Anti mullerian hormone and antral follicle count for prediction of ovarian reserve in female infertile patients: A cross sectional study

Aakriti Aryal
Department of Pharmacy Practice, SRM College of Pharmacy, Faculty of Medicine & Health Sciences, SRM Institute of Science and Technology, Kattankulathur, 603203, Chengalpattu (DT), TN, India

Vishnu Vandana G.
Department of Pharmacy Practice, SRM College of Pharmacy, Faculty of Medicine & Health Sciences, SRM Institute of Science and Technology, Kattankulathur, 603203, Chengalpattu (DT), TN, India

Meghana C. V.
Department of Pharmacy Practice, SRM College of Pharmacy, Faculty of Medicine & Health Sciences, SRM Institute of Science and Technology, Kattankulathur, 603203, Chengalpattu (DT), TN, India

Dasari Himaja
Department of Pharmacy Practice, SRM College of Pharmacy, Faculty of Medicine & Health Sciences, SRM Institute of Science and Technology, Kattankulathur, 603203, Chengalpattu (DT), TN, India

Sandeep D.
Department of Pharmacy Practice, SRM College of Pharmacy, Faculty of Medicine & Health Sciences, SRM Institute of Science and Technology, Kattankulathur, 603203, Chengalpattu (DT), TN, India

Sarumathy S.
Department of Pharmacy Practice, SRM College of Pharmacy, Faculty of Medicine & Health Sciences, SRM Institute of Science and Technology, Kattankulathur, 603203, Chengalpattu (DT), TN, India

Melvin George
Department of Cardiology, SRM Medical College Hospital and Research Centre, Faculty of Medicine & Health Sciences, SRM Institute of Science and Technology, Kattankulathur, 603203, Chengalpattu (DT), TN, India

Roopini R.
Department of Pharmacy Practice, SRM College of Pharmacy, Faculty of Medicine & Health Sciences, SRM Institute of Science and Technology, Kattankulathur, 603203, Chengalpattu (DT), TN, India

Abstract---Ovarian reserve (OR) determines the quantity and quality of follicles present in the ovary. It is independent of menstrual cycle in women of reproductive age. The chances of conceiving in infertile women can be predicted by understanding and determining the OR. The aim of the study was to assess and determine whether Anti-Mullerian Hormone (AMH) with Antral Follicle Count (AFC) or AMH alone is a better predictor of ovarian reserve in female infertile patients. Methods: In this prospective, cross-sectional study, a total of 60 patients were measured for AMH levels of which 30 patients was assessed along with AFC. The demographic details were collected and 5ml of venous blood samples were taken on day 2 of menstrual cycle. Serum was separated and stored at -20oC. Serum AMH levels were analysed using enzyme-linked immune sorbent assay (ELISA) whereas Antral Follicle Count with ultrasonography. Finally, 54 patients completed the analysis with the mean age of 26.32±3.95 years and body mass index of 24.09±4.62. The average AMH levels and AFC were found to be 2.49±4.05 ng/ml and 14.92±8.69 follicles respectively. AMH showed negative correlation with both FSH and LH and no correlation with estradiol. AFC showed statistically significant positive correlation with AMH. Receiver operating characteristic curve of AMH compared to AFC was plotted which suggested that AMH alone is not a better predictor of ovarian reserve. Anti-Mullerian Hormone as adjuvant with Antral Follicle Count proved to be better predictors of ovarian reserve rather than Anti-Mullerian Hormonealone.

Keywords---infertility, ovarian reserve, anti mullerian-hormone, antral follicle count.

Introduction

Infertility is defined as the inability to conceive even after 12 months or more of unprotected regular sexual intercourse [1,2]. Infertility can be categorized into primary and secondary infertility. A woman's inability to conceive at all is classified as primary infertility whereas a woman's inability to conceive after misconception or after carrying a pregnancy to live birth is classified as secondary infertility. Infertility is estimated to affect around 8 – 12% of the couples worldwide and varies across regions of the world. The prevalence of primary infertility in India, as estimated by the WHO, is between 3.9 and 16.8 percent [3].

Ovarian reserve (OR) is defined as the number and quality of follicles left in the ovary at any given time [4] and is measured accurately by counting the number of follicles in both the ovaries. OR helps in predicting response to ovarian stimulation with exogenous gonadotropins and therefore determines the chances of conceiving [5]. Poor Ovarian Reserve (POR) indicates a reduction in the quantity of ovarian follicular pool in women of reproductive age group and is an important cause of infertility in many couples. [7]. Fecundability markedly declines as the woman crosses early 30s and the prevalence of infertility increases above 35 years with 99% of patients expected to be infertile with the age of 45 years [8]. One of the biomarkers to assess OR is Anti-mullerian hormone (AMH), a transforming growth factor – β (TGF – β). AMH is produced by the granulosa cells of pre-antral and small antral follicles in measurable amounts during the reproductive age [9]. AMH correlates with antral follicles and age thereby acting as a novel marker for ovarian aging [10]. AFC represents the number of ovarian follicles with a diameter of 2-10 mm when observed on ultrasonography during early follicular phase (primordial follicles) i.e., $D_2 – D_5$ of the menstrual cycle. This count can be correlated with the amount of the remaining follicles, remaining ovarian follicle pool as well as ovarian response to a controlled ovarian stimulation. The endocrine function of the follicle depends upon the size of the follicle. Although AFC is a better predictor in predicting the ovarian response, the combination of AFC+AMH is considered highly predictive of oocyte maturity, fertilization and embryo cleavage [11].

Diagnosis of infertility requires a basic workup which includes detailed history and physical examination, evidence of ovulation i.e., day 2, day 3 gonadotropins and day 21 progesterone, cervical smear screening, susceptibility to Rubella virus, screening for Chlamydia trachomatis, serum prolactin, Thyroid function tests and hysterosalpingography. The diagnostic evaluation is done with the aid of parameters such as Gonadotropin-Releasing Hormone Agonist Stimulation Test (GAST), ovulatory function, ovarian reserve tests (cycle day-2 to day-4 (D_2-D_4)), serum FSH and E_2 levels assessed in plasma with AxSYMimmunoanalyser, Clomiphene Citrate Challenge Test (CCCT), Antral Follicle Count (AFC), serum Anti Mullerian Hormone (AMH) level, cervical factors, uterine abnormalities, tubal patency, Chlamydia Antibody Test (CAT) and transvaginal ultrasonography [5]. It was recently found that CCCT, GAST and methodology for Exogenous FSH Ovarian Reserve Testing is far from considering as uniform parameters and should be avoided in determining OR for infertile patients. [6]

There is a strong association between smoking and fertility. It also affects the success rates of Assisted Reproductive Technology (ARTs) [12]. Age related reduction in the quality of oocytes and quantity of primordial follicles defines ovarian aging. Fertility is at its peak at the age of 20 years after which there is loss in 3/4th of follicular reserve at the age of 30-40 years followed by decline in fertility until menopause. This indicates that women's potential of reproduction does not depend on age but also on OR [13]. The vulnerability of young age must be taken into account when assessing patients during therapy, especially when cycle cancellation is being considered. [14]

Methodology

Study site, study design and ethical considerations

A total of 60 infertile women diagnosed with primary infertility as per American Congress of Obstetricians and Gynaecologists (ACOG) were included in this prospective, cross-sectional, comparative study which was conducted for a period of 6 months in a multidisciplinary hospital. Six patients were lost to follow up and did not complete the study. Finally, 54 infertile women were included in the analysis. This study was conducted according to the standards of the International Committee on Harmonization on Good Clinical Practice and the revised version of the Declaration of Helsinki.

Patient selection

The inclusion criteria for subject recruitment were i) Female patients of age group 20-40 years with infertility as diagnosed by ACOG. ii) Patients failing to conceive after 12 months of unprotected sexual intercourse. Any subject who has undergone any previous ovarian surgery or with premature ovarian follicle or unwilling to participate in the study were excluded from the study.

Study procedure

A total of 60 patients were screened and finally 54 patients were selected for the analysis based on the inclusion and exclusion criteria. The demographic details such as age, BMI, occupation, social history, infertility duration etc. were collected from the patients. 5 ml of venous blood samples were collected from the patients on day 2 of menstrual cycle and serum was separated and stored at $-20C$ for further analysis. The results of biochemical parameters such as Follicular Stimulating Hormone (FSH), Luteinizing Hormone (LH), LH: FSH ratio and Estradiol (E2) and Thyroid Stimulating Hormone (TSH) were obtained from the records of patient population. All 54 patients were tested for AMH levels of which 27 patients were assessed both for AMH values and AFC levels. Pelvic anatomy of organs was found by transvaginal ultrasonography. Serum AMH values were analysed using human AMH ELISA kit.

ELISA procedure

Measurement of serum AMH levels was performed using AMH/MIS enzyme-linked immune sorbent assay kit. (R&D Systems, Inc., Mn, USA). AMH is added to the wells pre-coated with AMH monoclonal antibody. After incubation a biotin-conjugated anti-human AMH antibody is added and binds to human AMH. The unbound biotin-conjugated anti-human AMH antibody is washed away during a washing step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human AMH antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and the colour develops in proportion to the amount of human AMH. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm. Then, patients were referred to the radiology clinic of the hospital for a vaginal ultrasound. All ultrasound examinations were performed by a radiologist, who

counted the follicles with 2 to 9 mm diameters and recorded them as AFC. The laboratory results and ultrasound measurements were recorded and used for analysis. Then the patients were scheduled for the treatment plan, based on the gynecologists' opinion on AMH and AFC counts.

Statistical analysis

The data was analysed using Social Science Software program (SPSS) - Version 17 software. The data was expressed in percentage and continuous data was expressed as mean ± SD changes. The available AMH and AFC parameters were statistically related using receiver operating characteristic (ROC) curve.

Results

The mean value of age of study population was 26.32±3.95 years. The mean marital age was 3.42±2.39 months (Table 1). The mean value of BMI of study subjects was found to be 24.0910±4.62 Kg/m^2. The mean values of FSH and LH were found to be 5.5376±2.19 and 7.1173±5.66 IU/L respectively. Mean estradiol, AMH, and AFC value were found to be 39.1983±1.97 pg/ml, 2.4864±4.05 ng/ml and 7.42±4.42 respectively (Table 2). The results suggest that there is no statistical correlation between age, marital age, BMI, FSH, LH, Estradiol, AFC, co morbidities like hypothyroidism and PCOD among study population with normal and low AMH values (<0.5ng/ml). There was a statistically significant positive correlation (r=0.4580,p=0.021) between AFC and AMH (Table 3) while AMH showed statistically insignificant(p>0.05) negative correlation with FSH(r=-0.030),LH (r=-0.098)and no correlation with estradiol (r=1) (Table 4). The area under curve (AUC)when ROC (receiver operating characteristic curve) was plotted for AMH, AUC was found to be 0.486 (Table 5 and Figure 1) which was lesser than AFC (0.887) at 95% CI.

Table 1
Mean values of baseline parameters of study population

Parameters	Mean ± SD values (N = 54)
Age(years)	26.3200±3.95
Marital age(months)	3.4200±2.39
BMI(Kg/m^2)	24.0910±4.62
FSH(IU/L)	5.5376±2.19
LH(IU/L)	7.1173±5.66
Estradiol(pg/ml)	39.1983±1.97
AMH (ng/ml)	2.49±4.05
AFC (n = 27)	14.92±8.69

Table 2
Comparison of patient characteristics based on low and high AMH values

Patient characteristics	AMH (n = 54)		P value
	LOW (n=26)	NORMAL (n=28)	
Age(years)	26.4±4.4	26.1±3.5	0.815
Marital age(months)	3.8±2.6	3.08±2.0	0.201
BMI(Kg/m^2)	24.4±49	23.8±4.3	0.648
FSH(IU/L)	5.1±2.6	5.9±1.5	0.500
LH(IU/L)	8.3±6.3	6.3±4.9	0.385
Estradiol(pg/ml)	34.4±20.8	43.7±2.25	0.629
Total AFC	13.1±6.6(n=10)	16.1±9.8(n=15)	0.404
Hypothyroidism	0.291±0.46	0.0385±1.196	0.014
PCOD	6.20±0.41	0.192±6.4	0.890

* AMH value of less than 0.5ng/ml is considered as low AMH for a given patient.

Table 3
Correlation of Total AFC with AMH

Parameter	Pearson correlation (r)	P value
Total AFC (n = 27)	0.458	0.021

Table 4
Correlation of AMH with other parameters

Variable	FSH	LH	Estradiol
R P	-0.030 0.909	-0.098 0.729	1

Table 5
Area under curve of AMH

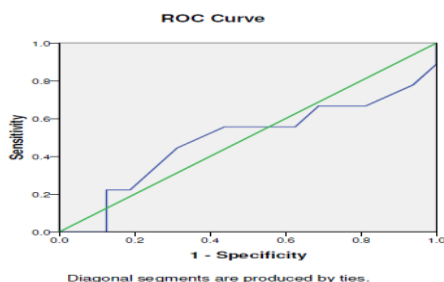| Area	Std error	Asymtotic significance	Asymtotic 95% CI	
			Upper bound	Lower bound
0.486	0.131	0.910	0.229	0.723

Figure 1. ROC Area under Curve of Plotted AMH Values

Discussion

AMH is synthesized by the granulosa cells present in ovaries. [15]. It is also produced by the Sertoli cells of the foetal testes and is known to induce regression of the Mullerian ducts, theanlagen of the female reproductive tract. [16] It is known to inhibit the recruitment of follicles from the primordial pool by altering the FSH sensitivity of antral follicles thereby reflecting the non-FSH dependent follicular growth. [17]. AMH levels increases from infancy up to adolescence and a plateau is observed until the age of 25. There onwards, the levels inversely correlate with age. This implies that serum AMH can be used as predictor of OR in women aged 25 years and above. Hence, the totality of ovarian follicles is determined early and reduction of this pool leads to reproductive aging. [18] AMH decreases with advancing age, whereas other markers such as serum levels of FSH, inhibin B and number of antral follicles, do not show any changes with advancing age. [19] According to the most recent studies, the function of AMH has been commended to decrease the cost, psychological burden and cycle cancellation risk which is experienced by the infertile couple. [20] In a study conducted by Tremellen KP in 2005, results showed that AMH assessment can be useful adjunct with FSH/estradiol and AFC count while assessing OR.[21]

As the reproductive age advances, basal serum FSH concentration increases on D2-D4 of the menstrual cycle. There may be inter-cycle variability where the basal FSH level can vary with each reproductive cycle. So, a single FSH value has limited reliability in measuring ovarian response. [22] Patients aged greater than 40 years with prior elevations in basal FSH levels have both compromised ovarian response and embryo quality. [23] This shows that FSH is a factor that is dependent of advancing age and due to these limitations FSH cannot alone be used as a better predictor of OR. The total AFC and AMH are found to correlate significantly with ovarian response with p-values < 0.001 and 0.03 respectively, indicating that they are good predictors of OR. The basal FSH and ovarian volume do not correlate with ovarian response indicating their poor value as predictors of OR. [20]

Estradiol is a primary female sex hormone produced by the ovaries during follicular development. [24] Low predictive accuracy, lack of high sensitivity and specificity cut off levels suggest that E_2 is not an effective prognostic tool for in-

vitro fertilization. [25] Studies failed to correlate E_2 as OR marker to follicular development or to predict the chances of conceiving [26]. Treatment for infertility in females usually aims to improve the chances of conceiving. Infertility treatment depends on the cause, age, duration of infertility and personal preferences because infertility is a complex disorder, treatment involves significant financial, physical, psychological and time commitments. The general hypothesis is that ovarian aging is a process where oocyte decreases in both in quantity and quality. [27]

Early screening of OR helps in identifying patients who are the risk for diminished ovarian reserve and show a poor response to gonadotropin stimulation with less chances of conceiving. It can therefore be concluded that correct assessment of OR is a central issue in management of patients with infertility. [28]. A comprehensive awareness about ovarian reserve testing and its significance helps in providing better treatment options to the patient. The suitable treatment for a patient presenting with poor ovarian reserve would be in-vitro fertilization whereas, pharmacotherapy would serve as the best option for a patient presenting with a better ovarian reserve. There was no significant correlation found between AMH, age, BMI, FSH, LH, and PCOS in the current study. There was a significant correlation of AMH with Estradiol and AFC.

The current study showed that AMH is independent of age contrary to the results obtained in an earlier study by Joseph van Helden in 2017 which stated that AMH is strongly correlated with increasing age.[29] The present study showed that AMH and FSH are independent of each other and do not have any correlation similar to the study by David H Barad in 2009. In this study, AMH does not show statistical specificity and sensitivity towards OR contrary to some studies.[30] In the current study, the AFC and AMH were found to correlate significantly with the ovarian reserve and the *p* value is 0.021 which is less than <0.05 but AMH alone had an insignificant value. The current study showed a correlation (positive correlation) between AFC and AMH, whereas, other parameters did not show any significant correlation with AMH. Also, the AUC for AMH is lesser than the AUC of AFC, thereby showing that AMH along with AFC is a better predictor of ovarian reserve than AMH alone.

Study limitations

This study was conducted for a short period of 6 months. The study was conducted only in single study centre and with a lesser sample size. Therefore, future studies with large sample size should be conducted.

Conclusion

In conclusion, we found that AMH along with AFC showed more significance in predicting OR than AMH alone. MH assessment as adjunct with AFC proved to be a predictor of ovarian reserve.

Availability of data and material

 The data that support the findings of this study are available on request from the corresponding author, Dr. S. Sarumathy. The data are not publicly available due to [restrictions e.g., their containing information that could compromise the privacy of research participants].

Ethics approval and consent to participate

This study was conducted according to the standards of the International Committee on Harmonization on Good Clinical Practice and the revised version of the Declaration of Helsinki (IEC No: 1059/IEC/2016).

Competing interest

The authors declared that they have no conflict of interest among themselves.

Funding

None

Author contributions

All the authors have equally contributed for the study concept, design and data analysis of the article.

Acknowledgements

References

1. AS Fauci, DL Kasper, DL Longo, E Braunwald, SL Hauser, JL Jameson, *et al.* Harrison's Principles of Internal Medicine. 17th edition, New York: The McGraw-Hill Companies, Inc; 2008; 2330-31.
2. F Zegers-Hochschild, G D Adamson, J de Mouzon, O Ishihara, R Mansour, K Nygren, *et al.* The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009.
3. Adamson PC, Krupp K, Freeman AH, Klausner JD, Reingold AL, Madhivanan P. Prevalence and correlates of primary infertility among young women in Mysore, India. Indian J Med Res. 2011;134(4): 440-446.

4. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update. 2006;12(6):685-718.
5. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile female: a committee opinion. Fertil Steril. 2015 Jun;103(6):e44-50.
6. Ramalho de Carvalho B, Gomes Sobrinho DB, Vieira AD, Resende, M. P., Barbosa, A. C., Silva, A. et al. Ovarian reserve assessment for infertility investigation. ISRN Obstet Gynecol. 2012;2012:576385.
7. Padma Rekha Jirge. Poor ovarian reserve. J Hum Reprod Sci. 2016; 9(2): 63–69.
8. Menken J, Trussell J, Larsen U. Age and infertility. Science. 1986;233(4771):1389-94.
9. van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, et al. Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. Hum Reprod. 2002; 17(12):3065-71.
10. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimüllerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002; 77(2):357-62.
11. Jayakrishnan Krishnakumar, Akansha Agarwal, Divya Nambiar, Shankar Radhakrishnan. Comparison of antral follicle count, antimullerian hormone and day 2 follicle stimulating hormone as predictor of ovarian response and clinical pregnancy rate in patient with an abnormal ovarian reserve test. Int J Reprod Contracept Obstet Gynecol. 2016; 5(8):2762-67.
12. Vivekanandan K, Bhavya E, Harikrishnan N, Epidemiological Study On Diabetes Patients Along With Patient Interaction And Counselling, Asian J Pharm Clin Res, Vol 13, Issue 3, 2020; p. 165-167.
13. Grande M, Borobio V, Bennasar M, Stergiotou I, Mercadé I, Masoller N, et al. A. Role of ovarian reserve markers, follicle count, as aneuploidy markers in ongoing pregnancies and miscarriages. Fertil Steril 2015;103(5):1221-7.e2..
14. Bishop LA, Richter KS, Patounakis G, Andriani l, Moon K, Devine K et al. Diminished ovarian reserve as measured by means of baseline follicle-stimulating hormone and antral follicle count is not associated with pregnancy loss in younger in vitro fertilization patients. Fertil steril. 2017;108(6):980-987.
15. Barut MU, Agacayak E, Bozkurt M, Aksu T, Gul T. There is a Positive Correlation Between Socioeconomic Status and Ovarian Reserve in Women of Reproductive Age. Med Sci Monit. 2016;22:4386-4392.
16. Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Müllerian hormone: a new marker for ovarian function. Reproduction. 2006;131(1):1-9.
17. Ibrahim A Abdelazim, Belal Mostafa, Makhlouf Hanan., Anti-Mullerian hormone and antral follicle count as predictors of ovarian reserve and successful IVF. Asian Pacific Journal of Reproduction. 2012;1:89-92.
18. Verma AK, Rajbhar S, Mishra J, Mayank Gupta, Mratunjai Sharma, Geeta Deshmukh, et al. Anti-Mullerian Hormone: A Marker of Ovarian Reserve and its Association with Polycystic Ovarian Syndrome. J Clin Diagn Res. 2016;10(12):QC10-QC12.
19. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimüllerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002;77(2):357-62.

20. Yomna Islam, Mona M. Aboulghar, Alaa El-Din AlEbrashy, Omar Abdel-Aziz. The value of different ovarian reserve tests in the prediction of ovarian response in patients with unexplained infertility. Middle East Fertility Society Journal. 2016;21:69-74.
21. Tremellen KP, Kolo M, Gilmore A, Lekamge DN. Anti-mullerian hormone as a marker of ovarian reserve. Aust N Z J Obstet Gynaecol. 2005;45(1):20-4.
22. Kwee J, Schats R, McDonnell J, Lambalk CB, Schoemaker J. Intercycle variability of ovarian reserve tests: results of a prospective randomized study. Hum Reprod. 2004;19(3):590-5.
23. Roberts JE, Spandorfer S, Fasouliotis SJ, Kashyap S, Rosenwaks Z. Taking a basal follicle-stimulating hormone history is essential before initiating in vitro fertilization. Fertil Steril. 2005;83(1):37-41.
24. Kenneth J. Ryan. Biochemistry of aromatase: significance to female reproductive physiology. Cancer Res. 42, 3342s–3344s.
25. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update. 2006;12(6):685-718.
26. Ramalho de Carvalho B, Gomes Sobrinho DB, Vieira AD, Resende MP, Barbosa AC, Silva AA, *et al.* Ovarian reserve assessment for infertility investigation. ISRN Obstet Gynecol. 2012;2012:576385.
27. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Fertil Steril. 2012;98(6):1407-15.
28. Spressão M, Oliani AH, Oliani DC. Value of the Ultrasound in the Study of Ovarian Reserve for Prediction of Oocyte Recovery. Rev Bras Ginecol Obstet. 2016;38(10):499-505.
29. Iliodromiti S, Salje B, Dewailly D. Non-equivalence of anti Mullerian hormone automated assays - clinical implications for use as a companion diagnostic for individualised gonadotrophin dosing. Hum Reprod. 2017;32(8):1710-1715.
30. Barad D, Gleicher N., Less may, indeed, be less: multicollinearity in studies of ovarian reserve. Fertil Steril. 2009;91(5):e16.