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Original Research Article

Comparison Between AMH and AFC For Assessment of Ovarian Reserve

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Abstract: ***Background***: Anti-Müllerian hormone (AMH) is generally known to have a positive relationship with antral follicle count (AFC), and both markers are commonly used to assess ovarian reserve. Although AMH and AFC are considered useful predictors of ovarian response, their reliability and reproducibility remain subjects of ongoing evaluation. ***Methods:*** The present study included 50 patients who attended the hospital for infertility evaluation and treatment. The correlation between serum AMH levels and total AFC was analyzed using Karl Pearson's correlation coefficient. A p-value of less than 0.05 was considered statistically significant. ***Results:*** A total of 50 patients were enrolled in the study, with a mean age of 34.1 years. The mean body mass index (BMI) was 27.1 kg/m², and the average duration of infertility was 3.6 years. The mean ± SD of AFC was 6.41 ± 9.65 in the 26–30 years age group, 5.60 ± 4.42 in the 31–35 years age group, and 2.76 ± 2.34 in the 36–40 years age group, showing a statistically significant difference ($p = 0.012$). Similarly, the mean ± SD of AMH levels was 2.10 ± 1.98 ng/ml in the 26–30 years age group, 1.83 ± 2.19 ng/ml in the 31–35 years age group, and 0.49 ± 0.72 ng/ml in the 36–40 years age group. ***Conclusion:*** AMH is considered a reliable marker for evaluating ovarian reserve. However, due to the strong correlation between AMH and AFC, AFC may serve as an alternative marker in patients with financial constraints, as AMH testing is relatively more expensive.

Keywords: AMH, AFC, infertility, pregnancy.

INTRODUCTION

Ovarian reserve refers to a woman's reproductive potential and reflects both the number and quality of oocytes present in the ovaries.[1] It is influenced by several factors including age, genetic background, and environmental conditions.[2] Anti-Müllerian hormone (AMH) is predominantly produced by granulosa cells of secondary, pre-antral, and small antral follicles measuring up to 6 mm in diameter; therefore, AMH levels are considered to reflect the population of these developing follicles.[3] The number of antral follicles within the ovaries is proportionate to the size of the primordial follicle pool from which they originate.[4] Consequently, antral follicle count (AFC) is regarded as an indicator of the quantitative component of ovarian aging.[5]

Generally, AMH levels show a positive association with AFC. Women with a good ovarian reserve typically demonstrate higher AMH levels along with greater AFC values, whereas those with diminished ovarian reserve tend to have lower levels of both

markers. A meta-analysis has also demonstrated a negative relationship between body mass index (BMI) and AMH levels across different study populations.[6] Although AMH levels may fluctuate slightly during different phases of the menstrual cycle, with a modest reduction observed during the luteal phase, measurement of AMH is generally considered cycle-independent for clinical use.[7] Despite the steady age-related decline in AMH, considerable variability in AMH levels may still occur among women of the same age group.[8]

Similarly, AFC can vary during the menstrual cycle and is therefore ideally measured during the early follicular phase. Although some degree of inter-cycle variation exists, it is usually regarded as clinically insignificant when predicting ovarian response in IVF cycles.[9] In the present study, data were collected from patients undergoing treatment with in vitro fertilization or intracytoplasmic sperm injection (IVF/ICSI) at the reproductive center of our hospital. The study evaluated the relationship between serum AMH levels, ovarian antral follicle count (AFC), body mass index (BMI), age, and other factors in relation to ovarian response. Although AMH testing is simple and demonstrates minimal variation within the menstrual cycle, it is affected by assay variability and the absence of a universally standardized testing method.[10,11,12]

Assessment of ovarian reserve is an essential component in the evaluation and management of infertility, particularly in women undergoing assisted reproductive techniques. Accurate estimation of ovarian reserve helps clinicians predict ovarian response to stimulation and select the most appropriate ovulation induction protocol. Although both Anti-Müllerian Hormone (AMH) and Antral Follicle Count (AFC) are widely used markers for assessing ovarian reserve, variability in their measurements and differences in their predictive accuracy necessitate comparative evaluation. Therefore, comparing AMH and AFC may help determine the more reliable indicator for assessing ovarian reserve and guiding infertility treatment strategies, ultimately improving pregnancy outcomes in infertile women.

MATERIALS AND METHODS

The study was an observational study. It was conducted in SKIMS MCH Srinagar over a period of 4 months from January 2023 to April 2023. Total 50 patients who visited routine infertility centre of Department of Obstetrics and Gynaecology, SKIMS MCH Srinagar over a period of one month, were included in the study.

Data collected included age, BMI, AMH, AFC. The study was conducted over a period of 4 months from January 2023 to April 2023. Inclusion criteria: the patients included patients with age ranging from 26 to 40 years, duration of infertility more than 1 year.
Exclusion criteria: history of ovarian cystectomy, PID, endometriosis, POF, no underlying comorbidity, PCOD.

Blood samples from all patients were stored at -20°C and tested using an enzyme-linked immunosorbent assay kit .The correlation coefficient, $r \geq 0.9900$; the relative deviation of the assay results, within 10%; and the coefficient of variation (CV), ≤10%.
AFC measurement: The antral follicle count (AFC) numbers were counted using the Color Doppler ultrasonic diagnostic apparatus on day 2.

Statistical Methods: Total of 50 patients were included in the study with mean age of patients being 34.1 The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS Version 20.0 (SPSS Inc., Chicago, Illinois, USA). Statistical software SPSS (version 20.0) and Microsoft Excel were used to carry out the statistical analysis of data. Continuous variables were expressed as Mean±SD and categorical variables were summarized as percentages. Analysis of variance (ANOVA) test was used for comparing continuous variables. Karl Pearson's correlation coefficient was employed for assessing correlation between AMH levels and Total AFC. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1: Age distribution of study patients

Age	Number	Percentage
26-30 Years	14	28
31-35 Years	15	30
36-40 Years	21	42
Total	50	100
Mean±SD=34.1±4.27		

Table 1 shows the age distribution of the study participants. Out of 50 women included in the study, the majority belonged to the 36–40 years age group (21 patients, 42%), followed by 31–35 years (15 patients, 30%) and 26–30 years (14 patients, 28%). The mean age of the participants was 34.1 ± 4.27 years, indicating that most women were in the later reproductive age group. This age distribution is relevant because ovarian reserve markers such as AMH and AFC decline with increasing age, making their assessment important for selecting appropriate ovulation

induction strategies and improving pregnancy outcomes in infertile women.

Table 2: Baseline characteristics of study patients	
Parameter	**Mean±SD**
BMI (Kg/m^2)	27.1±2.43
Duration of infertility (Years)	3.6±1.89
Total ovarian volume	7.3±2.18

Table 2 presents the baseline characteristics of the study participants. The mean BMI of the patients was 27.1 ± 2.43 kg/m², indicating that most women were in the overweight range. The mean duration of infertility was 3.6 ± 1.89 years, suggesting that the majority of participants had experienced infertility for several years before evaluation. The mean total ovarian volume was 7.3 ± 2.18, reflecting the overall ovarian size among the study population. These baseline parameters provide important clinical background for assessing ovarian reserve using AMH and AFC, which can assist clinicians in selecting appropriate ovulation induction strategies.

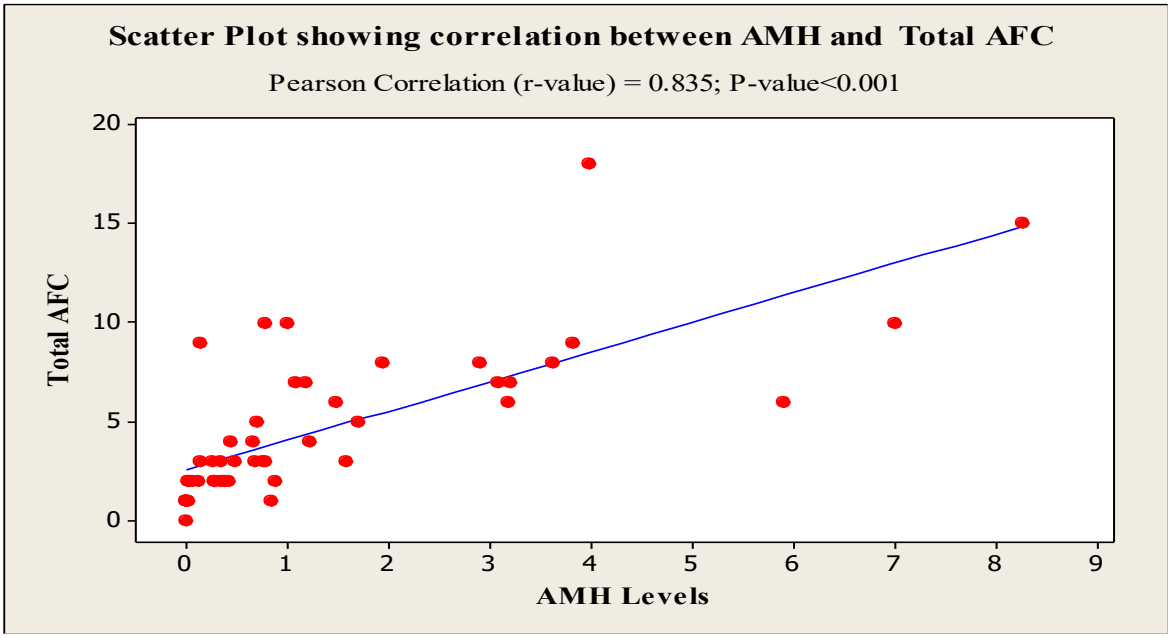
Table 3: Distribution of study patients according to serum AMH level							
Serum AMH (ng/ml)	**26-30 Years**		**31-35 Years**		**36-40 Years**		**P-value**
	No.	**%age**	**No.**	**%age**	**No.**	**%age**	
< 1.0 (Low)	6	42.9	8	53.3	18	85.7	0.017*
1.0-3.5 (Normal)	5	35.7	5	33.3	3	14.3	
> 3.5 (High)	3	21.4	2	13.3	0	0.0	
Mean±SD	2.10±1.98		1.83±2.19		0.49±0.72		

***Statistically Significant (P-value <0.05)**

Table 3 shows the distribution of study participants according to serum Anti-Müllerian Hormone (AMH) levels across different age groups. Low AMH levels (<1.0 ng/ml), indicating reduced ovarian reserve, were more frequently observed in the 36–40 years age group, where 18 patients (85.7%) had low AMH levels. In comparison, 8 patients (53.3%) in the 31–35 years group and 6 patients (42.9%) in the 26–30 years group had low AMH levels.

Normal AMH levels (1.0–3.5 ng/ml) were observed in 35.7% of women aged 26–30 years, 33.3% of women aged 31–35 years, and 14.3% of women aged 36–40 years. High AMH levels (>3.5 ng/ml) were seen only in younger age groups, with 21.4% in the 26–30 years group and 13.3% in the 31–35 years group, while none of the women aged 36–40 years had high AMH levels.

The mean AMH levels decreased with increasing age, being 2.10 ± 1.98 ng/ml in the 26–30 years group, 1.83 ± 2.19 ng/ml in the 31–35 years group, and 0.49 ± 0.72 ng/ml in the 36–40 years group. The association between age and AMH levels was statistically significant (p = 0.017), indicating that ovarian reserve declines significantly with advancing age.



The scatter plot demonstrates the correlation between serum Anti-Müllerian Hormone (AMH) levels and Total Antral Follicle Count (AFC) among the study participants. The analysis shows a strong positive correlation

between AMH and AFC (r = 0.835), which is statistically significant (p < 0.001). This indicates that women with higher AMH levels tend to have a higher antral follicle count, reflecting better ovarian reserve. Conversely, lower AMH levels are associated with lower AFC values, suggesting diminished ovarian reserve.

Therefore, the strong correlation observed in the present study supports the usefulness of AMH as a reliable biochemical marker of ovarian reserve, which closely corresponds with the ultrasonographic parameter AFC in the assessment of ovarian function in infertile women.

Table 4: Distribution of study patients according to total AFC level

Total AFC	26-30 Years		31-35 Years		36-40 Years		P-value
	No.	%age	No.	%age	No.	%age	
< 5 (Low)	6	42.9	8	53.3	17	81.0	0.012*
5-10 (Normal)	5	35.7	4	26.7	3	14.3	
> 10 (High)	3	21.4	3	20.0	1	4.8	
Mean±SD	6.41±9.65		5.60±4.42		2.76±2.34		

***Statistically Significant (P-value<0.05)**

Table 4 shows the distribution of study participants according to total Antral Follicle Count (AFC) across different age groups. Low AFC (<5), indicating reduced ovarian reserve, was most commonly observed in the 36–40 years age group, where 17 patients (81.0%) had low AFC. In comparison, 8 patients (53.3%) in the 31–35 years group and 6 patients (42.9%) in the 26–30 years group had low AFC.

Normal AFC (5–10) was observed in 35.7% of women aged 26–30 years, 26.7% of women aged 31–35 years, and 14.3% of women aged 36–40 years. High AFC (>10) was more frequently seen in younger women, with 21.4% in the 26–30 years group, 20.0% in the 31–35 years group, and only 4.8% in the 36–40 years group.

The mean AFC decreased with increasing age, being 6.41 ± 9.65 in the 26–30 years group, 5.60 ± 4.42 in the 31–35 years group, and 2.76 ± 2.34 in the 36–40 years group. The association between age and AFC levels was statistically significant (p = 0.012), indicating that AFC declines significantly with advancing age.

DISCUSSION

Assessment of ovarian reserve is a fundamental component in infertility evaluation and in predicting the response to assisted reproductive technologies such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Several biochemical and ultrasonographic markers have been proposed for the evaluation of ovarian reserve; however, Anti-Müllerian Hormone (AMH) and Antral Follicle Count (AFC) are currently regarded as the most reliable predictors of ovarian response and follicular reserve.

In the present study, the majority of patients belonged to the 36–40 years age group, followed by 31–35 years and 26–30 years age groups. The mean age of the study population was 34.1 years, indicating that most women seeking infertility treatment were in the later reproductive age group. Similar age distribution was reported by Anuradha K et al., (2022)13, where the largest proportion of respondents (36.7%) belonged to the 35–40 years age group, followed by 25–29 years (30.8%) and 30–34 years (24.2%). Likewise, Bhowmik J et al., (2021)14 also reported that the highest proportion of infertility patients belonged to the 36–40 years age group (36.5%), indicating that many women seek infertility evaluation during the later reproductive years. These findings support the observation that advancing maternal age is strongly associated with declining ovarian reserve, which subsequently affects fertility potential. Previous studies by Tal R and Seifer DB (2017)15 have also emphasized that ovarian reserve testing becomes particularly important in women above 30 years of age due to the progressive depletion of the ovarian follicular pool.

In the present study, the mean BMI was 27.1 kg/m², and the mean duration of infertility was 3.6 years. Comparable findings were reported by Anuradha K et al., (2022)13, who observed a mean BMI of 26.61 ± 1.96 kg/m² and mean duration of infertility of 3.75 ± 1.64 years among infertile women undergoing ovarian reserve testing. Similarly, El-Shorbagy SH (2017)16 reported mean BMI values around 26.61 ± 1.96 kg/m² and a mean duration of infertility of 3.75 ± 1.64 years, which is consistent with the findings of the present study. These findings suggest that most infertility patients present with a relatively similar clinical profile in terms of body mass index and duration of infertility.

Etiological factors of infertility observed in the referenced study included tubal factor infertility (21.7%), male factor infertility (20%), PCOS (16.7%), endometriosis (5%), and unexplained infertility (18.3%). Similar distribution of etiological factors was reported by Shembekar CA et al., (2017)17 who found tubal factor infertility in 22%, male factor infertility in

20%, PCOS in 17%, endometriosis in 5%, and unexplained infertility in 18% of cases.

The present study demonstrated that serum AMH levels declined progressively with increasing age. The mean AMH values decreased from 2.10 ± 1.98 ng/ml in women aged 26–30 years to 0.49 ± 0.72 ng/ml in women aged 36–40 years, indicating a significant reduction in ovarian reserve with advancing age. Similar findings were reported by Anuradha K et al., (2022)13, who observed that low AMH levels (<1 ng/ml) were increasingly common with advancing age, particularly in the 35–40 years age group, where 47.7% of women exhibited low AMH levels. Their study also demonstrated a progressive decline in mean AMH values from 2.67 ± 0.80 ng/ml in younger women to 1.17 ± 1.06 ng/ml in women aged 35–40 years.

Further evidence supporting age-related decline of AMH was provided by Jain S et al., (2022)18, who reported a strong negative correlation between AMH and age ($r = -0.824$, $p<0.001$). Their study also demonstrated that AMH levels decline progressively during reproductive life due to gradual depletion of the primordial follicle pool. These findings are consistent with previous literature. Tal R and Seifer DB (2017)15 reported that AMH is a sensitive marker of ovarian reserve and declines progressively with age as the ovarian follicular pool decreases. Similarly, Moolhuijsen LME, Visser JA (2020)12 reported that AMH reflects the number of small growing follicles and therefore serves as an accurate biochemical indicator of ovarian reserve.

In the present study, AFC also demonstrated a decline with advancing age, with the mean AFC decreasing from 6.41 ± 9.65 in women aged 26–30 years to 2.76 ± 2.34 in women aged 36–40 years, and this difference was statistically significant ($p = 0.012$). Similar findings were reported by Anuradha K et al., (2022)13, where low AFC (<5) was most commonly observed in older women, particularly those aged 35–40 years (22.7%). Their study also showed that the mean AFC decreased progressively with age from 12.5 ± 1.7 in younger women to 7.5 ± 3.3 in older women. Likewise, Bhowmik J et al., (2021)14 reported that low AFC levels were more prevalent in women aged 36–40 years, supporting the concept that AFC decreases with advancing age due to depletion of the ovarian follicular pool. Similarly, Jain S et al., (2022)18 demonstrated a negative correlation between AFC and age ($r=-0.403$, $p < 0.001$), indicating that AFC declines progressively as women age. They also reported that age accounted for approximately 16.3% of the variation in AFC, suggesting that additional factors such as genetic, nutritional, and environmental influences may also affect follicular count.

In the present study, a strong positive correlation between AMH and AFC was observed ($r = 0.835$, $p < 0.001$). This indicates that women with higher AMH levels tend to have higher antral follicle counts, reflecting better ovarian reserve. Comparable findings were reported by Anuradha K et al., (2022)13, who observed that AMH and AFC are reliable predictors of ovarian reserve and are useful indicators in infertility evaluation. However, Jain S et al., (2022)18 reported a weak positive correlation between AMH and AFC ($r = 0.328$, $p<0.001$). They suggested that although both markers reflect ovarian reserve, they may represent different biological aspects of ovarian follicular development. Further evidence supporting this concept was provided by de Vet et al., (2002)19 who reported stronger correlations between AMH and AFC in smaller cohort studies. Differences in the strength of correlation between these markers across studies may be attributed to differences in study population, sample size, measurement techniques, and assay variability. Additionally, Bentzen JG et al., (2013)20 demonstrated that AFC may explain a large proportion of the variation in AMH levels, suggesting that both markers are closely related but not entirely interchangeable.

CONCLUSION

Anti-Müllerian Hormone (AMH) demonstrated a stronger association with age compared to AFC and may therefore serve as a better indicator of ovarian aging. Although AMH is widely regarded as a more effective predictor of ovarian response, several studies suggest that both AMH and AFC provide comparable accuracy and clinical usefulness in predicting ovarian response. Consequently, many authors propose that AFC can be used as an alternative to AMH, particularly in settings where the cost of AMH testing is a limiting factor.

Limitations: The study included as maller number of patients and had a shorter follow up period.For proper validation of these conclusions, a long term prospective clinical study with large sample size and longer follow up is required.

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