DOI - 10.21276/obgyn.2022.9.1.24 *ISSN Print – 2454-2334; ISSN Online – 2454-2342*

RESEARCH ARTICLE

A study to correlate serum anti mullerian hormone, basal follicle stimulating hormone and antral follicle count in primary infertility as a measure of ovarian reserve

Shruthi A, Sujatha Prabhu

Corresponding author: Dr. Shruthi A, Senior Resident, Dept of OBG, Yenepoya Medical College, Mangalore, Karnataka, India; Email: Shruthisr198@gmail.com

ABSTRACT

Objectives: This study aims to find the correlation between anti mullerian hormone (AMH) and follicle stimulating hormone (FSH) and to observe the above mentioned hormones' relation with antral follicle count (AFC) in patients with primary infertility. **Methodology:** This is a cross-sectional correlation study in which 60 patients with primary infertility meeting inclusion criteria, attending infertility clinic in ESIC MC PGIMSR, Rajajinagar between January 2017 and June 2018 were enrolled by simple random sampling. Detailed menstrual, obstetric, coital and medical history was obtained. On the third day of the spontaneous cycle, all patients were investigated with a transvaginal scan to assess the number of antral follicles and a fasting venous blood sample was obtained for the measurement of serum AMH and serum basal FSH level. **Results:** Basal serum FSH shows a moderately strong negative correlation with antral follicle count (AFC) ($r=0.65$; $p=<0.001$); and a strong negative correlation with anti mullerian hormone (AMH) ($r=0.69$ and $p=<0.001$). However, the strongest correlation between a biochemical marker and biophysical marker of ovarian reserve is between anti mullerian hormone (AMH) and antral follicle count (AFC) with a very strong positive correlation with a correlation co-efficient $r=0.89$ ($p=<0.001$). **Conclusion:** Serum AMH best correlates with the antral follicle count. Antral follicle counts although an efficient test to detect ovarian reserve is uncomfortable for the patient as it has to be done during menstrual flow. Serum AMH with minimal intracycle and intercycle variation is a more convenient marker to assess ovarian reserve while it maintains the accuracy of AFC.

Keywords: Anti mullerian hormone, follicle stimulating hormone, antral follicle count, ovarian reserve, primary infertility.

Infertility refers to the inability of a woman to become pregnant after having unprotected coitus for a specified amount of time, usually, a year.[1] One of the most important factors contributing to infertility is the quantity and quality of the ovarian reserve. The quantity of the ovarian follicular cohort and the quality of the oocytes contained within, determines an individual's ovarian reserve. The assessment of ovarian reserve aids in postulating the response to controlled ovarian stimulation (COH) and facilitates appropriate counseling and modification of an individual's treatment protocol in an attempt to maximize their therapeutic response.[2]

An individual's ovarian reserve can be assessed by age, estradiol (E2), basal follicle stimulating hormone (FSH) levels, serum inhibin B levels, anti mullerian hormone, antral follicle count, etc.[2,3] The woman's age and assays of serum FSH in the early follicular phase were among the earliest and most useful parameters used for the evaluation of ovarian reserve.[4,5] Low levels of FSH are seen during follicle development and high levels during ovulation. The variation in levels of FSH is due to a feedback loop between the hormones secreted from the ovaries and the pituitary gland.[6]

Follicle Stimulating hormone level also depends on the estradiol (E2) level. As antral follicles develop in the ovaries,

Received: 25th February 2021, Peer review completed: 4th April 2021, Accepted: 1st May 2021.

Shruthi A, Prabhu S. A study to correlate serum anti mullerian hormone, basal follicle stimulating hormone and antral follicle count in primary infertility as a measure of ovarian reserve. The New Indian Journal of OBGYN. 2022; 9(1): 127-31.

they secrete E2 and inhibin B. An increase in these hormones signals the gonadotropic cells in the anterior pituitary gland to reduce the release of FSH. Once ovulation occurs there is a decrease in E2, and this removes the negative feedback of E2 resulting in an increase of serum FSH, which stimulates the next cohort of follicles. This accounts for the variability of FSH during the menstrual cycle. Day three FSH has been the most used test of ovarian reserve and has been the standard way of determining ovarian reserve, providing the greatest accuracy.[1] However, it has intercycle and intracycle variation due to the feedback loop between pituitary and ovarian hormones.[7]

Another widely used ovarian reserve test is the antral follicle count (AFC) done by high resolution ultrasonography. Although there are well known difficulties to obtain a correct AFC, the ability of AFC to predict a poor response to ovulation induction therapy might be significantly better than basal FSH. Thus, AFC has been considered the "test of first choice" to assess ovarian reserve by some investigators.[8, 9]

Serum anti mullerian hormone (AMH) is a potential new test for ovarian reserve. AMH is a member of the transforming growth factor-β (TGF-β) family, just like inhibins and activins.[10] AMH is synthesized by granulosa cells residing within ovarian follicles and this production starts around birth itself. AMH is predominantly secreted into the intrafollicular compartment giving rise to high follicular fluid concentrations and some of it reaches the blood permitting detection of AMH in the circulation.[11,12] AMH is the best currently available measure of the ovarian reserve under a variety of clinical situations, such as infertility treatment (IVF), the prediction of reproductive lifespan, ovarian dysfunction (like polycystic ovary syndrome). Moreover, AMH may help to individualize dosing for ovarian stimulation thereby improving the efficiency and safety of IVF.[10] AMH levels do not change significantly throughout the menstrual cycle thus being a more reliable marker and time independent parameter.[2]

This study aims to find a correlation between biochemical (hormonal) markers of ovarian reserve such as serum AMH, and basal serum FSH with the biophysical marker of ovarian reserve i.e antral follicle count to better assess the patients with primary infertility.

Materials and methods

This is a cross-sectional correlation study involving 60 patients with primary infertility attending the infertility clinic in ESIC MC PGIMSR, Rajajinagar, between January 2017 and June 2018. The selection was done by simple random sampling. Only patients belonging to the age group of 25-40 years with regular menstrual cycles of 21-35 days with BMI of <25kg/m^2 were included in the study.

Patients with PCOS, abnormal uterine bleeding, evidence of endocrine disorders and current or past diseases affecting the ovaries were excluded from the study.

The patients were evaluated on an outpatient basis after obtaining detailed menstrual, obstetric, coital and medical history. On the third day of the spontaneous cycle, all patients were investigated with a transvaginal scan by the same investigator to assess the number of antral follicles. All follicles measuring 2-10mm size were counted in both ovaries and "Antral Follicle Count" was obtained. On the same day, a fasting venous blood sample was obtained for the measurement of serum AMH and serum basal FSH level. Serum levels of AMH were determined by enzyme linked immune sorbent assay. FSH measurement was done by standard techniques in the laboratory (Beckman coulter by chemi-luminescence immune assay). The correlation between parameters was analysed.

Statistical analysis: Descriptive analysis of variable data is expressed as mean and standard deviation (SD). Statistical analysis was done using SPSS software. The relationship between two different continuous variables was assessed by Pearson correlation. The Fisher r to z-test is used to determine if the coefficient of correlation (r) is significantly different from 0. A p value of less than 0.05 was considered as statistically significant.

Results

In the 60 patients involved in the study group 28 were below 30yrs of age and 32 were above 30 yrs of age, with the mean BMI of 22.3kg/m^2 (table 1).

Table 1: Average age and BMI of study participants (N=60)

Features	Mean (SD)
Age in years	31.1 (4.0)
BMI in Kg/m^2	22.3 (1.9)

The average mean hormonal levels and standard deviation of the hormonal levels in our study population is as mentioned in table - 2. The mean and SD of anti mullerian hormone level in patients above 30 years of age was 2.50ng/mL and 1.5ng/mL which was significantly less (p - 0.001) than in patients ≤30 years with a mean hormonal level of 3.81ng/mL and a SD of 1.4ng/mL respectively. It moderately strong negative correlation with age, thus indicating a gradual fall in AMH with advancing age (table 3).

The mean and SD of follicle stimulating hormone level in patients above 30years of age was 11.25mIU/mL and 3.8mIU/mL which was significantly more (p - 0.002) than in patients ≤30years with a mean hormonal level of 8.19mIU/mL and a SD of 3.3mIU/mL respectively. It moderately strong positive correlation with age, thus indicating a rise in FSH with advancing age (table 3).

Table 2: Average hormone levels, ovarian volume and antral follicle count of study participants (N=60)

Hormones	Age group in mean (SD)		P value#
	≤ 30 years	>30 years	
AMH(ng/mL)	3.81 (1.4)	2.50 (1.5)	0.001*
FSH(mIU/mL)	8.19 (3.3)	11.25 (3.8)	0.002*
LH(mIU/mL)	6.38 (3.7)	6.26 (2.3)	0.88
Prolactin(ng/mL)	14.70 (5.0)	12.10 (3.4)	0.03*
Antral follicular count	11.29 (4.1)	8.72 (3.7)	0.01*
Ovarian volume (cc)	6.95 (1.1)	7.43 (1.6)	0.20

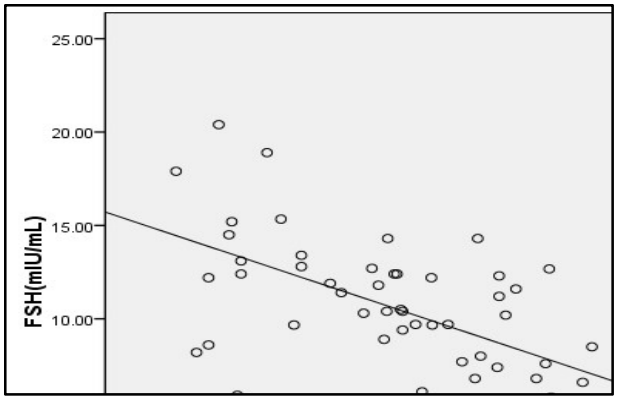
Note: # p value based on independent sample t test, * statistically significant (p<0.05)

Table 3: Correlation between various hormones related to infertility with age

Correlating factors	Correlation coefficient (r)	Direction	P value
AMH vs Age	0.65	Negative	<0.001*
AFC vs Age	0.54	Negative	<0.001*
FSH vs Age	0.58	Positive	<0.001*

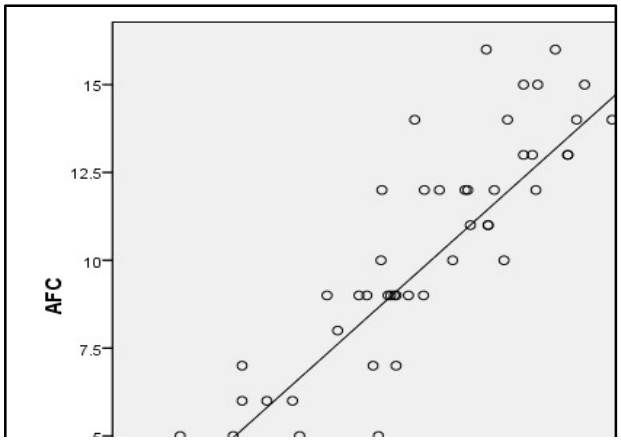
Note: P value based on Pearson correlation, * Statistically significant (p<0.05)

The antral follicle count on day 2 of menstrual cycle showed a significant fall in number with the advancing age. The mean (SD) of the two groups being 11.29 (4.1) and 8.72 (3.7) respectively. The decrease in AFC is statistically significant with the p value of 0.01. It moderately strong negative correlation with age, thus indicates a decrease in ovarian reserve with advancing age (table 3).



Graph1: Correlation between AMH and FSH

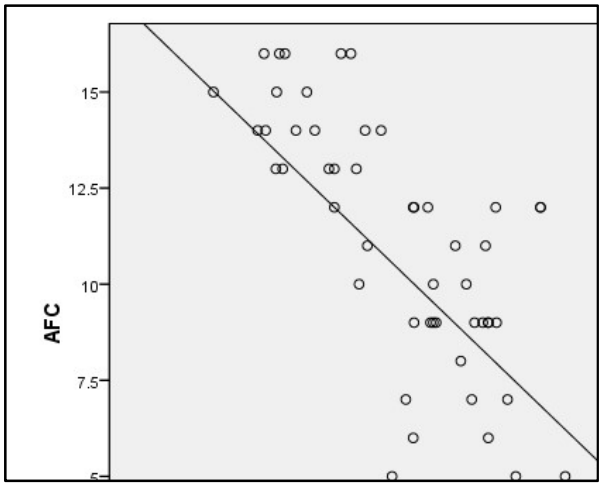
Further, results obtained from 60 patients with primary infertility were correlated for AMH, AFC, and basal FSH by Pearson correlation and correlation coefficient represented by "r". Serum AMH showed a strong negative correlation with serum basal FSH, with a correlation coefficient (r) of 0.69 and a p-value of <0.001 (graph 1). AFC showed a strong negative correlation with basal serum FSH, with a correlation coefficient (r) of 0.65 and a p-value of <0.001(graph 3). AMH shows a very strong positive correlation with AFC, with a correlation coefficient (r) of 0.89 and a p-value of <0.001(graph 2) (table 4) thus being strongest of the correlation between the biochemical and biophysical markers of ovarian reserve.



Graph 2: Correlation between AMH and AFC

Table 4: Correlation of various factors indicating ovarian reserve along with strength of correlation and direction

Correlating factors	Correlation coefficient	Direction and strength of correlation
AMH vs FSH	0.69	Strong negative
AFC vs AMH	0.89	Very strong positive
AFC vs FSH	0.65	Strong negative



Graph 3: Correlation between FSH and AFC related to infertility

Discussion

FSH and AMH are individually, widely used to assess functional ovarian reserve. At younger ages, abnormally elevated FSH levels have lower significance in the presence of good AMH levels, whereas in older women, especially those older than age 42years, AMH loses specificity in the presence of still decent FSH levels.[13] Hence we tried to find the correlation between serum FSH and AMH on the second day of the menstrual cycle to better understand the hormonal interplay involved in maintaining fertility. Our study showed a moderately strong negative correlation between the two hormones. Previous studies done by Bala et al in 2014 and Ludmila Barbakadze et al in 2015 also showed a moderately strong negative correlation between the two hormones. However, we achieved a much tighter negative correlation compared to the above two studies.[2,14]

Correlating serum basal FSH, one of the prominent biochemical (hormonal) marker of ovarian reserve with antral follicle count being the biophysical (measured by TVS) marker of ovarian reserve, we found a moderately strong negative correlation between the two. This result obtained in our study goes along with the study conducted by Ludmila Barbakadze et al in 2015 who also obtained a moderately strong correlation between the two ovarian reserve variables.[14] However study conducted by Göksedef et al, only found a moderate negative correlation between FSH and AFC and Bala et al, did not find a statistically significant correlation between the two variables [2,7].

AFC is one of the best biophysical markers of ovarian reserve, measured by transvaginal ultrasonography. It is considered the "test of the first choice" by some investigators for the assessment of ovarian reserve.[9] AMH, on the other hand, is a potential new test for the assessment of ovarian reserve. With its minimal intra and intercycle variation, it acts as one of the prominent biochemical (hormonal) tools in the assessment of ovarian reserve. Thus the correlation between these two parameters can help in better assessment of patients with infertility. In our study, we found a very strong positive correlation between the two markers. The results obtained in the study conducted by Ludmila Barbakadze et al is in support of our study, where the correlation between the two markers was also a very strong positive one.[14]

However studies conducted by Göksedef et al and Bala et al, derived a moderately strong positive correlation between these two novel markers of ovarian reserve.

Finally, in our study, we found that the AMH and AFC had a much tighter correlation when compared to the correlation between other markers of ovarian reserve. Serum AMH best correlates with antral follicle count and with its minimal intracycle and intercycle variation can be considered the best biochemical marker to assess changes occurring in ovarian function over time (i.e. reproductive aging).

Conclusion

In our study serum AMH best correlated with antral follicle count. Antral follicle count although an efficient test to detect ovarian reserve is uncomfortable for the patient as it has to be done during menstrual flow. Serum AMH with minimal intracycle and intercycle variation is a more convenient and accurate marker to assess ovarian reserve.

Conflict of interest: None. **Disclaimer:** Nil.

References

1. Iverson A, Younis A, Butler WJ, Roudebush WE. Inverse correspondence of AMH and FSH levels in women presenting for infertility treatment. J South Carol Acad Sci. 2011; 9:1-4.
2. Goksedef BP, Idis N, Gorgen H, Asma YR, Api M, Cetin A. The correlation of the antral follicle count and serum anti-mullerian hormone. Journal of the Turkish German Gynecological Association. 2010; 11(4): 212-15.
3. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: Implications for forecasting menopause. Hum Reprod. 1992; 7: 1342 - 6.
4. Tan SL, Royston P, Campbell S, Jacobs HS, Betts J, Mason B, et al. Cumulative conception and live birth rates after in-vitro fertilisation. Lancet. 1992; 339:1390-4.
5. Toner JP, Philput CB, Jones GS, Muasher SJ. Basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. Fertil Steril. 1991; 55: 784-91.
6. Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Müllerian hormone: A new marker for ovarian function. Reproduction. 2006;131:1-9.
7. Agrawal Y, Seth S, Goyal V, Kumar P, Bala J. Correlation between anti-Müllerian and follicle-stimulating hormone in female infertility. International Journal of Health and Allied Sciences. 2014; 3(4): 232-6.

8. Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD, te Velde ER. Predictors of poor ovarian response in in-vitro fertilization: a prospective study comparing basal markers of ovarian reserve, Fertil Steril. 2002; 77: 328-36.
9. Avril C. Antral follicle count and oocyte quality. J Gynecol Obstet Biol Reprod. 2006; 35: 42-3.
10. Brore SL, Broekmans FJ, Laven JS, Fauser BC. Anti Mullerian hormone: ovarian reserve testing and its potential clinical implications. Human Reproduction Update. 2014; 20(5): 688-701.
11. Jirge PR. Ovarian reserve tests. Journal of human reproductive sciences. 2011 Sep; 4(3):108.
12. Rajpert-De Meyts E, Jørgensen N, Graem N, Müller J, Cate RL, Skakkebaek NE. Expression of anti-Müllerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab. 1999; 84(10): 3836-44.
13. Gleicher N, Kim A, Kushnir V, Weghofer A, Shohat-Tal A, Lazzaroni E, et al. Clinical relevance of combined FSH and AMH observations in infertile women. The Journal of Clinical Endocrinology & Metabolism. 2013 May 1; 98(5): 2136-45.
14. Barbakadze L, Kristesashvili J, Khonelidze N, Tsagareishvili G. The correlations of anti-mullerian hormone, follicle-stimulating hormone and antral follicle count in different age groups of infertile women. Int J Fertil Steril. 2015; 8(4): 393-98.

Shruthi A [1], Sujatha Prabhu [2]

[1] Senior Resident, Dept of OBG, Yenepoya Medical College, Mangalore, Karnataka, India; [2] Professor, Dept of OBG, ESIC Medical College and PGIMSR, Bangalore, India.