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

Research Article

J Exp Clin Med
2022; 39(3): 719-722
doi: 10.52142/omujecm.39.3.24

Evaluation of ovarian reserve at late reproductive age

Deniz KULAKSIZ * , **Recep ERİN**

University of Health Sciences, Trabzon Kanuni Health Practice and Research Center, Department of Obstetrics and Gynecology, Trabzon, Turkey

Received: 01.02.2022 • **Accepted/Published Online:** 17.07.2022 • **Final Version:** 30.08.2022

Abstract

Although follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), antimullerian hormone (AMH), and ovarian volume are commonly used to assess ovarian reserve and ovarian response to stimulation, no test directly indicates this. We aimed to observe how ovarian reserve tests help us in the diagnosis and treatment of women of late reproductive age. We included 190 patients in this prospective cohort study. We took FSH, LH, E2 and AMH blood samples on the third day of menstruation to biochemically evaluate the ovarian reserve of the patients who were planned for hysterectomy and bilateral salpingo-oopherectomy at late reproductive age. We recorded antral follicle counts (AFC) and ovarian volumes by transvaginal ultrasonography for ultrasonographic evaluation and weighed ovarian weights postoperatively. The data of 190 women aged 39-44.9 years revealed that AMH and ovarian volume, ovarian volume and AFS show significant positive correlations, whereas FSH, LH, and E2 levels do not indicate a significant relationship with AFS. AMH, ovarian volume and ovarian weight, and AFC may show us the ovarian reserve with the highest reliability in women of late reproductive age.

Keywords: Antimullerian hormone, antral follicle, infertility, ovarian reserves

1. Introduction

The purpose of ovarian reserve tests is to determine the number of eggs remaining in the ovaries and thus the ovarian response to ovarian stimulation. Antimullerian hormone (AMH), a dimeric glycoprotein that suppresses the development of Müllerian ducts, was first described by Josso (1). Various studies revealed that granulosa cells in the ovaries produced moderate AMH amounts after birth (2). AMH plays a role in the negative regulation of aromatase and luteinizing hormone (LH) receptor genes, increasing androgen production by theca cells while inhibiting the stimulatory effects of follicle stimulating hormone (FSH) on small and preantral follicle growth in postnatal ovarian tissue (3). Although antimullerian hormone (AMH) has been used as the greatest helper in recent years, it should be kept in mind that it may change with the menstrual cycle and may yield different results when studied with different methods (4).

FSH, inhibin B, and estradiol (E2) levels measured on the 3rd day of the cycle were other tests used to evaluate ovarian reserve (5). Basal FSH level was expected to increase with age, possibly correlated with decreased inhibin-mediated feedback to the pituitary (6). Since inhibin B production was mainly from small antral follicles, its level decreased with age (7). Evaluation of E2 level together with FSH gave information about ovarian follicles (5).

Transvaginal ultrasound was also an observer-dependent method, helping directly determine ovarian reserve by measuring ovarian volume or antral follicle count (8). To determine the number of antral follicles, FSH, and AMH levels; ovarian volume was independent of the menstrual cycle, although it was easier to determine ovarian volume with two-dimensional (2D) transvaginal ultrasound (8). Evaluating the relationship between ovarian weight and ovarian volume, Rosendahl et al. stated that one cubic centimeter equaled one gram by measuring with a microbalance (8). Although no research revealed a relationship between smoking and ovarian reserve, various studies claimed the opposite (9–11).

The present study aimed to show the relationship between ovarian weight, volume, and number of follicles, AMH, LH, FSH, and E2. The primary aim was to observe to what extent we could rely on ovarian reserve testing for late fertility.

2. Material and Methods

We included in this study 190 patients scheduled for

*Correspondence: drdenizkulaksiz@gmail.com

hysterectomy in a tertiary hospital in Northern Turkey and excluded patients in the postmenopausal period, genital tract cancer patients, patients using hormones and patients scheduled for surgery because of ovarian mass. We recorded patients' age, number of pregnancies, smoking habits, and BMI (weight/height squared) and evaluated FSH, LH, AMH, E2, endometrial thickness, right ovarian volume and left ovarian volume on the third day of menstruation.

We carried out all hormone tests using a chemiluminescence immunoassay method (Abbott®); its normal ranges were 3.03-8.8 mIU/ml for FSH, 1.8-11.78 IU/L for LH, and 21-251 ng/L for E2 in the follicular phase. Another chemiluminescence immunoassay method (Beckman-Coulter®) determined the AMH levels; its normal ranges were 0.07 -7.35 ng/ml. We conducted ultrasonographic measurements transvaginally by ESAOTE S.p.A./Italy model EA720 and measured endometrial thickness in mm. By taking the width, height and depth measurements of both ovaries in three dimensions, we made volume calculations with the formula D1xD2xD3x0.523 (Fig. 1). We scanned both ovaries systematically and counted follicles 2 to 10 mm in diameter to determine the number of antral follicles. We measured the ovarian weights in grams with a microbalance after surgery (Denver Instrument APX-200®).

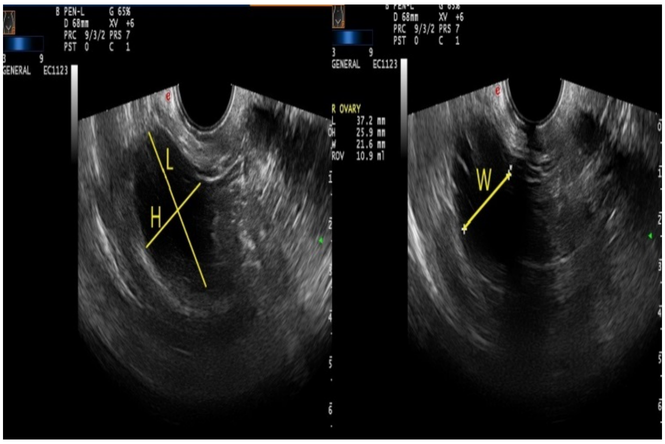

Fig. 1. Calculation of ovarian volume ultrasonographically

We obtained ethics approval number 2018/40 from the Clinical Research Ethics Committee of the University of Health Sciences Kanuni Training and Research Hospital. After informing all patients about the study, we obtained informed consent.

For power analysis, we reached the requirement of 120 patients to obtain a medium effect size (d = 0.5) with 80% power using version 3.01 of the G* Power software (Franz Faul, Kiel, Germany) at a significance level of 0.05. We performed the Kolmogorov-Smirnov test for conformity of the data to see the normal distributions. We used the Mann-Whitney U test to analyze the data to determine the differences between groups and the Pearson correlation test as the basis for the regression analysis. We analyzed all data obtained with IBM SPSS Statistics for Windows, Version 23.0, and p≤0.05 was significant.

3. Results

We included in this study 190 patients aged between 39 and 44.9 years whose number of pregnancies varied between 1 and 6. 72 were smokers while 118 were non-smokers. Table 1 shows the ovarian reserve test and age and BMI results. The mean endometrial thickness was 10.6 ± 1.9 cm. BMI, E2, right ovarian and left ovarian volumes showed normal distribution in the Kolmogorov-Smirnov statistical analysis. AMH, FSH, LH, E2, the number of follicles in the ovaries, ovarian volumes, and ovarian weights seemed to be statistically unrelated to age. Endometrial thickness and BMI, E2, FSH, LH, AMH were statistically unrelated. AMH levels decreased with an increase in BMI (r=-0.125). No statistical differences were present between FSH, LH, E2, AMH, follicle counts and ovarian volumes according to the smoking status by the Mann-Whitney U test.

Table 1. Biochemical and ultrasonographic ovarian reserve tests obtained on the third day of menstruation and age and body mass index data of patients

	Mean ± SD
Age (years)	42.2 ± 1.6
BMI (kg/m2)	29.4 ± 2.8
Follicle stimulating hormone (FSH) (mU/mL)	7.4 ± 2
Luteinizing hormone (LH) (mU/mL)	10.4 ± 4.4
Estradiol(E2) (ng/L)	75.3 ± 20.2
AMH (ng/ml)	1.2 ± 0.6
Right ovarian volume (cm³)	6.9 ± 0.5
Left ovarian volume (cm³)	6.8 ± 0.5
Right ovarian weight (grams)	6.9 ± 0.6
Left ovary weight (grams)	6.9 ± 0.6
Number of antral follicles in the right ovary	2.5 ± 0.8
Number of antral follicles in the left ovary	2.5 ± 0.9

AMH level was statistically unrelated to the number of pregnancies, FSH, LH, and E2. The number of follicles in the ovaries (r=0.982), ovarian volumes (r=0.817) and ovarian weights (r=0.798) were positively correlated with AMH levels. There was a significant positive correlation between ovarian volumes and ovarian weights and the number of follicles in the ovaries (r=0.912 and r=0.874, respectively). There was a significant positive correlation between ovarian volumes and ovarian weights (r=0.932).

4. Discussion

In recent years, the incidence of infertility has increased with an increase in the first gestational age (12). Therefore, we planned the patients included in this study to be women of late reproductive age. By resecting the ovaries, we could also evaluate their volumes and weights.

Ovarian reserve defines primordial follicles that increase in quality and quantity and decrease with age (13). Although no serum marker can directly measure the number of primordial follicles, AMH is an important helpful marker and decreases with age (14). AMH levels plateau around the age of 25 (15). Cui et al. reported median levels of AMH as 2.35 ng/mL for 20-31 years old, 1.58 ng/mL for 32-34 years old, 1.30 ng/mL, 0.96 for 35-37 years old. ng/mL for 38-40 years old, 1.05 ng/mL for 41-43 years old and 0.67 ng/mL for 43 years and older (16). The current study revealed the AMH level as 1.2 ± 0.6 ng/ml. While the AMH results were similar, they were slightly different. This difference may be due to the menstrual cycle and the patient profile included in the study.

AMH occurs in the 36th week of pregnancy and cannot be detected at menopause (17). Fanchin et al. found the AMH level to be more associated with antral follicle number than inhibin B, FSH, and E2 levels in infertile patients (18). The data in the present study supported the literature.

Sova et al. found higher AMH levels in obese polycystic ovarian syndrome (PCOS) patients than normal-weight patients while finding a negative correlation between serum AMH levels and BMI in the normal weight group (19). The current study revealed a negative correlation between the increase in BMI and AMH levels.

Although serum AMH is an important indicator of ovarian reserve, the lack of an international standard for AMH and multifactorial variability are the primary handicaps of the test (20). Furthermore, this review shows that AMH and the number of ovarian follicles are correlated in many studies, but results may vary depending on the population and technique chosen (20). The present study revealed a positive and significant correlation between follicles numbers and the AMH level.

The present study found no significant relationship between FSH, LH, E2 and AMH and smoking. Dafopoulos et al.'s research with 137 women found no significant relationship between smoking in FSH, LH, E2 progesterone and AMH levels (21).

The current study also found no relationship between endometrial thickness and FSH, E2, AMH and BMI. This may be explained by the multifactorial variability of the endometrial thickness (22). FSH and LH were biochemical markers reflecting aging in women, and they deteriorated significantly after the age of 35 due to the decrease in ovarian reserve (23). The Buyalos and Danesmand studies showed that the E2 level in infertile patients entered the abnormal range within two years of observation (22). Although FSH, LH, and E2 levels are consistent with the current literature (24), they did not change with age in the present study. The homogeneity of our study group and the narrow age range of the patients have an important effect on this.

In line with our findings, one cm^3 ovarian volume was equal to one gram ovarian weight in Rosendahl et al.'s study (8). Although a positive relationship was present between the number of antral follicles and ovarian volumes, the role of the ovarian volume in determining ovarian reserve has not been fully defined yet (25,26). The present study revealed a significant positive correlation between ovarian volumes and the total ovarian weight and follicle count.

AMH, ovarian volumes and ovarian weights, and AFC were crucial data for us in evaluating the ovarian reserve in women of late reproductive age.

Conflict of interest
The authors declared no conflict of interest.

Funding
No funding was used for the study.

Acknowledgments
The authors thank Prof Dr Suleyman GUVEN for their assistance in editing the study.

Authors' contributions
Concept: M.U., T.A., Design: M.U., T.A., Data Collection or Processing: M.U., T.A., Analysis or Interpretation: M.U., T.A., Literature Search: M.U., T.A., Writing: M.U., T.A.

References

1. Josso N, Legeai L, Forest MG, Chaussain JL, Brauner R. An enzyme linked immunoassay for anti-müllerian hormone: a new tool for the evaluation of testicular function in infants and children. J Clin Endocrinol Metab. 1990 Jan;70(1):23-7. doi: 10.1210/jcem-70-1-23. PMID: 1688440.
2. Hazout A, Bouchard P, Seifer DB, Aussage P, Junca AM, Cohen-Bacrie P. Serum antimüllerian hormone/müllerian-inhibiting substance appears to be a more discriminatory marker of assisted reproductive technology outcome than follicle-stimulating hormone, inhibin B, or estradiol. Fertil Steril. 2004 Nov;82(5):1323-9. doi: 10.1016/j.fertnstert.2004.03.061. PMID: 15533354.
3. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, Uilenbroek JT, Grootegoed JA, Themmen AP. Anti-Müllerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology. 2002 Mar;143(3):1076-84. doi: 10.1210/endo.143.3.8691. PMID: 11861535.
4. Melado L, Lawrenz B, Sibal J, Abu E, Coughlan C, Navarro AT, et al. Anti-müllerian Hormone During Natural Cycle Presents Significant Intra and Intercycle Variations When Measured With Fully Automated Assay. Front Endocrinol (Lausanne) [Internet]. 2018 Nov 27;9.
5. Ulrich ND, Marsh EE. Ovarian Reserve Testing: A Review of the Options, Their Applications, and Their Limitations. Clin Obstet Gynecol. 2019 Jun;62(2):228-237. doi: 10.1097/GRF.0000000000000445. PMID: 30998601; PMCID: PMC6505459.
6. Broekmans FJ, Scheffer GJ, Bancsi LF, Dorland M, Blankenstein MA, te Velde ER. Ovarian reserve tests in

infertility practice and normal fertile women. Maturitas. 1998 Oct 12;30(2):205-14. doi: 10.1016/s0378-5122(98)00075-9. PMID: 9871914.

7. Hall JE. Endocrinology of the Menopause. Endocrinol Metab Clin NA [Internet]. 2015;44(3):485–96.

8. Rosendahl M, Ernst E, Rasmussen PE, Andersen CY. True ovarian volume is underestimated by two-dimensional transvaginal ultrasound measurement. Fertil Steril. 2010 Feb;93(3):995-8. doi: 10.1016/j.fertnstert.2008.10.055. Epub 2008 Dec 23. PMID: 19108822.

9. Augood C, Duckitt K, Templeton AA. Smoking and female infertility: a systematic review and meta-analysis. Hum Reprod. 1998 Jun;13(6):1532-9. doi: 10.1093/humrep/13.6.1532. PMID: 9688387.

10. Bressler LH, Bernardi LA, De PJD. HHS Public Access. 2017;215(6):1–20.

11. Kee K, Flores M, Cedars MI, Reijo Pera RA. Human primordial germ cell formation is diminished by exposure to environmental toxicants acting through the AHR signaling pathway. Toxicol Sci. 2010 Sep;117(1):218-24. doi: 10.1093/toxsci/kfq179. Epub 2010 Jun 18. PMID: 20562217; PMCID: PMC2923286.

12. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem. 2018 Dec;62:2-10. doi: 10.1016/j.clinbiochem.2018.03.012. Epub 2018 Mar 16. PMID: 29555319.

13. te Velde E., Scheffer G., Dorland M, Broekmans F., Fauser BCJ. Developmental and endocrine aspects of normal ovarian aging. Mol Cell Endocrinol [Internet]. 1998 Oct;145(1–2):67–73.

14. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, Griesinger G, Kelsey TW, La Marca A, Lambalk C, Mason H, Nelson SM, Visser JA, Wallace WH, Anderson RA. The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update. 2014 May-Jun;20(3):370-85. doi: 10.1093/humupd/dmt062. Epub 2014 Jan 14. Erratum in: Hum Reprod Update. 2014 Sep-Oct;20(5):804. PMID: 24430863.

15. Lie Fong S, Visser JA, Welt CK, de Rijke YB, Eijkemans MJC, Broekmans FJ, et al. Serum Anti-Müllerian Hormone Levels in Healthy Females: A Nomogram Ranging from Infancy to Adulthood. J Clin Endocrinol Metab [Internet]. 2012 Dec 1;97(12):4650–5.

16. Zhou SJ, Zhao MJ, Li C, Su X. The comparison of evaluative effectiveness between antral follicle count/age ratio and ovarian response prediction index for the ovarian reserve and response functions in infertile women. Medicine (Baltimore). 2020 Sep 4;99(36):e21979. doi: 10.1097/MD.0000000000021979. PMID: 32899038; PMCID: PMC7478524.

17. de Vet A, Laven JSE, de Jong FH, Themmen APN, Fauser BCJM. Antimüllerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril [Internet]. 2002 Feb;77(2):357–62.

18. Fanchin R, Schonäuer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Müllerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprod. 2003 Feb;18(2):323-7. doi: 10.1093/humrep/deg042. PMID: 12571168.

19. Sova H, Unkila-Kallio L, Tiitinen A, Hippeläinen M, Perheentupa A, Tinkanen H, et al. Hormone profiling, including anti-Müllerian hormone (AMH), for the diagnosis of polycystic ovary syndrome (PCOS) and characterization of PCOS phenotypes. Gynecol Endocrinol [Internet]. 2019 Jul 3;35(7):595–600.

20. Moolhuijsen LME, Visser JA. Anti-Müllerian Hormone and Ovarian Reserve: Update on Assessing Ovarian Function. J Clin Endocrinol Metab [Internet]. 2020 Nov 1;105(11).

21. Dafopoulos A, Dafopoulos K, Georgoulias P, Galazios G, Limberis V, Tsikouras P, Koutlaki N, Maroulis G. Smoking and AMH levels in women with normal reproductive history. Arch Gynecol Obstet. 2010 Aug;282(2):215-9. doi: 10.1007/s00404-010-1425-1. Epub 2010 Mar 23. PMID: 20309569.

22. Buyalos RP, Ghosh K, Daneshmand ST. Infertile women of advanced reproductive age. Variability of day 3 FSH and E2 levels. J Reprod Med [Internet]. 1998 Dec;43(12):1023–6.

23. Mullen RD, Wang Y, Liu B, Moore EL, Behringer RR. Osterix functions downstream of anti-Müllerian hormone signaling to regulate Müllerian duct regression. Proc Natl Acad Sci [Internet]. 2018 Aug 14;115(33):8382–7.

24. Hale GE, Zhao X, Hughes CL, Burger HG, Robertson DM, Fraser IS. Endocrine features of menstrual cycles in middle and late reproductive age and the menopausal transition classified according to the Staging of Reproductive Aging Workshop (STRAW) staging system. J Clin Endocrinol Metab. 2007 Aug;92(8):3060-7. doi: 10.1210/jc.2007-0066. Epub 2007 Jun 5. PMID: 17550960.

25. de Vasconcelos GL, Maculan R, da Cunha EV, Silva AWB, Batista ALS, Donato MAM, Peixoto CA, Silva JRV, de Souza JC. Antral follicular count and its relationship with ovarian volume, preantral follicle population and survival, oocyte meiotic progression and ultrastructure of in vitro matured bovine cumulus-oocyte complexes. Zygote. 2020 Dec;28(6):495-503. doi: 10.1017/S0967199420000386. Epub 2020 Aug 19. PMID: 32811580.

26. Korsholm AS, Hvidman HW, Bentzen JG, Nyboe Andersen A, Birch Petersen K. Left-right differences in ovarian volume and antral follicle count in 1423 women of reproductive age. Gynecol Endocrinol. 2017 Apr;33(4):320-323. doi: 10.1080/09513590.2016.1259406. Epub 2016 Dec 2. PMID: 27910705.