Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Clinical Research

J. Exp. Clin. Med., 2018; 35(3): 57-61
doi: 10.5835/jecm.omu.35.03.001

Changes in antral follicle count and ovarian volume with age

Aysegul Kaya, Arif Kokcu, Emel Kurtoglu Ozdes*, Tayfun Alper, Mehmet Bilge Cetinkaya

Department of Obstetrics and Gynecology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey

ARTICLE INFO

Article History
Received 16 / 06 / 2016
Accepted 20 / 12 / 2016
Online Published Date 31 / 10 / 2019

*** Correspondence to:**
Emel Kurtoglu Ozdes
Department of Obstetrics and Gynecology,
Faculty of Medicine,
Ondokuz Mayis University,
Samsun, Turkey
e-mail: emel0022@mynet.com

Keywords:
Age
Ovarian reserve
Ovarian volume
Total antral follicle

ABSTRACT

We aimed to ascertain the chronologic age at which these sonographic measurements begin to show a significant difference. The study included 100 women (78, aged 19-44 and 22, aged 45-50) menstruated spontaneously and without thyroid disease, diabetes mellitus, hyperprolactinaemia, hypogonadotropic hypogonadism or polycystic ovary syndrome and any gynaecologic surgery. They all underwent sonographic measurement of total antral follicle (TAF) count and mean ovarian volume (MOV) on the second or third days of menstrual cycle. Then, the women were assigned to 5 groups: group 1, 19–24 years; group 2, 25–29 years; group 3, 30–37 years; group 4, 38-44 years; and group 5, 45-50 years. There was a significant association between chronological age and TAF number ($r^2 = 0.328$, $p < 0.001$). No significant difference was noted in TAF number among the first three groups ($r^2 = 0.58$, $p > 0.05$). TAF number was significantly different between groups 3 and 4 (Z: -3.463, $p < 0.001$), but not between groups 4 and 5 (Z: -1.698, $p > 0.05$). There was a significant relationship between chronological age and MOV ($r^2 = 0.149$, $p < 0.001$). MOV values did not show a significant difference among the first 4 groups ($r^2 = 0.58$, $p > 0.05$; $r^2 = 7.87$, $p > 0.05$ respectively). The MOV of group 5 was significantly different from those of groups 1, 2, 3, and 4 (Z: -2.75, $p < 0.01$; Z: -4.351, $p < 0.01$; Z: -2.722, $p < 0.01$; and Z: -2.829, $p < 0.01$ respectively). In conclusion, the TAF count decreases significantly beginning at 38 years of age, while MOV decreases significantly beginning at 45 years of age. Thus, there is no significant decrease in the ovarian follicular reserve until the age of 38 years, or in the ovarian volume until the age of 45 years.

1. Introduction

Oocyte numbers progressively reduce during the reproductive years by ovulation and atresia. Therefore, concentration of follicles in women > 35 years is only one-third of that in younger women. At a mean age of 45 years, menstrual cyles become irregular and the number of wasting follicles decrease below a few thousand and then 1000 at menopause (Lass et al., 1997; Johnson et al., 2006; Kwee et al., 2007; Hansen et al., 2011).

A mathematical linear model has been developed that predicts the rate of follicular decline with advancing age. A biexponential decline is noted in follicular depletion, with an acceleration in oocyte loss when the remaining oocyte number equals approximately 25.000. In this model, the decline starts at the age of 37 years and menopause occurs in approximately 10 years. At this point, the rate of follicular atresia accelerates. The number of follicles diminishes to a few thousand at approximately 45 years of age and menopause, the median age of which is 51 years in western countries, occurs when the number reaches 1000 follicles. This number is reported to be the critical threshold for maintaining the menstrual cycles (Faddy et al., 1992; Johnson et al., 2006; Kwee et al., 2007;Cedars and Evans, 2008; Hansen et al., 2011).

Antral follicle (AF) count and mean ovarian volume (MOV) measurement by ultrasound are frequently used to estimate ovarian reserve. AF count has been reported to be the most reliable sonographic test for estimating the primordial follicle cohort (Domingues et al., 2010; Hsu et al., 2011). It is better to interprete these tests in conjunction with chronological age of the women in order to identify the group who have diminished ovarian reserve (Sills et al., 2009). However, there are conflicting reports about whether there are significant age-related differences in sonographically measured ovarian volume during the period before the usual age of menopause (Andolf et al., 1987; Merz et al., 1996; Christensen et al., 1997; Pavlik et al., 2000; Oppermann et al., 2003).

In this prospective clinical study, which examines changes in AF number C and ovarian volume in women ranging from early reproductive to perimenopausal age, we aimed to ascertain the chronologic age at which these sonographic measurements begin to show a significant difference.

2. Materials and methods

The study was conducted at the Medical School Hospital of Ondokuz Mayis University, approved by the Local Ethics Committee. Informed consent was obtained from each subject prior to enrolment in the study.

Healthy, non-pregnant and non-menopausal women who visited to our outpatient clinic for routine gynaecologic control examinations were considered to be eligible for this study. The study included 100 women; 78 women were aged between 19 and 44 years and had regular spontaneous menstrual cycles, whereas 22 women were aged between 45 and 50 years and had regular or irregular spontaneous menstrual cycles. All the women menstruated spontaneously; had 2 intact ovaries; showed no evidence of thyroid disease, diabetes mellitus, significant hyperprolactinaemia, hypogonadotropic hypogonadism, or polycystic ovary syndrome; and had no history of any gynaecologic surgery.

The women underwent sonographic measurement of total antral follicle (TAF) count and MOV. Following these measurements, the women were assigned to 5 groups based on their age: group 1, 19–24 years; group 2, 25–29 years; group 3, 30–37 years; group 4, 38-44 years; and group 5, 45-50 years.

Sonographic measurements were performed on the second or third days of the menstrual cycle. For the AFC, round- or oval-shaped echo-free structures from 2 to 6 mm in diameter within the ovaries were considered to be follicles. TAF count was estimated as the sum of the number of antral follicles measured in both ovaries. Ovarian volume measurement and AFC were performed by using a two-dimensional (2D) abdominal probe (in virgin women) and an endovaginal probe of 6.5 MHz frequency (Siemens Medical Solutions, Issaquah, WA, USA). For each ovary (n = 200), a set of 2D images was stored to facilitate measurement of the standard 3 planes used for volume calculation. An ellipsoid formula ($D1 \times D2 \times D3 \times \pi/6$) which is composed of measurement of three perpendicular directions is used to calculate the volumes of the ovaries. The mean of the volumes of both ovaries was used as the MOV. All ultrasound scan procedures and measurements were performed by a single operator.

Statistical analysis was performed using the Student's t-test, Kruskal-Wallis variance analysis, the Mann-Whitney U Test, regression analysis. Receiver operating characteristic (ROC) analysis was used to determine the sensitivity and specificity and the data were presented as the mean ± standard deviation (SD). A p value of <0.05 was considered to be significant.

3. Results

A comparison of some demographic properties of the study groups was shown in Table 1. The TAF number was 10.0 ± 3.89 in group 1, 11.09 ± 4.30 in group 2, 10.38 ± 4.37 in group 3, 6.30 ± 2.97 in group 4, and

Table 1. Comparison of some demographical properties of the study groups (Mean±Standard Deviation). The different letters (a, b, c, d, e) show a statistically significant difference (p<0.05) between the groups.

Demographic Characteristics	Group-1 (n:13) (age:19-24)	Group-2 (n:21) (age:25-29)	Group-3 (n:21) (age:30-37)	Group-4 (n:23) (age:38-44)	Group-5 (n:22) (age:45-50)
Age (year)	22.0±1.73 (a)	26.61±1.35 (b)	34.04±2.08 (c)	41.39±1.80 (d)	47.54±1.71 (e)
BMI (kg/m^2)	22.0±3.24 (a)	22.23±3.12 (a)	25.52±5.10 (b)	26.60±4.96 (b)	28.81±4.61 (b)
Cycle length (day)	30.23±6.09 (a)	27.71±3.06 (a)	29.52±5.90 (a)	31.52±9.07 (a)	38.54±14.42 (a)
Bleeding duration (day)	5.84±1.28 (a)	5.76±1.67 (a)	5.71±2.81 (a)	6.21±2.85 (a)	10.13±4.43 (b)
Gravida	--	0.46±1.12 (a)	2.0±1.65 (b)	3.04±1.70 (b)	4.19±1.96 (c)
Parity	--	0.46±1.12 (a)	1.45±0.99 (b)	2.40±1.25 (c)	3.0±1.37 (c)
Basal FSH mIU/mll	7.12±5.52 (a)	6.47±1.60 (a)	5.68±2.50 (a)	9.22±5.05 (b)	17.84±13.64 (c)
MLP (ng/ml)	12.23±5.36 (a)	11.98±4.41 (a)	8.21±4.07 (b)	4.38 ±2.95 (c)	0.66±0.77 (d)

4.77 ± 2.11 in group 5. The MOV value was 6.16 ± 1.90 cm^3in group 1, 6.71 ± 3.77 cm^3 in group 2, 6.28 ± 3.77 cm^3 in group 3, 4.84 ± 1.62 cm^3 in group 4, and 3.77 ± 0.76 cm^3 in group 5.

There was a significant association between chronological age and TAF number (r^2 = 0.328, p < 0.001). As is seen in Fig. 1, TAF number began to decrease at approximately 25 years of age. Although a significant correlation was not found between chronological age and TAF number in women between the ages of 19 and 37 years (r^2 = 0.002, p > 0.05), there was a significant correlation between age and TAF number in women between the ages of 38 and 50 years (r^2 = 0.179, p < 0.05).

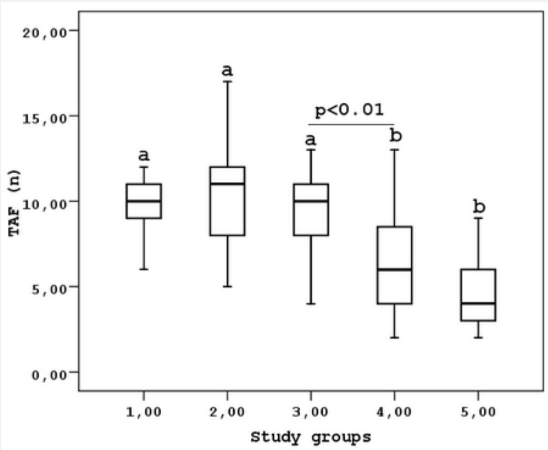

Fig. 1. Changes in TAF count with age.

No significant difference was noted in TAF number among the first three groups (r^2 = 0.58, p > 0.05) (Table 2). TAF number was significantly different between groups 3 and 4 (Z: -3.463, p < 0.001), but not between groups 4 and 5 (Z: -1.698, p > 0.05). The optimal cut-off value of the AF number for discrimination between groups 3 and 4 was determined through ROC analysis. The optimal cut-off value for the TAF count for discrimination between groups 3 and 4 was found to be 7. Based on this cut-off value, Table 3 shows the discriminative values for TAF number between groups 3 and 4.

Overall, there was a significant relationship between chronological age and MOV (r^2 = 0.149, p < 0.001). MOV began to decrease at 30 years of age. Although no significant correlation was noted between age and MOV in women between 30 and 44 years of age (r^2 = 0.081, p = 0.175), the correlation in women between 30 and 50 years of age was significant (r^2 = 0.146, p = 0.01) (Fig. 2).

MOV values did not show a significant difference among the first 4 groups (r^2 = 0.58, p> 0.05; r^2 = 7.87, p > 0.05 respectively) (Table 2). The MOV of group 5 was significantly different from those of groups 1, 2, 3,

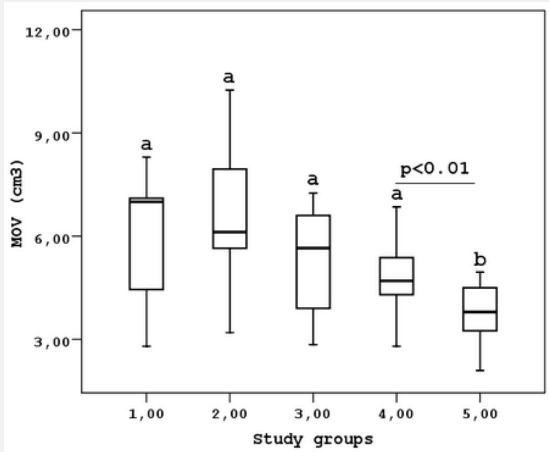

Fig. 2. Changes in MOV values with age.

and 4 (Z: -2.75, p < 0.01; Z: -4.351, p < 0.01; Z: -2.722, p < 0.01; and Z: -2.829, p < 0.01 respectively). The optimal cut-off value of MOV to discriminate between groups 4 and 5 was found to be 4 cm^3. With regard to this cut-off value, the discriminative features of MOV between groups 4 and 5 are shown in Table 3.

Table 2. Comparison of TAF count and MOV among the study groups.

Sonographic measurements	Study Groups	Mean Rank	X^2 / P	Mean Rank	X^2 / P	Mean Rank	X^2 / P
	1	64.00		44.19		26.15	
	2	70.29		49.45	19.74 <0.001	30.02	0.58 NS
TAF	3	65.79	42.84 <0.001	45.60		27.12	
	4	36.41		22.20			
	5	23.77					
	1	60.46		43.27			
	2	68.10		48.57	7.87 NS		
MOV	3	54.93	23.51 <0.001	38.60			
	4	46.33		29.91			
	5	27.95					

TAF: Total antral follicle, MOV: Mean ovarian volume, NS: Not significant (p>0.05).

Table 3. Sensitivity, specificity, PPV, NPV and AUC values of the TAF count and MOV according to their optimal cut off values for the discrimination of the study groups.

Sonographic measurements	Cut off value	Group discrimination	Sensitivity %	Specificity %	PPV %	NPV %	ROC AUC	P Value	95% CI
TAF (count)	7	Between 3 and 4	77.8	65.4	60.9	81	0.803	0.001	0.675 - 0.932
MOV (cm^3)	4	Between 4 and 5	82.6	63.6	70.4	77.8	0.746	0.005	0.598 - 0.894

TAF: Total antral follicle count MOV: Mean ovarian volume PPV: Positive predictive value, NPV: Negative predictive value, ROC-AUC: Area under the roc curve.

4. Discussion

Ovarian reserve has been found to be correlated with ovarian volume and antral folicle number measured by ultrasonography in the early follicular phase. However, interobserver variations reagrding AFC could be occur (Coccia and Rizzello 2008; Younis et al., 2010). Measurement of ovarian volume can be easily performed using ultrasound. Individual estimates of ovarian volume using 2D and three-dimensional (3D) ultrasound techniques do not differ in their precision (Brett et al., 2009). Real-time 2D pelvic ultrasonography is accepted more reliable method for determining ovarian volume and morphology. Transvaginal ultrasonography is shown to decrease the inter and intraobserver measurement differences (Kwee et al., 2007). Stored 3D data for predictive value of AFC which was calculated by 2D equivalent technique and two 3D techniques (multiplanar view, rendered inversion mode) was used in a study by Jayaprakasan et al. AFC measured by any of the 3 techniques was reported to be good predictor for poor ovarian response with optimal cut-off value of 6 or 7 follicles. In the present study, 2D pelvic ultrasonography was used.

There is reportedly a positive correlation between AFC and ovarian primordial follicle count; this is shown to remain significant even after adjustment for chronological age (Scheffer et al., 2003; Kline et al., 2005; Lutchman Singh et al., 2007; Hansen et al., 2011). It has been shown to be sufficient for predicting ovarian reserve if there are ≥ 5 antral follicles with a diameter of 2 to 5 mm on day 1 to 2 (Sills et al., 2009). The methodology for AFC differs among centres. Follicles of 2 to 5 mm or 2 to 10 mm predominantly are counted. When production of anti-Mullerian hormone (AMH) or inhibin B are compared according to the follicle sizes, pre-antral and smaller antral follicles of sizes up to 4 to 6 mm produce AMH, whereas inhibin B is produced by the follicles of sizes up to 13 mm. There is significant relationship between the number of small antral follicles (2–6 mm) and woman age; it is also significantly related to all endocrine ovarian reserve tests that we assessed, independent of age (Haadsma et al., 2007; Kwee et al., 2007). It was shown that functional ovarian reserve could be shown by the number of small antral follicles measuring 2 to 6 mm, independent of age. Therefore, it was proposed, only counted antral follicles of sizes 2 to 6 mm should be used to evaluate the outcome of endocrine ovarian reserve (Haadsma et al., 2007). Transvaginal ultrasonography can easily detect these follicles containing small amount of antral fluid in early antral phase (Kwee et al., 2007). Therefore, in our study, we counted the follicles between 2 and 6 mm.

In the present study, a significant difference was not noted among the TAF counts of the first 3 groups ($p > 0.05$) (Table 2 and Fig. 1). Optimal sensitivity and specificity values occurred at a cutoff TAF number of 7 to distinguish women younger than 37 years from those 37 years or older ($p < 0.01$) (Table 3). It was observed that the decrease in TAF count could be sonographically established beginning at age 25 years, and that this decrease was found to be significant beginning at the age of 38 years ($p < 0.05$) (Fig. 1).

MOV increase from 0.7 ml to 5.8 ml between 10 to 17 years. During reproductive period, there is no significant change in ovarian volume, however after 40 years old, a dramatic decline in ovarian volume which appears to be related with decreased oestrogen because when oestorgen is replaced decline in ovarian volume is not decreased (Kwee et al., 2007). In the present study, none of the participants underwent any oestrogen treatment and all had spontaneous menstruation.

An earlier study, no parity or age-related change was observed in ovarian volume of 155 premenopausal women aged 16 to 52 years (Merz et al., 1996). Moreover, the ovarian volume was measured in 428 women, aged 14 to 45 years, who visited a family planning clinic. It was reported that no correlation between the age and ovarian volume was found (Christensen et al., 1997). It was stated that, ovarian volume significantly decreases every decade from 30 to 70 years (Pavlik et al., 2000). A negative relationship between ovarian volume and age- not menopausal age- was found in a study including 337 women aged 40 to 70 (Andolf et al., 1987). In another study, ovarian volume was found to decrease beginning from 40 years and suggested to reach maximal value at the age of 39 years. It was found that ovarian volume was significantly less in all age groups over 40 years compared with the 35–39 years age group (Oppermann et al., 2003). In another former study, it was found that MOV was significantly less in women aged 35 years or older than in women younger than 35 years (Lass et al., 1997). In the present study, ovarian volume was found to reach a maximum value at approximately 30 years of age, and decrease became significant beginning at 45 years of age ($p < 0.05$) (Fig. 2). These results differ from the findings of Lass et al. Moreover, the fact that no significant difference was noted among the MOV of the first 4 groups strengthened the finding that a significant decrease in volume is noted after the age of 44 years (Table 2). In the present study, optimal sensitivity and specificity values were found at a cut-off MOV of 4 cm3 to distinguish the ovarian reserve between women younger than 45 years and those aged 45 years or older ($p < 0.01$) (Table 3).

There are several strengths of the present study. First, all participants younger than 45 years (78% of total) had regular spontaneous menstrual cycles. Second, the basal FSH level was less than 10 mIU/mL in 90% of the participants younger than 45 years. Third, none of the participants had any ovarian or endocrine disorder. Fourth, except for age, none had any factors that might affect the ovarian reserve. Fifth, sonographic measurements were assessed without knowledge of the woman age, which eliminated the possibility of bias.

In conclusion, we found that the TAF count decreases significantly beginning at 38 years of age, while MOV decreases significantly beginning at 45 years of age. Thus, there is no significant decrease in the ovarian follicular reserve until the age of 38 years, or in the ovarian volume until the age of 45 years.

REFERENCES

Andolf, E., Jorgensen, C., Svalenius, E., Sunden, B., 1987. Ultrasound measurements of the ovarian volume. Acta Obstet. Gynecol. Scand. 66, 387-389.

Brett, S., Bee, N., Wallace, W.H., Rajkhowa, M., Kelsey, T.W., 2009. Individual ovarian volumes obtained from 2-dimensional and 3-dimensional ultrasound lack precision. Reprod. Biomed. Online. 18, 348-351.

Cedars, M.I., Evans, M., 2008. Menopause. In: Gibbs, R.S., Karlan, B.Y., Haney, A.F., Nygaard, I.E., editors, Danforth's Obstetrics and Gynecology. Philadelphia: Lippincott Williams and Wilkins; pp. 725-742.

Christensen, J.T., Boldsen, J., Westergaard, J.G., 1997. Ovarian volume in gynecologically healthy women using no contraception, or using IUD or oral contraception. Acta Obstet. Gynecol. Scand. 76, 784-789.

Coccia, M.E., Rizzello, F., 2008. Ovarian reserve. Ann. N. Y. Acad. Sci. 127, 27-30.

Domingues, T.S., Rocha, A.M., Serafini, P.C., 2010. Tests for ovarian reserve: Reliability and utility. Curr. Opin. Obstet. Gynecol. 22, 271-276.

Faddy, M.J., Gosden, R.G., Gougeon, A., Richardson, S.J., Nelson, J.F., 1992. Accelerated disappearance of ovarian follicles in midlife — implications for forecasting menopause. Hum. Reprod. 7, 1342-1346.

Haadsma, M.L., Bukman, A., Groen, H., Roeloffzen, E.M., Groenewoud, E.R., Heineman, M.J., Hoek, A., 2007. The number of small antral follicles (2-6 mm) determines the outcome of endocrine ovarian reserve tests in a subfertile population. Hum. Reprod. 22, 1925-1931.

Hansen, K.R., Hodnett, G.M., Knowlton, N., Craig, L.B., 2011. Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil. Steril. 95, 170-175.

Hsu, A., Arny, M., Knee, A.B., Bell, C., Cook, E., Novak, A.L., Grow, D.R., 2011. Antral follicle count in clinical practice: Analyzing clinical relevance. Fertil. Steril. 95, 474-479.

Jayaprakasan, K., Hilwah, N., Kendall, N.R., Hopkisson, J.F., Campbell, B.K., Johnson, I.R., Raine-Fenning, N.J., 2007. Does 3D ultrasound offer any advantage in the pretreatment assessment of ovarian reserve and prediction of outcome after assisted reproduction treatment? Hum. Reprod. 22:1932-41.

Johnson, N.P., Bagrie, E.M., Coomarasamy, A., Bhattacharya, S., Shelling, A.N., Jessop, S., Farquhar, C., Khan, K.S., 2006. Ovarian reserve tests for predicting fertility outcomes for assisted reproductive technology: the International Systematic Collaboration of Ovarian Reserve Evaluation protocol for a systematic review of ovarian reserve test accuracy. BJOG. 113, 1472-14780.

Kline, J., Kinney, A., Kelly, A., Reuss, M.L., Levin, B., 2005. Predictors of antral follicle count during the reproductive years. Hum. Reprod. 20:2179-89.

Kwee, J., Elting, M.E., Schats, R., McDonnell, J., Lambalk, C.B., 2007. Ovarian volume and antral follicle count for the prediction of low and hyper responders with in vitro fertilization. Reprod. Biol. Endocrinol. 5,9.

Lass, A., Silye, R., Abrams, D.C., Krausz, T., Hovatta, O., Margara, R., Winston, R. M., 1997. Follicular density in ovarian biopsy of infertile women: A novel method to assess ovarian reserve. Hum. Reprod. 12,1028-1031.

Lutchman Singh, K., Muttukrishna, S., Stein, R.C., McGarrigle, H.H., Patel, A., Parikh, B., Groome, N.P., Davies, M.C., Chatterjee, R., 2007. Predictors of ovarian reserve in young women with breast cancer. Br. J. Cancer. 96, 1808-1816.

Merz, E., Miri-Tesanic, D., Bahlmann, F., Weber, G., Wellek, S., 1996. Sonographic size of uterus and ovaries in pre- and postmenopausal women. Ultrasound. Obstet. Gynecol. 7, 38-42.

Oppermann, K., Fuchs, S.C., Spritzer, P.M., 2003. Ovarian volume in pre- and perimenopausal women: A population-based study. Menopause. 10, 209-213.

Pavlik, E.J., DePriest, P.D., Gallion, H.H., Ueland, F.R., Reedy, M.B., Kryscio, R.J., Van Nagell, J.R., 2000. Ovarian volume related to age. Gynecol. Oncol. 77, 410-412.

Scheffer, G.J., Broekmans, F.J., Looman, C.W., Blankenstein, M., Fauser, B.C., teJong, F.H., teVelde, E.R., 2003. The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. Hum. Reprod. 18, 700–706.

Sharara, F.I., McClamrock, H.D., 2001. Use of microdose GnRH agonist protocol in women with low ovarian volumes undergoing IVF. Hum. Reprod. 16, 500-503.

Sills, E.S., Alper, M.M., Walsh, A.P., 2009. Ovarian reserve screening in infertility: Practical applications and theoretical directions for research. Eur. J. Obstet. Gynecol. Reprod. Biol. 146, 30-36.

Younis, J.S., Jadaon, J., Izhai, I., Haddad, S., Radin, O., Bar-Ami, S., Ben-Ami, M., 2010. A simple multivariate score could predict ovarian reserve, as well as pregnancy rate, in infertile women. Fertil. Steril. 94, 655-661.