded multiplanar views of the ovaries in the mid-sagittal, transverse and coronal planes. All calculations were done on these multiplanar images. The longitudinal view was used as the reference image. The rotation steps were 30 degrees, resulting in the definition of six contours of the ovary. Ovarian contours were manually drawn in all six sections using the computer mouse. Once all contours had been drawn, the total ovarian volume was calculated automatically. Using the histogram facility of VOCAL™ software, three vascular indices were generated: Vascularization index (VI), flow index (FI) and vascularization flow index (VFI).The ultrasonographic and flow data from the right and left sides were evaluated with student t-tests for paired samples.

Application of VOCAL program will be seen in the following figures

Figure (a): The multiplanar view of a polycystic ovary obtained by three dimensional ultrasound with power Doppler angiography (PDA)showing the ovary in the mid-sagittal, transverse and coronal planes.
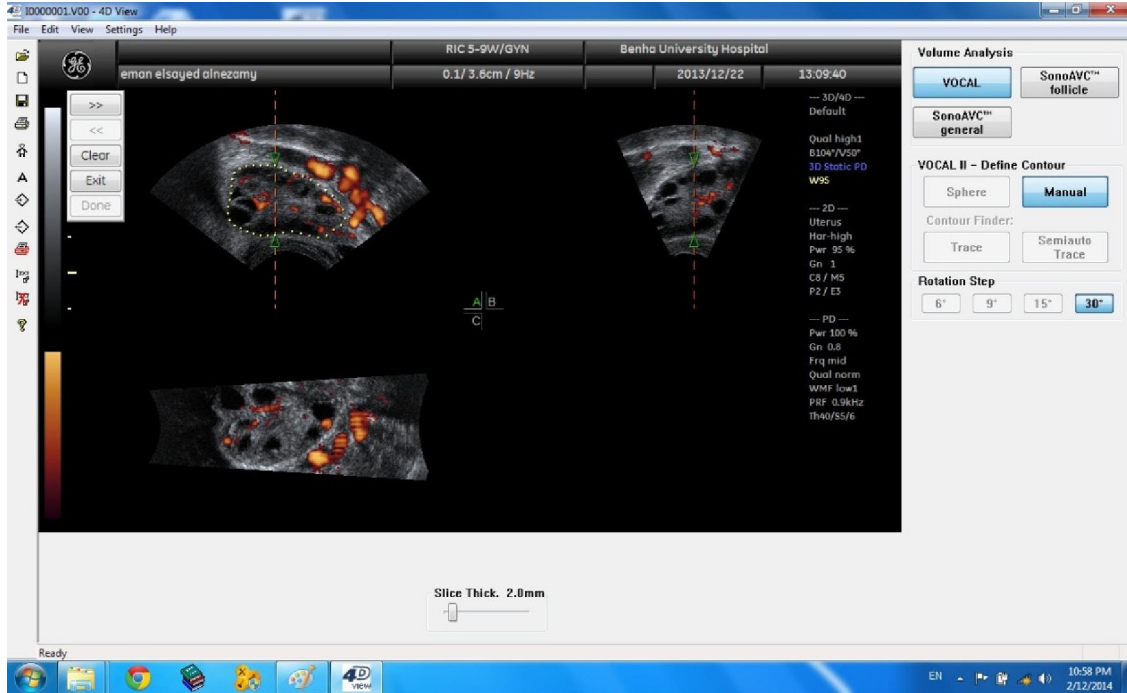
Figure (b): Starting the VOCAL program by selecting the manual method with a rotation step (30°)

Figure (c): The volume of the ovary was calculated automatically after the six contours had been drawn.
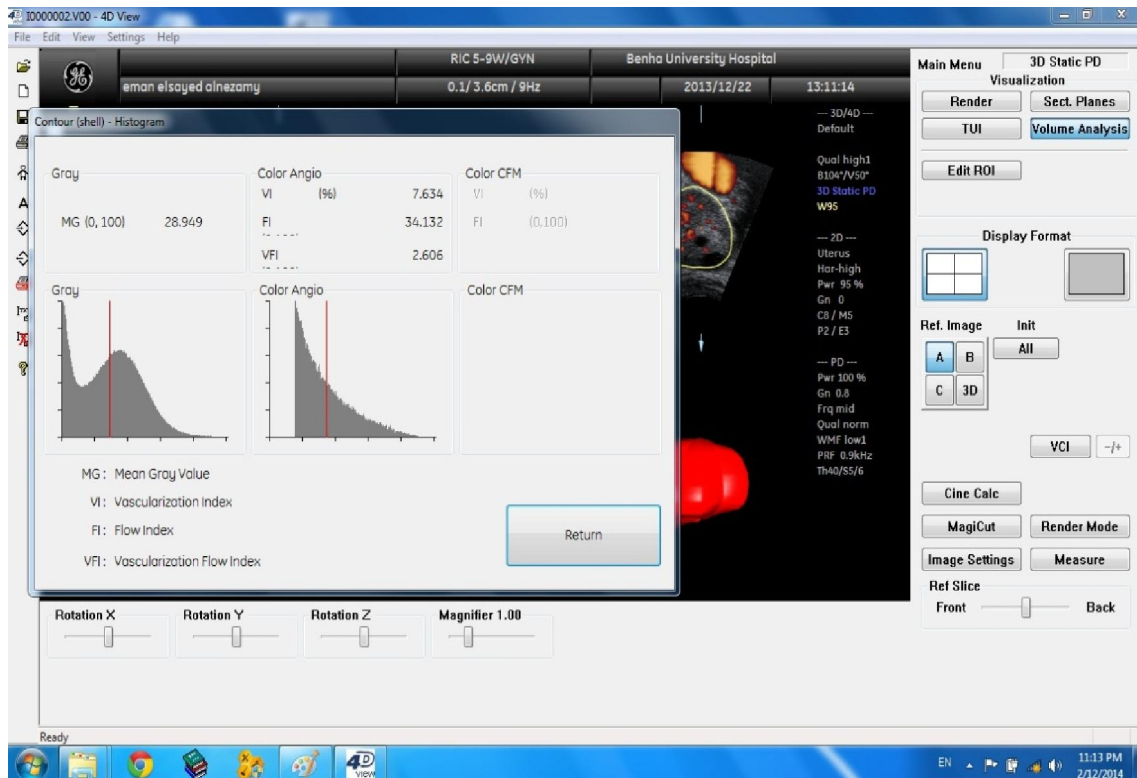
Figure (d): The power Doppler histogram showing the value of the three vascular Indices (VI, FI, VFI).

Figure (e): A multiplanar display of a polycystic ovary using the rendered inversion mode showing the follicles as hypo echoic structures.
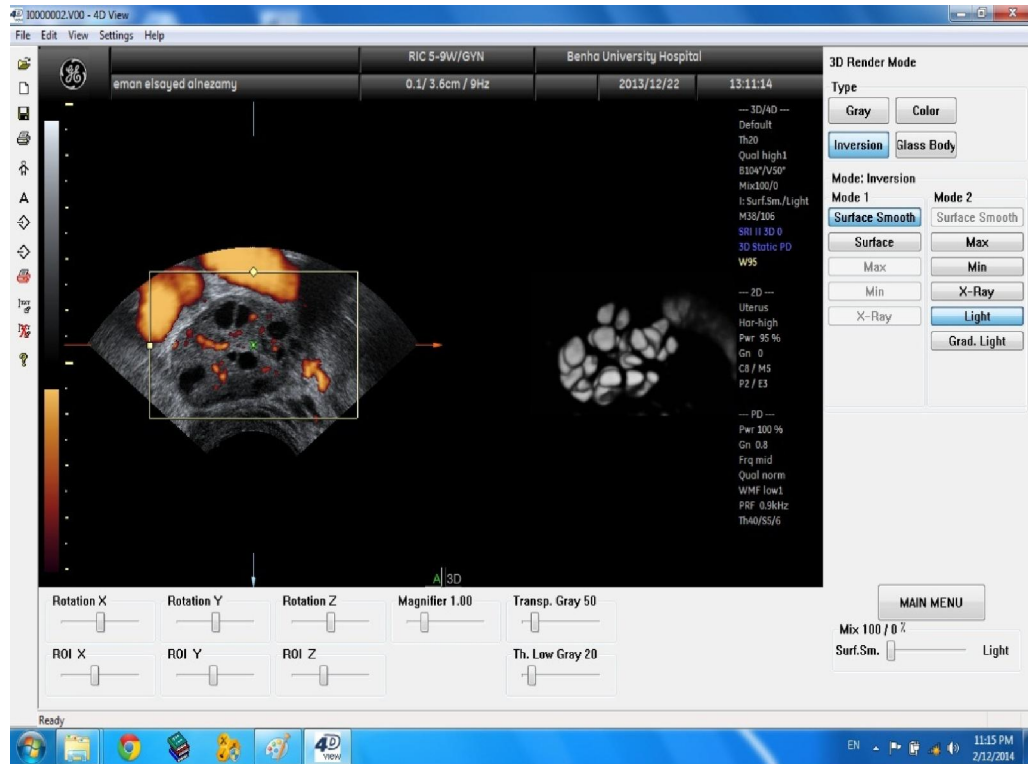
Figure (f): Another display of the same ovary using the rendered inversion mode showing the follicles as hypo echoic structures.
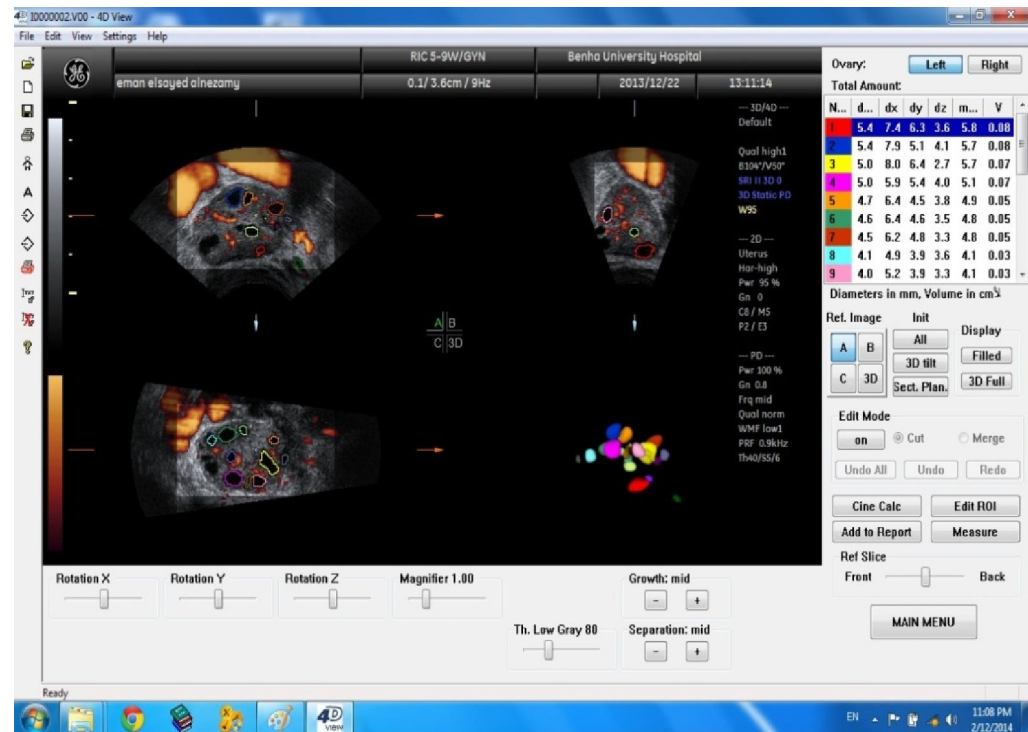

Figure (g): The application of (sonoAVC™) program to obtain follicular volumes. The dimensions as well as the volume of each single follicle is displayed in a table on the right side of the screen and a color coded display in 3D space refers to the measured follicle in the table.

3. Statistical analysis

Data were tabulated and analyzed using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 17 software, suitable statistical techniques were calculated (frequencies, mean, standard deviation and range). Student "t" tests were used as tests of significance. P value (< 0.05) was considered significant.

The following statistical tests were used for analysis of the results.

1. Arithmetic mean (X):
Was calculated as follows:

$$x = \frac{\sum x}{n}$$

X= arithmetic mean.

Σx= Sum of observations

n = number of observations

2. Standard deviation (SD): assess the variation of the observation around the mean.
Was calculated as follows:

$$SD = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

Σx² = sum of squared observations.

(Σx)² = square of the sum of observations.

n = number of observations

3. Student "t" test: analyzes the difference between two proportions from two means of two independent groups.

$$t = \frac{X_1 - X_2}{\sqrt{S_P^2 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}}$$

$$S^2 p = \frac{S_1^2 (n_1 - 1) + S_2^2 (n_2 - 1)}{n_1 - n_2 - 2}$$

Where:

S^2_p = pooled variance.

S^2_1 = Variance of sample (1).

S^2_2 = Variance of sample (2).

n_1 = Size of sample (1).

n_2 = Size of sample (2).

X_1 = Mean of sample (1).

X_2 = Mean of sample (2).

S_1 = Standard deviation of sample (1).

S_2 = Standard deviation of sample (2).

4. **Range**: represents the difference between the highest and the lowest observations.

"P" value: the probability of obtaining results by random chance when the Null hypothesis is true. The result of the test was considered significant when its value was equal to or less than 0.05 (< 0.05).

4. Results and discussion

The study population consisted of study & control groups; study group: One hundred women diagnosed as having infertility (more than 12 months of no conception) and control group: One hundred normal fertile women who had regular spontaneous menstrual cycles with a baseline transvaginal scan showing normal ovaries.

Patient characteristics are shown in *Table (1)*. As regarding the patient characteristics Mean ± SD for age were 24.6 ± 2.6 and 23.8±4.1 for infertile and fertile groups respectively and there was no significant differences in the mean ages between both groups (*P* > 0.05). As regarding BMI, Mean ± SD were 33.1±4.3 and 25.9±2.9 for infertile and fertile groups respectively and the mean BMI of infertile group was extremely significantly higher than the fertile group(*P* value <0.0001).

Table **(1):** Comparison between infertile group and fertile group regarding the age and BMI (Body Mass Index):

Group / Variable	Infertile group	Fertile group	"t"	*P*
Age (years)				
Range	(21-30)	(19-32)		
Mean ± SD	24.6 ± 2.6	23.8±4.1	1.6478	>0.05
BMI (Kg\m²)				
Range	(27-40)	(22-32)	13.8821	<0.0001*
Mean ± SD	33.1±4.3	25.9±2.9		

The mean BMI of infertile group was extremely significantly higher than the fertile group (*P* value <0.0001) but there was no significant differences in the mean ages between both groups (*P* > 0.05).

*SD: standard deviation

- **Ultrasound measurements:**

In Comparing between infertile and fertile groups regarding total Antral Follicle Count (AFC) as measured by 3 D ultrasound (sono AVC™ program), as shown in *Table (2)*, the Mean ± SD for total AFC were 4.570±3.736 and 13.6±4.5 for infertile and

fertile groups respectively and the mean AFC of fertile group was extremely significantly higher than

the infertile group (P value <0.0001).

Table (2): Comparison between infertile and fertile groups regarding Antral Follicle Count (AFC) as measured by 3 D ultrasound (sono AVC program):

Group Variable	infertile group	fertile group	"t"	P
Range(follicle)	(0-14)	(6-15)		
Mean ± SD	4.570±3.736	10.6±4.5	5.4392	<0.0001*

The mean AFC of fertile group was extremely significantly higher than the infertile group (P value <0.0001).

This agrees with a study that was carried out by **Elgindy et al.,**(13) on thirty-three patients undergoing their first intracytoplasmic sperm injection treatment cycle with a long protocol. On day 3 of the menstrual cycle, measurements of AMH, FSH, and LH and ultrasound evaluation of mean ovarian volume and antral follicle count were performed. They concluded that AFC with up to 10 mm in mean diameter is 10.1 ± 3.0 for normal responders and 5.7 ± 1.0 antral follicles, respectively, for poor responders.

This also agrees with a study that was carried out by **Muttukrishna et al.,**(11) where blood samples collected from 108 patients who had ovarian stimulation test for the assessment of their ovarian reserve were assayed. Basal ultrasound scan for AFC was performed on day 3 of the menstrual cycle, followed by a blood test for the assessment of serum FSH, oestradiol (E_2), inhibin β and AMH. Subsequently, 300 IU/L of recombinant FSH (Gonal F; Serono, Welland Garden City, Hertfordshire, UK) was administered. A second blood sample was taken on day 4 for the assessment of E_2 and inhibin β. The rise in E_2 after FSH stimulation (delta E_2: day 4 E_2 minus day 3 E_2) and the rise in inhibin β after FSH stimulation (delta inhibin β: day 4 inhibin β minus day 3 inhibin β) were calculated.

AFC on day 3 has a significant negative association with day 3 FSH ($r=-0.33$, $P= 0.001$) and age (-0.426, $P < 0.001$).

AFC on day 3 has a significant positive correlation with Basal inhibin β (0.29, $P < 0.01$) and AMH (0.487, $P < 0.001$).

AFCs were significantly correlated with delta E_2 ($r= 0.306$, $P= 0.01$), delta inhibin β ($r= 0.4$, $P= 0.001$).

Also that AFC at cut off 5 should allow identification of poor response with 89% sensitivity, in spite of low specificity 39%.

Maseelall et al.,(26)had reached a similar conclusion where they stated that women with AFC ≥ 11 are more likely to obtain a live birth if compared with those with less antral follicles, who should be advised about the increased risks of miscarriage, cycle cancellation, higher doses of gonadotropins, and fewer oocytes yielded.

In Comparing between infertile and fertile groups regarding small (2-6 mm) Antral Follicle Count (AFC) as measured by 3 D ultrasound (sono AVC program),as shown in ***Table (3)***, the Mean ± SD for small AFC were 4.2±5.3 and 7.5±2.2 for infertile and fertile groups respectively and the mean small AFC of fertile group was extremely significantly higher than the infertile group (P value <0.0001).

Table (3): Comparison between infertile and fertile groups regarding small (2- 6 mm) Antral Follicle Count(AFC) as measured by 3 D ultrasound (sonoAVC program):

Group Variable	Infertile group	Fertile group	"t"	P
Range(follicle)	(0-11)	(5-12)		
Mean ± SD	4.2±5.3	7.5±2.2	5.7507	<0.0001*

The mean of the small AFC of fertile group was extremely significantly higher than the infertile group (P value <0.0001).

This agrees with a study that was carried out by**Klinkert et al.,**(16)they stated that special attention has been given to small antral follicles where normal response to stimulus for ART was

significantly elevated for women with AFC≥ 5 with mean diameter up to 5 mm.

In Comparing between infertile and fertile groups regarding larger (7-9 mm) Antral Follicle

Count (AFC) as measured by 3 D ultrasound (sono AVC program), as shown in **Table (4)**, the Mean ± SD for larger AFC were 1.2±1.1 and 1±1.9 for infertile and fertile groups respectively and the difference between the mean of larger (6-9 mm) AFC of infertile group and fertile group was statistically insignificant (*P*value >0.05).

Table (4): Comparison between infertile and fertile groups regarding larger (7-9 mm) Antral Follicle Count (AFC) as measured by 3 D ultrasound (sonoAVC program):

Group \\ Variable	Infertile group	Fertile group	"t"	*P*
Range(follicle)	(0-3)	(1-3)		
Mean ± SD	1.2±1.1	1±1.9	0.9110	>0.05

The difference between the mean of larger (6-9 mm) AFC of infertile group and fertile group was statistically insignificant (*P*value >0.05).

This agrees with a study that was carried out by **Haadsma *et al.*,** (17) where a total of 474 subfertile, ovulatory patients, recruited from two fertility centers in The Netherlands, participated in this prospective cohort study. They found that the number of small follicles (2-6 mm) declined with age; the number of larger follicles (7-10 mm) remained constant. Independent of age, the number of small follicles was significantly related to all ovarian reserve tests (*P*<0.001, except bInhB *P*=0.005). The number of larger follicles was only significantly related to bInhB (*P*=0.009).

They concluded that the number of small antral follicles (2-6 mm) is significantly related to age and also, independent of age, to all endocrine ovarian reserve tests, suggesting that the number of small antral follicles represents the functional ovarian reserve.

In Comparing between infertile and fertile groups regarding the total ovarian volume measured by VOCAL™ program (3 D ultrasound),as shown in **Table (5)**, the Mean ± SD for the total ovarian volume were 15.28±12.11 and 12.6±4.8 for infertile and fertile groups respectively and the mean total ovarian volume of infertile group was significantly higher than the fertile group (*P*value <0.05).

Table (5): Comparison between infertile and fertile groups regarding the total ovarian volume measured by VOCAL program (3 D ultrasound):

Group \\ Variable	Infertile group	Fertile group	"t"	*P*
Range(cm^3)	(2.9-52.5)	(5.4-16.6)		
Mean ± SD	15.28±12.11	12.6±4.8	2.0573	0.0410*

The mean total ovarian volume of infertile group was significantly higher than the fertile group (Pvalue <0.05).

Opinions are divided on considering ovarian volume (OV) as an adequate ovarian reserve test.

Elgindy *et al.*,(13) did not observe OV differences in ART between young women with normal response (means of 4.1 ± 0.66 mL) and poor response (means of 3.36 ± 0.71 mL)

McIlven *et al.*,(19) reached similar results when evaluating women at high risk for cycle cancellation. There was a trend towards ovarian volume measurement being able to predict a poor response, but this trend did not reach statistical significance.

Hendriks *et al.*,(20) had performed a review of ten well-designed studies on ovarian volume for that purposes and concluded that OV presented little applicability in the prediction of poor response or pregnancy. The AFC performed statistically significantly better than ovarian volume in the prediction of poor response.

In contradiction, **Syrop *et al.*,** (27) studied women aged 23–46 years undergoing ART, associating diminished number of oocytes yielded and pregnancy rates with decreased ovarian volumes.

More recently, the study of **Gibreel *et al.*,**(14) observed 92.9% specificity for prediction of no pregnancy and 91.7% specificity for prediction of cycle cancellation with a 3.0 mL cutoff value for OV.

Finally, significant correlations had been previously found between reduced ovarian measures, increased age, and elevated serum FSH (28).

We believe that the higher mean OV for the infertile than the fertile group in our study was a result of cases of polycystic ovary syndrome that was not excluded in our study where the stromal volume

of the ovary is higher than cases of normal females even other infertile female ovaries.

This in agreement with **Lam *et al.*,**(29) who had a study including 40 women with PCOS and 40 subfertile women without PCOS, undergoing treatment cycles of intrauterine insemination(IUI) and\or ovulation induction for infertility due to male factor or unexplained infertility, the stromal volume was larger in PCOS group than the control group (mean ± SD of the PCOS group was 10.79± 2.94 cm3, while the mean ± SD of the control group was 4.69± 1.84 cm3) which was statistically highly significant ($P<0.001$).

Thus, OV alone should not be considered as a predictor of ovarian reserve, but because of its easy performance, it may be included as a routine in diagnostic procedures, adding information to the patient medical records and providing data for further study (18).

In Comparing between infertile and fertile groups regarding Vascularization Index (VI) measured by using Three-Dimensional power Doppler ultrasonography, as shown in ***Table (6)***, the Mean ± SD for the VI were 8.889±5.962 and 15.54±8.87 for infertile and fertile groups respectively and the mean Vascularization index was higher among cases of fertile compared to infertile group with an extremely statistical significant difference in between them ($P<0.0001$).

Table (6): Comparison between infertile and fertile groups regardingVascularization Index (VI) measured by using Three-Dimensional power Doppler ultrasonography:

Group / Variable	Infertile group	Fertile group	"t"	P
Range (%)	(0.134-24.240)	(0.66-22.68)		
Mean ± SD	8.889±5.962	15.54±8.87	6.2232	0.0001*

The mean Vascularization index was higher among cases of fertile compared to infertile group with an extremely statistical significant difference in between them ($P<0.0001$).

In Comparing between infertile and fertile groups regarding Flow Index (FI) measured by using Three-Dimensional power Doppler ultrasonography, as shown in ***Table (7)***, the Mean ± SD for the VI were 32.058±5.144 and 30.85±8.69 for infertile and fertile groups respectively and the difference of the mean flow index was statistically non-significant among cases of infertile compared to fertile group ($P >0.05$).

Table (7): Comparison between infertile and fertile groups regarding Flow Index (FI) measured by using Three-Dimensional power Doppler ultrasonography:

Group / Variable	Infertile group	Fertile group	"t"	P
Range	(18.074 -42.383)	(20.23-49.83)		
Mean ± SD	32.058±5.144	30.85±8.69	1.1962	0.2330(NS)

The difference of the mean flow index was statistically non-significant among cases of infertile compared to fertile group ($P>0.05$).

In Comparing between infertile and fertile groups regarding Vascularization Flow Index (VFI) measured by using Three-Dimensional power Doppler ultrasonography, as shown in **Table (8)**,the Mean ± SD for the VFI were 2.51±1.04 and 1.19±0.72 for infertile and fertile groups respectively and the mean Vascularization Flow index was higher among cases of fertile compared to infertile group with an extremely statistical significant difference in between them ($P<0.0001$).

Table (8): Comparison between infertile and fertile groups regarding Vascularization Flow Index (VFI) measured by using Three-Dimensional power Doppler ultrasonography:

Group / Variable	Infertile group	Fertile group	"t"	P
Range	(0.85-4.12)	(0.33-2.44)		
Mean ± SD	2.51±1.04	1.19±0.72	10.4355	<0.0001*

The mean Vascularization Flow index was higher among cases of fertile compared to infertile group with an extremely statistical significant difference in between them (P<0.0001).

Similar results were reached by **Jarvela *et al.,*** (24)they used three-dimensional power Doppler angiography after pituitary 'down-regulation' and during gonadotrophin stimulation to compare ovarian vascularity in 33 women with normal ovarian reserve, as judged by antral follicle counts, to 12 women who had demonstrated a previous poor response. All three indices of vascularity were shown to increase significantly during gonadotrophin stimulation in the group with normal ovarian reserve only but this was related to the antral follicle count, but the number of oocytes retrieved had not correlated with ovarian vascularity. Lowering the importance of this marker (ovarian vascularity) as an independent variable.

Similarly **Gibreel *et al.,***(14) conducted a systematic review of studies evaluating the diagnostic accuracy of all the ultrasound based tests of ovarian reserve, including antral follicle count (AFC), ovarian volume and stromal blood flow in predicting fertility outcomes and, where appropriate, performed a meta-analysis to determine the predictive value at each cut-off value described in the literature. They claimed that the clinical value of Doppler studies for ovarian stromal blood flow was unclear. This is why ovarian vascular flow may not be used to determine inclusion of infertile couples in ART programs or to estimate its results.

In Comparing between the right (RT) and the left (LT) ovaries in the infertile group:

Regarding all three dimensional ultrasound markers of the ovarian reserve,as shown in **Table (9)**, the Mean ± SD for the AFC were 2.54±1.7 and 3.2±1.4 for RT and LT ovaries respectively and the Mean ± SD for the FI were 30.088±6.146 and 34.058±5.844 for RT and LT ovaries respectively and the Mean ± SD for the VFI were 2.28±1.34 and 2.81±1.12 for RT and LT ovaries respectively, all of them show a statistical significant difference in between the RT and the LT ovaries.

While the Mean ± SD for the ovarian volume were 8.26±7.22 and 7.2±5.43 for RT and LT ovaries respectively and the Mean ± SD for the VI were 8.224±6.012 and 9.324±5.634 for RT and LT ovaries respectively and both of them show a statistical non-significant difference in between the RT and the LT ovaries in the infertile group.

Table (9): Comparison between the right (RT) and the left (LT) ovaries in the infertile group regarding all three dimensional ultrasound markers of the ovarian reserve.

GROUP	Infertile group			
Side	RT OVARY	LT OVARY		
variable	Mean ± SD	Mean ± SD	"t"	*P*
AFC	2.54±1.7	3.2±1.4	2.9969	0.0031*
Ovarian volume OV	8.26±7.22	7.2±5.43	1.1733	>0.05
VI	8.224±6.012	9.324±5.634	1.3351	>0.05
FI	30.088±6.146	34.058±5.844	4.6811	<0.0001*
VFI	2.28±1.34	2.81±1.12	3.0348	0.0027*

In Comparing between the right (RT) and the left (LT) ovaries in the fertile group:

Regarding all three dimensional ultrasound markers of the ovarian reserve, as shown in **Table (10)**,the Mean ± SD for the AFC were 6.8±2.4 and 6.1±2.8 for RT and LT ovaries respectively and the Mean ± SD for the total ovarian volume were 6.2±3.2 and 5.4±4.2 for RT and LT ovaries respectively and the Mean ± SD for the VI were 14.54±8.41 and 15.98±7.87 for RT and LT ovaries respectively and the Mean ± SD for the FI were 29.85±8.69 and 31.65±8.65 for RT and LT ovaries respectively and the Mean ± SD for the VFI were 1.14±0.52 and 1.28±0.78 for RT and LT ovaries respectively and all of them show a statistical non-significant difference in between the RT and the LT ovaries in the fertile group.

Table (10): Comparison between the right (RT) and the left (LT) ovaries in the fertile group regarding all three dimensional ultrasound markers of the ovarian reserve.

GROUP	Fertile group			
Side	RT OVARY	LT OVARY		
variable	Mean ± SD	Mean ± SD	"t"	*P*
AFC	6.8±2.4	6.1±2.8	1.8981	>0.05
Ovarian volume (OV)	6.2±3.2	5.4±4.2	1.5151	>0.05
VI	14.54±8.41	15.98±7.87	1.2502	>0.05
FI	29.85±8.69	31.65±8.65	1.4680	>0.05
VFI	1.14±0.52	1.28±0.78	1.4934	>0.05

No much studies compared between the ovaries on either side but we have found only two studies that tried to do so.

Deb *et al.,*(6) have evaluated differences in the three-dimensional (3D) ultrasound markers of ovarian reserve between the ovaries within an individual undergoing investigation for subfertility. Two hundred seventy women undergoing three-dimensional ultrasound scan in early follicular phase between days 2 and 5 of the menstrual cycle. Variations in 3D ultrasound markers of ovarian reserve between the two ovaries within same individual were estimated. Two hundred fifteen subjects were analyzed for ovarian volume and antral follicle count, and 205 subjects for 3D power Doppler indices.

Significant differences were noted (median, range) in the AFC especially seen in follicles measuring more than 6.0 mm (similar to our study).

Significant differences were noted (median, range) in ovarian volume (in contrast to our study).

Significant correlation was noted between the two ovaries in 3D power Doppler indices (similar to our study regarding the fertile not the infertile groups).

The difference between this study and ours may be due to that it only included sub fertile females and to a different sample size.

Earlier, Chow *et al.,*(25) had a study to estimate side-to-side variation in antral follicle counts. Forty-one patients between the ages of 20 and 42 years undergoing monitoring for in vitro fertilization-embryo transfer (IVF-ET) were evaluated ultrasonographically for antral follicle number. The antral follicle counts were determined for each ovary by experienced ultra-sonographers at the time of suppression check ultrasonography.

They stated that there was no significant difference between right and left antral follicle counts (P =.30).

This different result that was concluded by them can be explained by that they used a smaller sample size (41 infertile cases only including cases of male and other factors of infertility as indications for IVF-ET) and the technique they had depended on was two dimension ultrasound not 3D sono AVC program that we depend on, this software can identify and quantify the total number of antral follicles in an unstimulated ovary and is more reliable than manual two- and three-dimensional ultrasound techniques in this respect(30)**.**

5. Conclusions and Recommendations

Three-dimensional ultrasonography is an imaging modality that can be used as a complementary method for other endocrine markers for the assessment of ovarian reserve. It allows excellent evaluation of the ovaries with direct quantitative estimation of antral follicle count, ovarian volume and with power Doppler angiography; ovarian vascularity can also be assessed with the help of 4D view program including sonoAVC™ and VOCAL™ programs.

Antral follicle count especially small (≤6 mm) follicles are the best ultrasound marker of the ovarian reserve.

Ovarian volume alone should not be considered as a predictor of ovarian reserve, but because of its easy performance, it may be included as a routine in diagnostic procedures, adding information to the patient medical records and providing data for further study.

Evaluation of the ovarian stromal vascularity by 3 D power Doppler ultrasound may be used as an ultrasound marker of ovarian reserve but further research is needed.

Evaluation of the right and the left ovaries separately is recommended in every patient to overcome the possibility of the existence of a significant difference between them but further studies are needed to confirm that.

6. Conflict of interest
None.

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