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Recent Dynamic Ovarian Reserve Modalities

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ABSTRACT

Background: the pool of primordial follicles in the ovary or ovarian reserve is a major factor in the human fertility potential. The ageing ovary is characterized by reduction of the number of primordial follicles and this loss accelerates in the late 30's and precedes the menopause by 10-12 years. Woman's age alone or with combination of biochemical markers, dynamic tests and ultrasound measurement fail to predict this loss accurately. **Aim of the Work:** this study aimed to assess the recent dynamic ovarian reserve tests including antral follicle count, ovarian volume and ovarian blood flow (OBF) as a predictive value and less cheap method for evaluation of ovarian reserve. **Patients and Methods:** this randomized controlled trial was conducted on 200 women having unexplained infertility in outpatient clinic at Sohag General Hospital and private clinics during the period from 2016 to 2017 after taking a verbal consent from each case after explanation of the purpose of the study was taken. **Results:** mean baseline ovarian volume in cycle day 3 of infertility group was 15.28±12.1, while it was 12.6±4.8 in the control group. It also showed that the mean baseline total antral follicle count in cycle day 3 of infertility group was 5.98±1.74. While, it was 10.1±2.5 in the control group. The mean baseline resistance index of ovarian arteries of infertility group was 0.56±0.12. While, it was 0.47±0.058 in the control group. It also showed that the mean baseline pulsatility index of ovarian artery of infertility group was 0.75±0.15, while it was 0.9±0.26 in the control group. The differences between the two groups were significant regarding baseline total AFC, baseline OV and the rest of variables. It was even found that AFC compared to other predictors was considered the strongest predictor. **Conclusion:** antral follicular count and the mean ovarian volume measured through the transvaginal ultrasonography is a non-invasive method and easy to perform and can be considered as an accurate method for assessment of the ovarian reserve. Ovarian stromal blood flow may also become one of the parameters. **Recommendations:** perform transvaginal ultrasound at third day of the cycle in women undergoing infertility treatment is a good predictor for ovarian reserve. Early evaluation of ovarian reserve is very important step in the infertility work up especially in infertile women above the age of 35 years, women with unexplained infertility and women with poor response to ovulation induction.

Keywords: dynamic reserve modalities, antral follicle count, ovarian volume, ovarian blood flow.

INTRODUCTION

Considering modern trends of maternity postponement and the increasing demand for assisted reproduction technologies (ART), the evaluation of functional ovarian reserve has arisen to better advise interested couples, helping physicians in the inference of follicular response and success rates and guiding the elaboration of individualized stimulation protocols, with a reduction of emotional and financial burdens of hard and stressful therapeutic processes. In this context, the identification of women with a lower reproductive potential is a great challenge for reproductive medicine specialists [(1)].

Sharara and Scott [(2)] emphasized that an ideal ovarian reserve parameter should be easily measurable, minimally invasive, inexpensive and should have good predictive values [(1)].

Serum and ultrasonographic markers have been tested to infer the gonadal reserve of infertile women, but none of them has been proven to confidentially reflect the complex follicular dynamics or to be strongly correlated with the size and/or quality of primordial follicles remaining in the gonads after each wave of follicular growth. In other words, those tests do not ideally reflect the pool of unrecruited follicles, which may be responsible for the continuity of ovulatory cycles and therefore, for the long-term reproductive potential. The most commonly used tests for the evaluation of ovarian reserve in infertile women were divided into static (endocrine and ultrasonographic tests performed in the early follicular phase) and dynamic (endocrine tests assessing ovarian response to exogenous gonadotropic stimulus) [(1)].

Ovarian reserve assesses the quality and quantity of remaining oocytes in an attempt of predicts the reproductive potential [(3)].Ovarian reserve is done to identify those individuals who are at risk of decreased or diminished ovarian reserve as women older than 35 years old who have not conceived after 6 months of attempting pregnancy, women with history of cancer were treated by gonadotoxic therapy, pelvic irradiation or ovarian surgery for endometriomas [(4)].

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Available tests of ovarian reserve include biochemical markers (Basal follicle stimulating hormone, estradiol, anti-Mullerian hormone, clomiphene citrate challenge test, inhibin B and ovarian ultrasound imaging (Antral follicle count, ovarian volume and ovarian Doppler) [(5)]. It has been suggested that ovarian blood flow may play a crucial role in the development of ovarian follicles. Better stromal blood flow may lead to greater delivery of gonadotropins to the granulosa cells, while the perifollicular blood flow may be capable of influencing and mediating oocyte maturation, its potential ability to be fertilized and develop, as well as oocyte quality [(6)]. Ovarian blood flow (OBF) has been extensively assessed in natural and stimulated reproductive cycles, a study of **Shrestha *et al.*** [(7)] demonstrated high pregnancy rate among women who presented with highly vascularized follicles in early follicular phase. Despite the foregoing, a recent meta-analysis assessed OBF as a predictor of IVF outcomes, but clinical value was unclear, because of different flow-derived predictors used in literature. Therefore, ovarian vascular flow may not be used to determine inclusion of infertile couples in ART programs or to infer its results [(1)].

Aim of the study: this study aimed to assess the recent dynamic ovarian reserve tests including antral follicle count, ovarian volume and ovarian blood flow (OBF) as a predictive value and less cheap method for evaluation of ovarian reserve.

PATIENTS AND METHODS

Study Design

Randomized controlled trial.

Subjects

Women having unexplained infertility.

Setting

The study was performed in outpatient clinic at Sohag General Hospital and private clinics during the period from 2016 to 2017.

Selection of the patients

The study was done on 200 cases who were selected from the out-clinic infertility department. A verbal consent was obtained from each case after explanation of the purpose of the study will be taken.

Inclusion criteria

The cases have the following inclusion criteria:

-With unexplained infertility.
-Age: 22-37 years old.
-BMI: 18-25.
-Normal baseline LH and FSH levels [(6)].

Exclusion criteria

The cases have the following exclusion criteria:

-Present Family and History of chronic medical diseases.
-Age less than 22 and more than 37 years old.
-History of ovarian surgery or chemotherapy.

The cases in the first visit were subjected to:

-Careful history taking.
-Careful clinical examination.
-BMI to detect underweight and obesity.
-Routine laboratory investigation (Hemoglobin level, blood sugar, urine analysis…).
-Regular blood pressure measurement.
-Notification of each studied case in individual clinical sheet.

Grouping of the cases

The studied cases were divided into control and case groups:

Group (1): Control group (100 cases) were normal and did not have an infertility problem.

Group (2): Case group (100 cases) had an unexplained infertility.

Patients monitoring

Ultrasonographic assessment (Antral Follicle Count, Ovarian volume, Ovarian Doppler).

- All transvaginal sonographic examinations were performed by the same experienced operator using a 9.5 MHz probe for B – Mode and color imaging as well as pulsed Doppler spectral analysis.
- All subjects underwent a baseline transvaginal sonography on day 2 or day 3 of the cycle and morphology of uterus and both ovaries was noted.
- The ovarian stromal blood flow was also recorded in the form of Resistance Index (RI) and Pulsatility Index (PI).

Technique of Transvaginal ultrasound

Then transvaginal ultrasonography was done using a 9.5 MHz probe for B – Mode. Each lady must evacuate the urinary bladder, and lay in lithotomy position where transvaginal ultrasound was done. Localization of each ovary done in longitudinal section adjacent to iliac vessels lateral to uterus.

Ovarian Volume

The volume of each ovary was calculated by measuring the three perpendicular diameters (longitudinal, anteroposterior and transverse diameter) and applying the formula for ovarian Volume=D1×D2×D3×0.523 where D1×D2 and D3 represent maximal longitudinal, anteroposterior and transverse diameters

respectively. Mean ovarian volume was the reference value calculated in this study [8].

Antral follicle count

After localization of both ovaries, any round or oval sonolucent structures in the ovaries were regarded as follicles. Follicles measuring smaller than 10mm were counted from lateral to medial margins of the ovary to determine the antral follicle count of each ovary. The total antral follicle count in both ovaries was calculated.

Ovarian stromal blood flow

After visualization of pelvic anatomy by B-mode, the equipment was switched to color Doppler mode to locate blood flow in normal or newly formed pelvic vessels. The color flow of interest explored with Doppler sample volume until the typical spectral waveform was seen. Awareness of normal vascular anatomy in the pelvis is essential for evaluation of the ovarian arteries [9]. The peak systolic and end diastolic frequency recorded and A/B ratio, resistance index (RI), or pulsatility index (PI) calculated.

Table 1: showing information date form

1- Name
2- Hospital number
3- Age
4- Weight
5- Height
6- BMI
7- Duration of infertility
8- Previous medical treatment
9- Baseline mean ovarian volume in cycles day 3
10- Baseline total antral follicle count in cycle day 3
11- Baseline mean resistance index of ovarian arteries
12- Baseline mean pulsatility index of ovarian arteries

The study was approved by the Ethics Board of Al-Azhar University.

RESULTS

Table 2: demographic data of the studied groups

	Group	Mean	SD	t Test	P value
Age	Infertility Group	27.9	3.84	0.662	0.509
	Control Group	27.6	3.85		
Height	Infertility Group	158.6	4.13	0.219	0.827
	Control Group	158.5	4.26		

The above table showed that the mean age of infertility group was 27.9 ± 3.84, while it was 27.6 ± 3.85 in the control group. The table showed that the mean height of infertility group was 158.6 ± 4.13, while it was 158.5 ± 4.26 in the control group.

Table 3: demographic distribution of weight

	Mean	SD	t Test	P value
Infertile group	66.6	9.79	0.533	**<0.001**
Control group	60.4	6.31		

This table showed that the mean weight of infertility group is 66.6 ± 9.79, while it was 60.4±6.31 in the control group.

Table 4: demographic distribution of BMI

	Mean	SD	t Test	P value
Infertile group	26.6	4.04	5.097	**<0.001**
Control group	24.05	2.8		

This table showed that the mean BMI of infertility group was 24.05 ± 2.8, while it was 26.6 ± 4.04 in the control group.

Table 5: baseline mean ovarian volume in cycle day 3

	Mean	SD	t Test	P value
Infertile group	15.28	12.1	2.059	0.041
Control group	12.6	4.8		

This table showed that the mean baseline mean ovarian volume in cycles day 3 of infertility group was 15.28 ± 12.1, while it was 12.6 ± 4.8 in the control group.

Table 6: baseline mean antral follicle count in cycle day 3

	Mean	SD	t Test	P value
Infertile group	5.98	1.74	13.43	**<0.001**
Control group	10.1	2.5		

This table showed that the mean baseline total antral follicle count in cycle day 3 of infertility group was 5.98 ± 1.74, while it was 10.1 ± 2.5 in the control group.

Table 7: baseline mean resistance index of ovarian arteries

	Mean	**SD**	**t Test**	**P value**
Infertile group	0.56	0.12	6.37	**<0.001**
Control group	0.47	0.058		

This table showed that the mean baseline resistance index of ovarian arteries of infertility group was 0.56 ± 0.12, while it was 0.47 ± 0.058 in the control group.

Table 8: baseline mean pulsatility index of ovarian arteries

	Mean	SD	t Test	P value
Infertile group	0.75	0.15	4.997	**<0.001**
Control group	0.9	0.26		

This table showed that the mean baseline pulsatility index of ovarian artery of infertility group was 0.75 ± 0.15, while it was 0.9 ±0.26 in the control group**.**

Table 9: previous medication for infertility

		Group		**Total**
		Infertility Group	**Control Group**	
Previous medical treatment for infertility	No	39 19.5%	100 50%	139 69.5%
	Yes	61 30.5	0	61 30.5%
Total		100 50%	100 50%	200 100%

Chi square = 87.77, p value = **0.001 (S)**

This table showed that the 2/3 of cases received medical treatment for infertility and there was a significant difference between both groups.

DISCUSSION

Delayed childbearing, voluntary or involuntary, is a common feature in couples visiting fertility clinics. Majority of the fertility clinics perform ovarian reserve tests (ORTs) as a part of the evaluation of women with infertility prior to *in vitro* fertilization. Diminishing ovarian reserve is a phenomenon noted in women during mid to late thirties and at times earlier, reflecting the declining follicular pool and oocyte quality [(10)]. This age-related decline of follicles in the human ovary is believed to more than double when numbers fall below a critical figure of 25,000 at ~37.5 years of age [(11)]. Assuming fixed time differences between reproductive milestones, fertility will not be lost completely for 4 years, on average, following the onset of this phase [(12)]. ORTs provide an indirect estimate of a woman's remaining follicular pool. An ideal ORT should be easy to perform, reproducible and the decisions based on their results should help differentiate women with a normal and poor ovarian response. This should in turn help identify and counsel couples with negligible chance of conception against any expensive and repeated treatment. However, the availability of multiple ovarian reserve markers suggests that none was ideal [(13)]. Their role in the assessment of ovarian reserve in subfertile women not necessarily undergoing IVF or in general population, to identify those at the risk of diminished ovarian reserve, is still poorly understood [(14)].

The initial evidence suggested that various ORTs have a good predictive value for pregnancy [(15)]. However, in the recent years it has been understood that these tests were effective in predicting the ovarian response to stimulation and

not for the prediction of pregnancy or its outcome [16].

Aim of our study was assessment of recent dynamic ovarian reserve tests including antral follicle count, ovarian volume and ovarian blood flow (OBF) as a predictive value and less cheap method for evaluation of ovarian reserve. In our study, the mean age of the infertility group was 27.9±3.84 years, while it was 27.6±3.85 years in the control group , this was similar to study done by **Islam *et al.*** [17] as they found that there was no significant difference between the age among 2 groups. Also, results of this study showed that the mean weight of infertility group was 66.6±9.79 Kg compared to 60.4±6.31 Kg in the control group. The mean height of infertility group was 158.6±4.13 cm,while it was 158.5±4.26 cm in the control group. Finally, the mean BMI of infertility group was 26.6±4.04, while it was 24.05±2.8 in the control group. There were significant differences between the two groups regarding the demographics of the participants regarding weight and BMI.

More recent studies showed that ovarian volume despite correlating with other predictors is not better than them. So, ovarian volume alone should not be considered as a predictor of ovarian reserve, but because of its easy performance, and no added cost to the routine ultrasound scan, it may be included as a routine in the initial diagnostic investigations for infertility patients. These results are in line with various studies that showed its insignificant role [1,18,19].

In this study we demonstrated that mean baseline mean ovarian volume in cycles day 3 of infertility group was 15.28±12.1, while it was 12.6±4.8 in the control group. It also showed that the mean baseline total antral follicle count in cycle day 3 of infertility group was 5.98±1.74, while it was 10.1±2.5 in the control group. The mean baseline resistance index of ovarian arteries of infertility group was 0.56±0.12, while it was 0.47±0.058 in the control group. It also showed that the mean baseline pulsatility index of ovarian artery of infertility group was 0.75±0.15, while it was 0.9±0.26 in the control group. The differences between the two groups were significant regarding baseline ovarian volume and highly significant regarding the rest of variables. The 2/3 of cases received medical treatment for infertility and there was a significant difference between the both groups. In studies of **Mutlu *et al***. [20], **He *et al***. [21] it was found that total antral follicle count (AFC) being increased in good responders indicated that it was a good predictor of ovarian reserve. It was even found that AFC compared to other predictors was considered the strongest predictor. This was agreed upon by other studies attributing this to AFC represents the follicle cohort in the ovaries and it was accepted as a direct marker of the recruitable follicular cohort.

A retrospective analysis was performed of two prospective studies consisting of 465 an ovulatory patients undergoing ovulation induction. Baseline ovarian volume was assessed on day 2 to 5 and data on ovarian response to stimulation, ovulation, cancellation rate, pregnancy rates and hyperstimulation syndrome were collected. The authors concluded that medium-to-large sized ovaries were at a higher risk of ovarian hyperstimulation than smaller ovaries during ovulation induction by gonadotropins. Women with small ovaries (OV <7.25 cm3) had a probability of conceiving that was equal to that of the women with large ovaries (mean ovarian volume x=11.55±6.0 cm3) [22,23,24].

In another meta-analysis, 11 studies on AFC were compared to 32 studies on basal FSH. Because P values for both the AFC and the basal FSH were less than 0.001, their homogeneity was rejected. For this reason, the evaluation of the summary point estimate for both sensitivity and specificity was found to be meaningless. Logistic regression analysis found no study to be statistically significant. However, current evidence suggested that the ability of AFC to predict poor ovarian response was high and its ability to predict nonpregnancy was low. Antral follicle count might be considered the test of choice in predicting ovarian reserve before IVF as it was easy to perform, was noninvasive and has a better predictive value than basal FSH [25].

Seventy-one women with median age of 36 years were included in a retrospective study of **Lorusso *et al***. [26] with strict exclusion criteria. The authors summarized that AFC can efficiently predict a woman's response to ovarian stimulation by determining the total number of oocytes retrieved and the number of mature oocytes. For this reason, AFC proved to be good marker of ovarian reserve before IVF, particularly in older patients. In a prospective study that included 110 patients between the ages of 18 and 39 years with regular menstrual cycles, the number of antral follicles was compared with other techniques for estimating ovarian reserve to assess ovarian hyperstimulation during IVF treatment. The AFC was the best predictor [19].

A meta-analysis included 10 studies looking at ovarian volume and 17 studies looking at antral follicle count showed that due to significant

heterogeneity the sensitivity and specificity of these studies were inaccurate. However, the authors concluded that the antral follicle count was a better predictor of ovarian reserve compared to the measurement of ovarian volume via ultrasound [(27)]. One of the major challenges with ultrasonography is reproducibility. A study looking at 29 women revealed that three-dimensional ultrasonography and power Doppler angiography created excellent intra and inter observer reproducibility. In this study, the authors mainly evaluated the assessment of ovarian response and oocyte quality. The results revealed that the coefficients for both groups were close to unity in the ovarian volume category, and 0.964 for intra observer and 0.978 for inter observer in reference to the antral follicle count measurements [(28)].

CONCLUSION

Antral follicular count and the mean ovarian volume measured through the transvaginal ultrasonography is a non-invasive method and easy to perform and can be considered as an accurate method for assessment of the ovarian reserve. Ovarian stromal blood flow may also become one of the parameters.

RECOMMENDATIONS

Perform transvaginal ultrasound at third day of the cycle in women undergoing infertility treatment is a good predictor for ovarian reserve. Early evaluation of ovarian reserve is very important step in the infertility work up especially in infertile women above the age of 35 years, women with unexplained infertility and women with poor response to ovulation induction.

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