

Correlation of Follicle-stimulating Hormone, Anti-Mullerian Hormone, and Antral Follicle Count with Age in Ovarian Reserve Testing

Abstract

Objective: Ovarian reserve and hence ovarian response has a key role in assisted reproductive technology and predicting response to gonadotrophins in controlled ovarian hyperstimulation. Various tools, namely follicle-stimulating hormone (FSH), anti-Mullerian hormone (AMH), antral follicle count (AFC), estradiol, etc., have been studied to discover the best determinant of ovarian reserve. The aim of our study is to correlate different reproductive hormones with age of women to estimate ovarian reserve and to evaluate reliable marker for aiding infertility treatment.

Materials and Methods: It is an observational study performed for 6 months, with 88 women (aged 21–39 years) having a complaint of infertility, enrolled in the infertility clinic of a tertiary care hospital. Baseline scan for AFC was done for every patient and their blood was sent for serum FSH, AMH analysis. Statistical procedures were employed to determine the association between age and reproductive hormones (i.e. FSH and AMH) as independent variables and AFC as a dependent variable. **Results:** A strong negative correlation was noted between FSH and AMH and between age and AMH ($r = -0.492$ and $r = -0.498$, respectively). A weak negative correlation was seen between AMH and total AFC ($r = -0.241$). A moderate positive correlation was seen on comparing age and FSH ($r = 0.331$), whereas no correlation was seen on comparing FSH with AFC and AMH with AFC. The presence of ovarian cyst did not affect AMH or AFC but reduced FSH values significantly.

Conclusion: In the quest to determine a panel test for ovarian reserve testing we conclude, FSH and AFC should perform fairly in poor resource and low socioeconomic setting. The combination of FSH with AMH and AFC might aid in better determination of ovarian reserve in tertiary centers with available resources.

Keywords: Anti-Mullerian hormone, antral follicle count, follicle-stimulating hormones, infertility, ovarian reserve

Subrata Das,
Namrata
Bhattacharya,
Ritu Mahata,
Sudip Ghosh¹,
Anindya Sundar Bhar,
Pragati Srivastava

Department of Obstetrics and
Gynaecology, ESI PGI MSR,
ESIC Medical College and
ESIC Hospital and ODC (EZ),
Kolkata, ¹Department of
Community Medicine, Burdwan
Medical College, Burdwan,
West Bengal, India

Introduction

The ovarian response to gonadotrophins has a crucial role in assisted reproductive technology (ART) to get success of infertility treatment. Ovarian reserve has a key role in predicting the response to gonadotrophins and other ovulation-inducing drugs in controlled ovarian hyperstimulation (COH).^[1] There are a number of investigations available for knowing the ovarian reserve. The day 3 serum follicle-stimulating hormone (FSH) and basal E2 (estradiol) levels are used for the last two decades to know the ovarian reserve.^[2,3] However, serum FSH level has both inter-cycle and intra-cycle variability. Ovarian reserve decreases with an increase in the level of serum FSH and progress of age, and consequently, treatment response to gonadotrophins also decreases.^[4]

In recent times, another biochemical marker named anti-Mullerian hormone (AMH) has been identified to assess ovarian reserve. It is a dimeric glycoprotein that belongs to transforming growth factor- β family.^[2,3] It is more specific for the detection of ovarian reserve and predicts the response to gonadotrophins. The low AMH level can predict poor ovarian reserve and possibly poor response to COH, and excess gonadotrophin is required to get an adequate response.^[5] Serum FSH is required to be measured in early follicular phase, but AMH can be tested at any time of the menstrual cycle. In younger women, serum level variation of AMH is also seen in different blood samples of the same menstrual cycle. However, AMH has 80% sensitivity and 93% specificity in random blood samples and is more reliable to predict poor ovarian reserve.^[2]

Submitted: 27-Feb-2024
Revised: 11-Jul-2024
Accepted: 16-Jul-2024
Published: 24-Aug-2024

Address for correspondence:
Dr. Subrata Das,
J/6, Banerjee Para, Kamdahari,
Garia, Kolkata - 700 084,
West Bengal, India.
E-mail: drsubrata02@gmail.com

Access this article online

Website:
<https://journals.lww.com/IJAB>

DOI:
10.4103/ijabmr.ijabmr_81_24

Quick Response Code:



How to cite this article: Das S, Bhattacharya N, Mahata R, Ghosh S, Bhar AS, Srivastava P. Correlation of follicle-stimulating hormone, anti-Mullerian hormone, and antral follicle count with age in ovarian reserve testing. Int J App Basic Med Res 2024;14:162-8.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

The two distinct hormones, i.e., FSH and AMH, are representatives of two different stages of follicular developments. Antral and postantral follicular developments are expressed by FSH level, whereas AMH hormones are produced by postprimordial preantral follicular pool. Maximum portion of AMH is produced by secondary, preantral, and small antral follicles of granulosa cells of up to 6 mm in diameter.^[3,6] Therefore, the level of AMH is correlated with available antral follicles of ovaries. This antral follicle is believed to be recruited for ovulation during COH. Now with the advancement of high-resolution ultrasonography and transvaginal transducers, it is possible to count the antral follicular number precisely on days 2–3 of menstrual cycle. Many infertility centers believe that antral follicular count (AFC) is the gold standard for predicting response to gonadotrophins for ovulation inductions.^[3,7]

Conventionally, serum FSH is in use for many years and AMH is used in recent times in a similar way for estimating ovarian reserve.^[8] However, there is also concordance and discordance found in their values.

Ovarian reserve testing is of utmost importance as it can help estimate ovarian aging and hence diagnose ovarian insufficiency. It helps to determine patients' response to ovarian stimulation for ART and provides information regarding patients' reproductive lifespan and menopausal timing. It is beneficial in counseling and planning treatment strategy for young female cancer survivors undergoing gonadotoxic therapy. It also aids in establishing the diagnosis of polycystic ovarian syndrome (PCOS) and gives insightful information regarding disease severity. The ideal ovarian reserve test should be easily available, reproducible, display little or no intra-cycle and inter-cycle variability, and be highly specific so as to minimize the risk of wrongly diagnosing women with poor ovarian reserve and accurately identifying those at greatest risk of developing ovarian hyperstimulation syndrome before fertility treatment. While there is currently no perfect ovarian reserve test, both AFC and AMH levels have a good predictive value and are superior to day 3 FSH. AMH has the convenience of untimed sampling, standardization, age-specific values, and availability of an automated platform, which makes this test the preferable biomarker for the evaluation of ovarian reserve in most women; however, it has the disadvantage of being costly, and hence, AFC becomes a better tool with similar specificity in poor socioeconomic settings. Compared to AMH and AFC, FSH is easily available in poor resource setting but its value is not stable throughout menstrual cycle.

At present, there is no set battery of tests which might correctly indicate optimal ovarian reserve. The aim of our study is to find out the correlation of different reproductive hormones and its relation with age of women to estimate ovarian reserve and to evaluate reliable marker for making strategy for infertility treatment.

Materials and Methods

Ethics approval

All human studies have been reviewed by the Institutional Ethics Committee and the Committee approved our study (registered no.-ESIC/42/IEC/[JOKA]/2021). The study has been performed in accordance with the ethical standards described in an appropriate version of the 1964 Declaration of Helsinki, as revised in 2013.

Study sample and setting

It is an observational study that was conducted at the department of obstetrics and gynecology of a tertiary care hospital of the eastern part of India. A total of 88 women were enrolled in an infertility treatment clinic of the hospital from March 2022 to August 2022 with a complaint of infertility (i.e., not being able to conceive even after 1 year or more of unprotected coitus). All persons who gave their informed consent were only included in the study.

Women of 21–39-year age groups of both primary and secondary infertility were included as study subjects. Women with any hormonal medications or PCOS or premature ovarian failure were excluded from the study. Women with a history of ovarian surgery or partial or complete removal of one or both ovaries or any history of radiotherapy or chemotherapy were also excluded from the study. Women with a complaint of infertility during the study period who fulfilled the inclusion/exclusion criteria were our study sample. Women were included in the study after not being able to conceive even after 1 year or more of unprotected coitus.

Baseline scan for AFC was performed for each woman and their serum FSH and serum AMH was measured on days 2-3 of menstrual cycles. Blood samples were obtained from each woman on the 2nd/3rd day of their menstrual cycle, and serum FSH and serum AMH were analyzed and recorded. Blood for serum FSH and AMH were collected in clotted vials on an outpatient department basis using the enzyme-linked immunosorbent assay method. The FSH range for our laboratory was 1.4–9.9 U/L. The range for AMH for our laboratory was 1.0–4.0 ng/mL. Each woman had undergone high-resolution transvaginal sonography by 4–9 MHz transducers. Follicles were sorted by size with color codes. Antral follicles of 2–10 mm on day 2nd or 3rd of menstrual cycle were counted. Follicles that may have been missed on automatic counting were added manually, postprocessing. A sum of antral follicle numbers from both ovaries were considered AFC. Ovarian cyst was said to be present at the mean diameter of clear lesion size >30 mm or lesion size <30 mm having the presence of septae and/or ground-glass appearance and/or solid components. Out of 88 total patients included, 7 patients were diagnosed to have ovarian cysts.

Subgroup analysis involves examining how the effects of an intervention vary across different categories within the

study population. These categories, or criteria, include demographic factors such as age, gender, ethnicity, and socioeconomic status, which can influence treatment outcomes. Baseline characteristics such as initial disease severity, comorbid conditions, and baseline measurements provide insight into how these factors interact with the intervention. Other criteria, such as variations in dosage, treatment duration, adherence levels, and clinical symptoms, help assess specific responses to the intervention. Behavioral factors such as smoking, alcohol use, and lifestyle habits, as well as environmental influences and geographical differences, also play roles in determining treatment effectiveness. Time-related factors, psychosocial variables, and responses to treatment, including adverse effects, further refine subgroup analyses to better understand and optimize interventions for diverse patient groups. A sample size of 76 was calculated using the following formula: Sample size (n) = $z^2 \times pq/d^2$, where the value of p (expected proportion of cases) was taken as 11% and value of d (precision) was taken as 7% from a previous study.^[9]

Outcome measure and statistical analysis

Data were recorded in the schedule form (predesigned, semi-structured). The collected data were transferred to Microsoft Excel and analyzed using SPSS (Statistical Package for the Social Sciences, version 26th, IBM Corp., Armonk, N.Y., USA). In this study, independent variables were age, FSH, and AMH, whereas AFC count was dependent variable. We estimated the correlation between FSH and AMH hormones with Pearson correlation coefficient. We considered values to be statistically significant and nonsignificant with a value of $P < 0.05$ and $P > 0.05$, respectively. The statistical procedures were employed to analyze data in a multiple linear regression model to determine the association between age and reproductive hormones (i.e. FSH and AMH) as independent variables and AFC count as a dependent variable.

Results

In our study, we observed that the mean (standard deviation [SD]) age was 33.02 years (4.98), the median (interquartile range [IQR]) age was 34 years (29.25–37.75), and the range of age was from 21.0 to 39.0 years. The mean (SD) value of FSH was 11.53 IU/L (17.49), the median (IQR) FSH was 6.9 IU/L (4.65–10.09), and the range for FSH was 0.89–41.42 IU/L. The mean (SD) value of AMH was found to be 2.78 ng/mL (3.50), the median (IQR) AMH was 1.38 ng/mL (0.72–4.11), and the range of AMH was 0.01–18.37 ng/mL. The mean (SD) value of AFC was 7.52 (4.13), the median (IQR) of total AFC was 7.5 (4.25–9.75), and the range of AFC was 0.00–16.00. We observed that the maximum mean AFC count was 9.83 in the age group of 30–34 years, the minimum mean AFC count was 6.62 in the age group of 25–29 years, the maximum mean FSH was 12.42 IU/L in the age group

of 35–39 years, and the minimum mean value of FSH count was 5.67 IU/L in the age group of 21–24 years. We also noted that the maximum mean value of AMH was 11.64 ng/mL in the age group of 21–24 years and the minimum mean value of AMH count was 1.78 ng/mL in the age group of 35–39 years [Table 1].

In our study, we have found a strong negative correlation between FSH (day 2nd/3rd) and AMH (day 2nd/3rd) and also between age and AMH (day 2nd/3rd) ($r = -0.492$ and $r = -0.498$, respectively). A weak negative correlation was noted between AMH (day 2nd/3rd) and total AFC (2–10 mm) count ($r = -0.241$). A moderate positive correlation was seen on comparing age and FSH (day 2nd/3rd) ($r = 0.331$). It was also noted that there was no correlation whatsoever on comparing FSH (day 2nd/3rd) with total AFC (2–10 mm) count and AMH (day 2nd/3rd) with AFC (2–10 mm) count [Table 2].

There clearly depicts a strong negative correlation ($r = -0.492$) between FSH (day 2nd/3rd) and AMH (day 2nd/3rd). On plotting a scatter diagram and drawing a regression (best fitted) line based on scatter plots with $R^2 = 0.054$, the following equation was established:

$$\text{FSH} = 14.77 - 1.16 \times \text{AMH} \text{ [Figure 1].}$$

FSH was normally distributed into two subgroups of variable group. Thus, parametric tests (t -test) were used to make group comparisons. The mean (SD) of FSH in the ovarian cyst absent group was 12.72 IU/L (18.86). The mean (SD) FSH in the ovarian cyst present group was 5.31 IU/L (1.54). The median (IQR) FSH in the ovarian cyst absent group was 8.46 IU/L (5.81–11.03). The median (IQR) of FSH in the ovarian cyst present group was 5.60 IU/L (3.96–6.89). FSH in the ovarian cyst absent group ranged from 0.89 to 41.42 IU/L. The FSH in the ovarian cyst present group ranged from 2.77 to 6.96 IU/L. There was a significant difference between the two groups in terms of FSH ($t = 2.365$, $P = 0.001$).

Age (years) was normally distributed in the two subgroups of the variable group. Thus, parametric test (t -test) was used

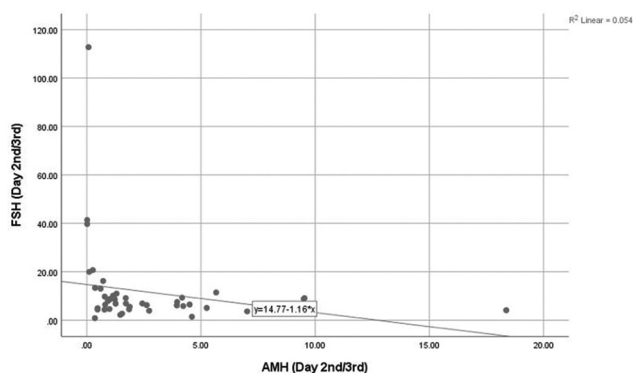


Figure 1: Scatter plot of follicle-stimulating hormone (day 2nd/3rd) and anti-Mullerian hormone (day 2nd/3rd) according to the study ($n = 88$). FSH: Follicle-stimulating hormone, AMH: Anti-Mullerian hormone

Table 1: Descriptive statistics of Age, FSH, AMH, AFC (n=88)

Descriptive Statistics				
	Age (Years)	FSH (IU/L) (Day 2 nd /3 rd)	AMH (ng/ml) (Day 2 nd /3 rd)	Total AFC (2-10 mm) count
Mean	33.02	11.53	2.78	7.52
Median	34.00	6.95	1.38	7.50
Mode	38.00	0.89	0.01	9.00
Std. Deviation	4.98	17.49	3.50	4.13
Minimum	21.00	0.89	0.01	0.00
Maximum	39.00	41.42	18.37	16.00
Percentiles				
25	29.25	4.65	0.72	4.25
50	34.00	6.95	1.38	7.50
75	37.75	10.09	4.11	9.75
	Mean AFC Count	Mean FSH	Mean AMH	
Age group (years)				
21-24	7.33	5.67	11.64	
25-29	6.62	6.50	3.51	
30-34	9.83	7.36	1.85	
35-39	6.57	12.42	1.78	

FSH: Follicle-stimulating hormone; AMH: Anti-Mullerian hormone; AFC: Antral follicle count

Table 2: Correlation coefficients of FSH, AMH and AFC (n=88)

Variables	Correlation	P	Remarks
FSH (Day 2 nd /3 rd) Vs. AMH (Day 2 nd /3 rd)	-0.492	0.0367**	Strongly Negative Correlation
FSH (Day 2 nd /3 rd) Vs. Total AFC (2-10 mm) count	0.072	0.491	No correlation
AMH (Day 2 nd /3 rd) Vs. Total AFC (2-10 mm) count	-0.241	0.0243	Weakly Negative Correlation
Age Vs. FSH (Day 2 nd /3 rd)	0.331	<0.001**	Moderate Positive Correlation
Age Vs. AMH (Day 2 nd /3 rd)	-0.498	<0.001**	Strongly Negative Correlation
AMH (Day 2 nd /3 rd) Vs. AFC (2-10 mm) count	0.072	0.379	No Correlation

**Correlation is significant at the 0.05 level (2-tailed)

to make group comparisons. The mean (SD) age (years) in the ovarian cyst absent group was 33.49 (5.02). The mean (SD) age (years) in the ovarian cyst present group was 30.57 (4.1). The median (IQR) of age (years) in the ovarian cyst absent group was 35 (31–38), whereas the median (IQR) of age (years) in the ovarian cyst present group was 32 (27–35). Age (years) in the ovarian cyst absent group ranged from 21 to 39 years. The age (years) in the ovarian cyst present group ranged from 25 to 36 years. There was no significant difference between the two groups in terms of age (years) ($t = 4.853$, $P = 0.641$).

AMH was normally distributed into two subgroups of the variable group. Thus, parametric test (t -test) was used to make group comparisons. The mean (SD) AMH (ng/mL) in the ovarian cyst absent group was 3 (3.78). The mean (SD) AMH (ng/mL) in the ovarian cyst present group was 1.68 (0.57). The median (IQR) AMH (ng/mL) in the ovarian cyst absent group was 1.22 (0.60–4.22). The median (IQR) AMH in the ovarian cyst present group was 1.71 (1.26–1.87). The AMH in the ovarian cyst absent group ranged from 0.01 to 18.37. AMH (ng/mL) in the ovarian cyst present group ranged from 0.8 to 2.73. There was a significant difference between the groups in terms of AMH ($t = 2.918$, $P = 0.001$).

Total AFC was normally distributed in the two subgroups of the variable group. Thus, parametric tests (t -test) were used to make group comparisons. The mean (SD) of total AFC in the ovarian cyst absent group was 7.76 (4.29). The mean (SD) of total AFC in the ovarian cyst present group was 6.29 (3.07). The median (IQR) of total AFC in the ovarian cyst absent group was 8 (5–10). The median (IQR) of total AFC in the ovarian cyst present group was 7 (3–9). The total AFC in the ovarian cyst absent group ranged from 0.0 to 16.00. The total AFC in the ovarian cyst present group ranged from 2.0 to 11.0. There was no significant difference between the groups in terms of total AFC ($t = 7.544$, $P = 0.591$) [Table 3].

The presence of ovarian cysts had no significant effect on hormone levels used for ovarian reserve testing.

Discussion

Approximately 10% of general female population is estimated to undergo accelerated loss of ovarian reserve and hence a loss of fertility starting from their 30s, thereby reaching early menopause by the age of 45 years.^[9,10] The goal of ovarian reserve testing is to aid prognostically in the planning and counseling process of couples in order to help them select appropriate treatment options, since ovarian

Table 3: Parametric tests of the following study variables (n=88)

	Ovarian cyst		t	P
	Ovarian cyst absent	Ovarian cyst present		
FSH (day 2nd/3rd)				
Mean	12.72	5.31	2.365	0.001*
SD	18.86	1.54		
Median	8.46	5.60		
Percentile 25	5.01	3.96		
Percentile 75	11.03	6.89		
Minimum	0.89	2.77		
Maximum	41.42	6.96		
Age (years)				
Mean	33.49	30.57	4.853	0.641
SD	5.02	4.11		
Median	35.00	32.00		
Percentile 25	31.00	27.00		
Percentile 75	38.00	35.00		
Minimum	21.00	25.00		
Maximum	39.00	36.00		
AMH (day 2nd/3rd)				
Mean	3.00	1.68	1.544	0.001*
SD	3.78	0.57		
Median	1.22	1.71		
Percentile 25	0.60	1.26		
Percentile 75	4.22	1.87		
Minimum	0.01	0.80		
Maximum	18.37	2.73		
Total AFC (2–10 mm) count				
Mean	7.76	6.29	7.544	0.591
SD	4.29	3.07		
Median	8.00	7.00		
Percentile 25	5.00	3.00		
Percentile 75	10.00	9.00		
Minimum	0.00	2.00		
Maximum	16.00	11.00		

*Significant at $P < 0.05$. FSH: Follicle-stimulating hormone; AMH: Anti-Mullerian hormone; AFC: Antral follicle count; SD: Standard deviation

reserve testing is largely a test of quantity and not quality of the remaining pool of oocytes. However, it is noteworthy that ovarian reserve tests are not unerring and should not be dependent upon solely to deny access to patients to various ARTs and similar treatments.^[5,11] In our study, we have compared the various parameters used for ovarian reserve testing and attempted to find correlation among them.

For ovarian reserve testing, FSH has been studied more extensively than other variables. It has been observed that women in similar age group with higher FSH levels are likely to have lower fecundability.^[12] However, women with elevated FSH levels but younger age often have a much better chance of conception than older women with comparable levels of FSH^[13] and age can predict outcome

in terms of fertility better than FSH alone.^[14] In our study, we found a moderate positive correlation on comparing age and FSH (day 2nd/3rd) ($r = -0.331$), i.e., with increasing age, FSH value was found to be increased. Although high and low FSH can prognosticate differences in pregnancy outcome, there is a suboptimal sensitivity in assay for both ovarian response and pregnancy rates, as shown in some studies.^[15]

AMH secreted by granulosa cells (preantral and antral follicular cells) is relatively stable during the hormonal variation of menstrual cycle. The absolute value of AMH is directly proportional to the number of primary follicles and it has regulatory function overdevelopment and maturation of follicles, and thus, it can be of prime importance as a serological marker for the assessment of ovarian reserve function and ovarian responsiveness during ovulation.^[16,17] In our study, we have found a strong negative correlation between FSH (day 2nd/3rd) and AMH (day 2nd/3rd) and also between age and AMH (day 2nd/3rd) ($r = -0.492$ and $r = -0.498$, respectively). Hence, we may conclude that in our study, with increasing age, AMH reduced and FSH increased.

AFC can be measured by a sonologist or clinician, compared to AMH, which can be evaluated in a laboratory only. There is a high inter- and intra-observer variability seen in AFC measurements which is its primary drawback and might lead to discrepancy in evaluating ovarian reserve.^[18,19] The advantage of using AMH over AFC is that ultrasound is not required. Furthermore, AMH levels remain constant in response to gonadotrophins and thus show little or no variability when measured throughout the cycle compared to other parameters which are labile to the changes of gonadotrophins throughout the cycle. Hence, AMH is advantageous for both patients and clinicians.^[20,21] Tremellen *et al.* in their study for the estimation of ovarian reserve concluded that AMH should be used as an adjunct to AFC or FSH or estradiol.^[22] de Vet *et al.* also established that AMH concentrations showed a correlation with age, FSH, and number of antral follicles but not with inhibin B levels.^[23] Elgindy *et al.* also opined in their study that early follicular and mid-luteal AMH values may show an excellent prognostic value for clinical pregnancy.^[24] Panchal and Nagori, however, suggested that AFC is independently sufficient for estimating ovarian reserve.^[6] Jain *et al.* found a significant correlation between AMH and AFC. They showed that AMH increased with age till the third decade after which showed a negative correlation with AFC. AMH decreased with age and showed a positive correlation with AFC.^[25] Suardi *et al.* found a negative correlation between serum AMH and an ovarian volume containing endometrioma although it was not statistically significant.^[26] In our study, we found a weak negative correlation between AMH (day 2nd/3rd) and total AFC (2–10 mm) count ($r = -0.241$). It was also noted in our study that there was no correlation whatsoever on

comparing FSH (day 2nd/3rd) with total AFC (2–10 mm) count and AMH (day 2nd/3rd) with AFC (2–10 mm) count.

Anuradha *et al.* concluded that AMH is the most reliable investigation in testing for ovarian reserve but is costlier than comparatively low-cost AFC, and hence, AFC can be beneficial for poor patients to test ovarian reserve.^[27] This might be applicable particularly in settings with low socioeconomic status and in developing countries. Muttukrishna *et al.* established in their study that AMH is the single best marker of ovarian reserve. Any combination of AMH with FSH and inhibin B will only moderately enhance the diagnostic value.^[28] Permadi *et al.* showed a significantly positive correlation between AMH ($P \leq 0.001$, $r = 0.530$), AFC ($P \leq 0.001$, $r = 0.687$), and combination of AMH-AFC ($P \leq 0.001$, $r = 0.652$).^[29] Tran *et al.* studied that AMH has disadvantages since it only reflects growing follicular pool which is responsive to gonadotropins and, hence, may not solely reflect the underlying primordial pool.^[30] Arvis *et al.* concluded that the faster decline in AMH with age compared to AFC suggested that their correlation changes with age.^[31] AMH seemed better reproducible in terms of prediction of ovarian response.^[31] Iwase *et al.* in their study found that AMH level monitoring is useful in a varied array of clinical situations like infertility treatments, diagnosing ovarian failure, assisted reproductive technology, evaluating iatrogenic ovarian damage, polycystic ovarian syndrome, ovarian tumours like granulosa cell tumor, for reproductive health management, improving prediction of pregnancy and live birth, etc.^[32] In our study, it was found that the presence of ovarian cyst did not affect the values of AMH or AFC, but FSH showed significantly reduced values in the presence of an ovarian cyst.

While there is currently no perfect ovarian reserve test, both AFC and AMH levels have good predictive value and are superior to day 3 FSH. AMH has the convenience of untimed sampling, standardization, age-specific values, and availability of an automated platform, which makes this test the preferable biomarker for the evaluation of ovarian reserve in most women; however, it has the disadvantage of being costly, and hence, AFC becomes a better tool with similar specificity in poor socioeconomic settings. FSH analysis is an easily available and cheap test, but it shows variable values in different days of menstrual cycles and in presence of ovarian cysts.

Our study is the first to be performed in this population in the eastern region on an insured group of patients. Furthermore, till date, there is no standardized panel of tests for determining ovarian reserve. This is our attempt to establish a set panel of tests for ovarian reserve so as to aid in the early diagnosis and prompt management of an exponentially increasing problem that is subfertility.

Our study has some limitations as well. We have measured serum AMH on day 2nd/3rd of menstrual cycle along with serum FSH for the ease of samplings and improved

compliance of patients. Hence, we could not demonstrate the advantage of AMH remaining stable throughout the menstrual cycle or any time of day. Availability of AMH and ultrasonographic assessment for AFC is limited to tertiary care center setting and are expensive tests. Hence, reproducibility of the same in low socioeconomic setting would be difficult. Finally, the study was performed on a smaller number of subjects. More such studies with larger sample size are required for establishing the ideal tests for ovarian reserve.

Conclusion

FSH, AMH, and AFC are optimal tools of measuring ovarian reserve with AMH and AFC having advantage of showing no significant change in the presence or absence of ovarian cysts. The combination of FSH with AMH and AFC might aid in the better determination of ovarian reserve in tertiary centers with available resources. FSH and AFC should perform fairly in poor resource and low socioeconomic setting. No tool can be deemed absolutely when it comes to ovarian reserve testing. More studies with larger sample size are required to establish the best mode of ovarian reserve testing.

Ethical approval

The study protocol was approved by the Independent Ethics Committee (IEC) of the institution (research subject number: ESIC/42/IEC/[JOKA]/2021).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Valvekar U, Lakshmi S, Kumar AN. Study of ovarian reserve parameters in infertile women of different age groups. *Int J Reprod Contracept Obstet Gynecol* 2016;5:2116-21.
2. Battikhi MN, Zqlam W, Banat E, Battikhi Q. Correlation of antimullerian hormone (AMH) and follicle stimulating hormone (FSH). *EC Microbiol* 2016;4:617-22.
3. 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;8:393-8.
4. 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.
5. Tal R, Seifer DB. Ovarian reserve testing: A user's guide. *Am J Obstet Gynecol* 2017;217:129-40.
6. Panchal S, Nagori C. Comparison of anti-mullerian hormone and antral follicle count for assessment of ovarian reserve. *J Hum Reprod Sci* 2012;5:274-8.
7. Nelson SM, Klein BM, Arce JC. Comparison of antimüllerian hormone levels and antral follicle count as predictor of ovarian response to controlled ovarian stimulation in good-prognosis

- patients at individual fertility clinics in two multicenter trials. *Fertil Steril* 2015;103:923-30.e1.
8. Vaze-Parab A, Bhatt P, Parihar M, Baldawa P. Anti-mullerian hormone, follicular phase follicle stimulating hormone and antralfollicular count as predictors of ovarian response in assisted reproductive technique cycles. *IVF Lite* 2014;1:32-5.
 9. Nayak PK, Mukherjee C, Mitra S. The relevance of antral follicle count (AFC) as a marker of ovarian reserve in normal and infertile women in a tertiary care centre in central India. *Int J Reprod Contracept Obstet Gynecol* 2018;7:4711-4.
 10. Lin YH, Chiu WC, Wu CH, Tzeng CR, Hsu CS, Hsu MI. Antimüllerian hormone and polycystic ovary syndrome. *Fertil Steril* 2011;96:230-5.
 11. Sjaarda LA, Mumford SL, Kissell K, Schliep KC, Hammoud AO, Perkins NJ, *et al.* Increased androgen, anti-müllerian hormone, and sporadic anovulation in healthy, eumenorrheic women: A mild PCOS-like phenotype? *J Clin Endocrinol Metab* 2014;99:2208-16.
 12. Tobler KJ, Shoham G, Christianson MS, Zhao Y, Leong M, Shoham Z. Use of Anti-mullerian hormone for testing ovarian reserve: A survey of 796 infertility clinics worldwide. *J Assist Reprod Genet* 2015;32:1441-8.
 13. Broer SL, Dólleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: A meta-analysis. *Hum Reprod Update* 2011;17:46-54.
 14. Caroppo E, Matteo M, Schonauer LM, Vizziello G, Pasquadibisceglie A, Vitti A, *et al.* Basal FSH concentration as a predictor of IVF outcome in older women undergoing stimulation with GnRH antagonist. *Reprod Biomed Online* 2006;13:815-20.
 15. Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, *et al.* Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: An individual patient data approach. *Hum Reprod Update* 2013;19:26-36.
 16. Anckaert E, Denk B, He Y, Torrance HL, Broekmans F, Hund M. Evaluation of the Elecsys® anti-Müllerian hormone assay for the prediction of hyper-response to controlled ovarian stimulation with a gonadotrophin-releasing hormone antagonist protocol. *Eur J Obstet Gynecol Reprod Biol* 2019;236:133-8.
 17. Sadruddin S, Barnett B, Ku L, Havemann D, Mucowski S, Herrington R, *et al.* Maternal serum concentration of anti-müllerian hormone is a better predictor than basal follicle stimulating hormone of successful blastocysts development during IVF treatment. *PLoS One* 2020;15:e0239779.
 18. Deb S, Jayaprakasan K, Campbell BK, Clewes JS, Johnson IR, Raine-Fenning NJ. Intraobserver and interobserver reliability of automated antral follicle counts made using three-dimensional ultrasound and SonoAVC. *Ultrasound Obstet Gynecol* 2009;33:477-83.
 19. te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum Reprod Update* 2002;8:141-54.
 20. 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;17:3065-71.
 21. Cook CL, Siow Y, Taylor S, Fallat ME. Serum müllerian-inhibiting substance levels during normal menstrual cycles. *Fertil Steril* 2000;73:859-61.
 22. Tremellen KP, Kolo M, Gilmore A, Lekamge DN. Error! Hyperlink reference not valid. *Aust NZ J Obstet Gynaecol* 2005;45:20-4.
 23. 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;77:357-62.
 24. Elgindy EA, El-Haieg DO, El-Sebaey A. Anti-Müllerian hormone: Correlation of early follicular, ovulatory and midluteal levels with ovarian response and cycle outcome in intracytoplasmic sperm injection patients. *Fertil Steril* 2008;89:1670-6.
 25. Jain S, Shukla RC, Jain M, Singh U, Singh TB. A correlation between antral follicle count and anti-müllerian hormone in healthy Indian women of reproductive age. *J South Asian Fed Obstet Gynaecol* 2022;14:1-5.
 26. Suardi D, Permadi W, Djuwantono T, Hidayat YM, Bayuaji H, Edo Gautama GP. Correlation of serum anti-Mullerian hormone (AMH) level on ovarian volume in women with endometrioma. *Int J Gen Med* 2021;14:1-8.
 27. Anuradha K, Partha Majumder S, Shiffin R. Correlation of anti mullerian hormone and antral follicular count in ovarian reserve testing. *Obstet Gynecol Res* 2022;5:170-4.
 28. Muttukrishna S, Yussoff H, Naidu M, Barua J, Arambage K, Suharjono H, *et al.* Serum anti-müllerian hormone and inhibin B in disorders of spermatogenesis. *Fertil Steril* 2007;88:516-8.
 29. Permadi W, Wahyu Ferdian M, Tjahyadi D, Ardhana Iswari W, Djuwantono T. Correlation of anti-mullerian hormone level and antral follicle count with oocyte number in a fixed-dose controlled ovarian hyperstimulation of patients of *in vitro* fertilization program. *Int J Fertil Steril* 2021;15:40-3.
 30. Tran ND, Cedars MI, Rosen MP. The role of anti-müllerian hormone (AMH) in assessing ovarian reserve. *J Clin Endocrinol Metab* 2011;96:3609-14.
 31. Arvis P, Rongières C, Pirrello O, Leher P. Reliability of AMH and AFC measurements and their correlation: A large multicenter study. *J Assist Reprod Genet* 2022;39:1045-53.
 32. Iwase A, Nakamura T, Osuka S, Takikawa S, Goto M, Kikkawa F. Anti-müllerian hormone as a marker of ovarian reserve: What have we learned, and what should we know? *Reprod Med Biol* 2016;15:127-36.