ORIGINAL ARTICLE

A Correlation between Antral Follicle Count and Anti-Müllerian Hormone in Healthy Indian Women of Reproductive Age

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Abstract

Aim: To determine which of the two parameters between antral follicle count (AFC) and anti-Müllerian hormone (AMH) had better correlation with age in healthy females, and also to estimate the strength of correlation between AMH and AFC.

Materials and methods: This was a prospective, cross-sectional study comprising 1,181 fertile women of age 20–40 years, who were divided into four age groups, i.e., group I (20–24 years), group II (25–29 years), group III (30–34 years), and group IV (35–40 years). AFC and AMH were measured on third day of menstrual cycle. Pearson correlation and linear regression analysis were used. Statistical Package for Social Sciences, trial version 20, was used for the statistical analysis. A *p*-value of <0.05 was considered statistically significant.

Results: The correlation coefficients between AFC and age, AMH and age, AMH and AFC were $r = -0.403$, $r = -0.824$ and $r = 0.328$; $p < 0.001$, respectively. A strong positive correlation ($r = 0.986$, $p < 0.001$) was noted between AMH and age in group I, while strong negative correlations ($p < 0.001$) were noted in other groups. The correlations between AFC and age ($r = -0.177$) and AMH and AFC ($r = 0.175$) were significant ($p < 0.05$) only in group IV. Age accounted for 16.3% variation in AFC and 67.8% variation in AMH.

Conclusion: AMH correlated better with age than AFC. There was a weak correlation between AMH and AFC.

Clinical significance: The counselling of a woman about her reproductive potential should be based on both AFC and AMH taken together, apart from chronological age, to avoid false sense of security or unnecessary alarm.

Keywords: Anti-Müllerian hormone, Antral follicle count, Cross-sectional study, Fertile women, Reproductive aging.

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Introduction

There is an ongoing demand among women for the estimation of ovarian reserve and reproductive potential, primarily due to delayed childbearing.[1,2] Among various ovarian reserve tests, antral follicle count (AFC) and anti-Müllerian hormone (AMH) are preferred over others.[3–5] Their levels vary with age and so have been used as markers of ovarian aging in normal healthy females.[6,7] Though the two parameters have been found to be positively associated with each other in normo-ovulatory premenopausal women,[6] reports of discordance between the two parameters have been reported in infertile women.[8,9] AFC has standard recommendations for its measurement,[10] but is operator-dependent and shows clinically significant variability during the menstrual cycle.[3] Although AMH is simple to measure and shows minimal intracycle variability, it suffers from assay variability and lack of standardized international assay.[3,11,12] Therefore, the objectives of this study were to determine which of these two parameters, i.e., AFC and AMH, had better correlation with age in healthy females and also to estimate the strength of correlation between AFC and AMH.

Materials and Methods

This cross-sectional study was performed at the Department of Radiodiagnosis and Imaging, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The study was done in accordance with the declaration of Helsinki guidelines on good clinical practice and after approval of the institute ethical committee. The normal healthy females (n = 1,471) with proven natural fertility (at least one-term pregnancy) were prospectively recruited from the outpatient department (OPD) of the Department of Obstetrics and Gynaecology where they had come for preconcepptional counseling, contraception, family planning, or routine health check-up.

The inclusion criteria were regular menstrual cycle (length: 25–35 days) with <5 days' difference between cycles, age 20–40 years, no hirsutism, serum luteinizing hormone (LH)/follicle stimulating hormone (FSH) <2, and presence of both ovaries.[13] The exclusion criteria were history of hormone administration in

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the previous 6 months, pelvic inflammatory disease or ovarian surgery, ovarian endometrioma, premature ovarian failure, uterine malformations or uterine pathology, known systemic, metabolic, and endocrine disease including hyperandrogenism. Although women with polycystic ovarian syndrome (PCOS) were excluded on the basis of clinical and biochemical criteria, those having only polycystic ovarian (PCO) morphology on ultrasound [presence of ≥12 follicles, 2–9 mm in diameter, and/or increased ovarian volume (>10 mL)] were included.[13] Women with poor ultrasound visualization of ovaries, because of retrouterine or abnormal position and the presence of at least one of cysts ≥20 mm, were excluded subsequently. Finally, 1,181 fertile females were included in the study. The subjects were further divided into four age groups, i.e., group I (20–24 years), group II (25–29 years), group III (30–34 years), and group IV (35–40 years) (Table 1). Informed consent was obtained from all the subjects.

All the relevant clinical data of the study subjects including biometry were acquired. The hormonal assays and transvaginal ultrasound (TVUS) were done on the second or third day of the menstrual cycle. Among the biochemical parameters, LH, FSH, triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH), free testosterone, and prolactin levels were documented. The serum AMH was measured using Gen II AMH Enzyme-linked Immunosorbent Assay (ELISA; Beckman Coulter, USA). All sonographic measurements were performed by the same investigator using a 7.5-MHz transvaginal transducer (Diagnostic ultrasound, iU22, Philips Medical System, California, United States). Thorough survey of each ovary was done by scanning from the outer to the inner margin. All follicles having adequate morphology as described for a healthy follicle (i.e., 2–10 mm size range of well-defined anechoic cysts with smooth margins and absence of internal septations or nodularity) were measured and counted in each ovary.[10] The sum of both the counts was labelled as the antral follicle count (AFC). Follicular size was measured using the internal diameters of the area. The mean of two perpendicular measurements was taken as the follicular size.

Statistical Analysis

Descriptive statistics was calculated in the form of mean ± standard deviation. Student's *t*-test/Mann-Whitney *U* test was applied to find out the significant difference in the mean values between the groups. Pearson correlation and linear regression analysis were done to find out the relationship between the study variables. The statistical analysis was done by SPSS (Statistical Package for Social Sciences), trial version 20. A *p*-value of <0.05 was considered statistically significant at two-tailed test.

Results

Of 1,181 fertile females included in the study, there were 251 (21.3%) females in group I, 399 (33.8%) in group II, 282 (23.9%) in group III, and 249 (21.0%) in group IV. They had mean age of 29.28 ± 5.47 years with mean BMI 23.93 ± 2.48 kg/m^2, mean AFC 13.66 ± 5.98 follicles, and mean AMH 2.78 ± 1.19 ng/mL. The mean AFC and mean AMH in different age groups have been shown in Table 1. AFC showed a linear pattern of decline with age (Fig. 1) whereas AMH demonstrated a nonlinear pattern of age-related decline, best-fitted by a polynomial function (Fig. 2). There was a modest negative correlation of AFC with age ($r = -0.403$, $p < 0.001$; Fig. 1). AMH showed strong negative correlation with age ($r = -0.824$, $p < 0.001$; Fig. 2) and weak positive correlation with AFC ($r = 0.328$, $p < 0.001$; Fig. 3). The correlation between age, AFC, and AMH in different age groups has been shown in Table 2. Age explained 16.3% variation in AFC and 67.8% variation in AMH.

Discussion

AMH has been labeled as "molecule of the moment" in the field of reproductive endocrinology due to its role in the management of the various clinical conditions associated with female reproduction.[14] However, determination of normative value of AMH in healthy Indian women especially in relationship with reproductive aging

Table 1: Comparison of mean AFC and mean AMH in different age groups of fertile women (*n* = 1,181)

	Age groups				*Total*
	I (20–24 years)	*II (25–29 years)*	*III (30–34 years)*	*IV (35–40 years)*	*20–40 years*
Number (%)	251 (21.3)	399 (33.8)	282 (23.9)	249 (21.0)	1,181 (100.0)
AFC (*n*) (mean ± SD)	16.85 ± 6.45	14.66 ± 5.64	12.54 ± 5.10	10.14 ± 4.57	13.66 ± 5.98
AMH (ng/mL) (mean ± SD)	3.35 ± 0.53	3.79 ± 0.24	2.59 ± 0.50	0.80 ± 0.39	2.78 ± 1.19

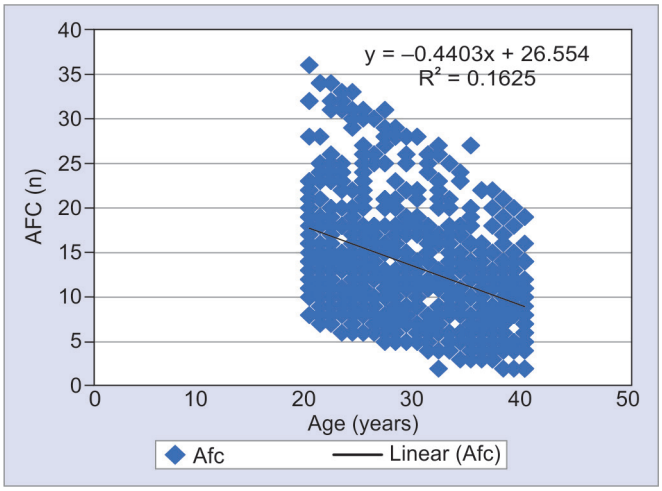

Fig. 1: Relationship between AFC and age in fertile women

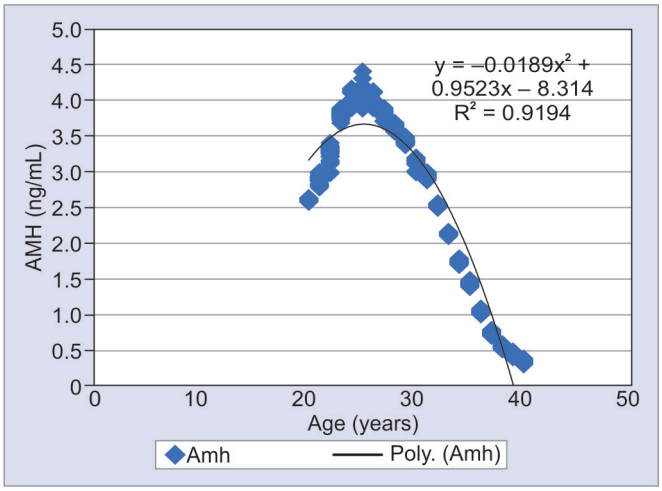

Fig. 2: Relationship between serum AMH and age in fertile women

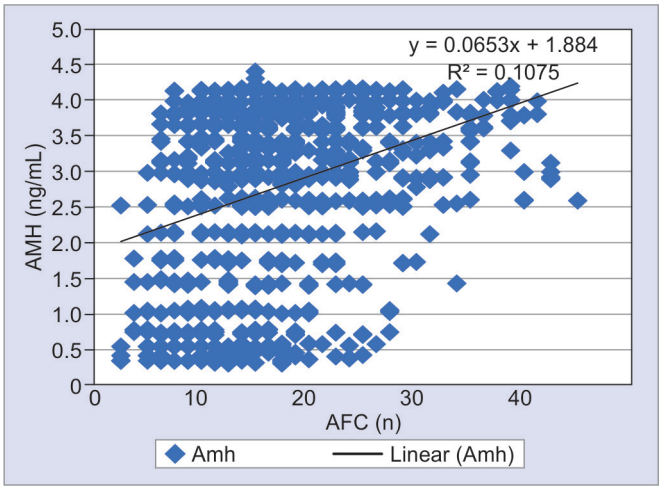

Fig. 3: Relationship between serum AMH and AFC in fertile women

Table 2: Correlation between age, AFC and AMH in different age groups of fertile women ($n = 1,181$)

Group I (20–24 years)		**AGE**	**AFC**	**AMH**
AGE	*r*	1	−0.103	0.986
	p	—	0.103	0.000
AFC	*r*	−0.103	1	−0.090
	p	0.103	—	0.153
AMH	*r*	0.986	−0.090	1
	p	0.000	0.153	—
Group II (25–29 years)				
AGE	*r*	1	−0.089	−0.976
	p	—	0.077	0.000
AFC	*r*	−0.089	1	0.055
	p	0.077	—	0.270
AMH	*r*	−0.976	0.055	1
	p	0.000	0.270	—
Group III (30–34 years)				
AGE	*r*	1	-0.116	-0.990
	p	—	0.052	0.000
AFC	*r*	−0.116	1	0.099
	p	0.052	—	0.098
AMH	*r*	−0.990	0.099	1
	p	0.000	0.098	—
Group IV (35–40 years)				
AGE	*r*	1	−0.177	−0.964
	p	—	0.005	0.000
AFC	*r*	−0.177	1	0.175
	p	0.005	—	0.006
AMH	*r*	−0.964	0.175	1
	p	0.000	0.006	—

"*r*" represents Pearson correlation coefficient; "*p*" value indicates significance of change (*t*-test)

(age dependent loss of female fertility due to decline in oocyte/follicle pool) has not been established. Since chronological age at the final stage of reproductive aging (menopause) showed a great difference, it might be due to the difference in stock of oocyte/follicle at a given age. In order to evaluate the reproductive aging, i.e., ovarian reserve (oocyte/follicle pool), we have assessed the endocrinal (AMH) and sonographic markers (AFC) in healthy female population and correlated with chronological age in different age groups.

We also included the women having polycystic ovaries on ultrasound but with normal clinical and biochemical parameters, thus ruling out PCOS.[13,15,16] The mean AFC of the subjects was 13.66 ± 5.98 follicles and their mean AMH was 2.78 ± 1.19 ng/mL.

The linear pattern of decline of AFC with age observed in our study was similar to that reported by La Marca et al.[14] In this study, AFC showed a weak negative correlation with age ($r = -0.403$, $p < 0.001$). However, in a longitudinal study conducted on 81 healthy women at mean time interval of 4 years, AFC was noted to have strong negative correlation with age at both the visits ($r = -0.74$, $p \leq 0.001$ at visit 1 and $r = -0.79$, $p \leq 0.001$ at visit 2).[7] This might be due to the smaller sample size studied by the authors and inclusion of subjects with polycystic ovarian morphology by us.[15,16] For separate age groups in our study, the correlation was significant only for group IV (35–40 years) and that was also weak ($r = -0.177$, $p = 0.005$; Table 2). Age accounted for 16.3% variation in AFC in this study. However, it varied from 12 to 37% in other studies.[14,17] On the one hand, this highlighted the technical limitations involved in counting of antral follicles. On the other hand, this indicated the presence of many factors like genetic, nutritional, and environmental besides age that could influence the quantity of the antral follicles.

We noted a nonlinear pattern of decline of AMH with age and the polynomial function was the best-fit. This pattern of age-related decline in AMH was similar to that reported by other authors.[18–21] Though some researchers found the quadratic function to render the best-fit,[22–25] all agreed to the fact that the age-related decline of AMH followed a nonlinear pattern. In the current study, AMH showed strong negative correlation with age ($r = -0.824$, $p < 0.001$). This was comparable to the findings of Rooij et al. who also noted strong negative correlation between AMH and age at both the visits ($r = -0.66$, $p \leq 0.001$ at visit 1 and $r = -0.65$, $p \leq 0.001$ at visit 2).[7] However, some authors had reported even less degree of correlation between the two parameters.[6,26] Among our age groups, the correlation was positive for group I (20–24 years) and negative for the rest, though it was very strong ($p < 0.001$) for all the age groups (Table 2). Lie Fong et al.[26] found weak positive correlation for <15.8 years ($r = 0.18$, $p = 0.007$) and modest negative correlation ($r = -0.47$, $p < 0.001$) for ≥25 years groups. The correlation for 15.8–25 years was not significant ($r = -0.33$, $p = 0.66$) in their study. Such variations in the degree of correlation between AMH and age might be attributed to the variations in the AMH assays, sample size, and characteristics of the study population. However, the consistent observation from these studies was the decline of AMH in a woman after the age of about 25 years. This finding was in agreement with other studies where the investigators had noted that the decline in AMH started in the third decade of life and continued till menopause.[19,27–29] This decline in AMH with age suffered a rapid drop at 35–36 years of age[28] or accelerated after 40 years of age.[30] Iyer et al. in their retrospective study also found a significant inverse relationship between age and AMH levels between 27 and 41 years of age, and stressed the need for AMH testing after 27 years in order to assess the ovarian reserve.[28]

Several authors studied this age-related behavior of AMH in detail and claimed that AMH levels got considerably reduced, sometimes becoming undetectable, about 5–10 years prior to

menopause and thus could be used for prediction of the age at menopause about a decade earlier.[18,31]

In our study, age explained 67.8% variation in AMH. While Lie Fong et al.[26] and Kelsey et al.[19] reported 41 and 34% variations, respectively, only 9% variation was reported by Bentzen et al.[17] This finding suggested that AMH levels were affected by various genetic, nutritional, and environmental factors other than age. Adding to this, de Kat et al.[30] in their longitudinal study on 3,326 subjects demonstrated that there was considerable variation in the levels of AMH in women of the same age, and the differences between low and high age-specific AMH levels were less at higher age.

It was clear from our study that AMH showed better correlation with age than AFC. However, as per Rooij et al.,[7] this cross-sectional relationship could not be used as the sole criterion to infer which marker was better for the assessment of ovarian aging. According to them, in order to decide that whether a marker was ideal for evaluation of age-related reproductive decline, it needed to fulfil four requirements. First, it should have the ability to reflect the entire follicle pool. Second, it should be clearly associated with age. Third, it should change with time, preferably during 30–50 years of age. Fourth, it should show consistent variation from the mean in a woman. In their longitudinal study, the correlation between AFC and age was greater than that between AMH and age at both the visits of the subjects ($r = -0.74$ vs $r = -0.66$ at visit 1, $r = -0.79$ vs $r = -0.65$ at visit 2 after mean time interval of 4 years), but AMH fared better than AFC in all other requirements. Considering the fourth requirement of consistent variation from the mean as the most important one, they proposed AMH as a better marker of reproductive decline than AFC. However, de Kat et al. opined that there was more to fertility than ovarian reserve, and in their recent review, they raised concern regarding the use of AMH and other markers as "fertility tests" for prediction of the menopausal age which might unfortunately mislead a woman about her remaining reproductive potential.[30,32]

In the current study, AMH showed weak positive correlation with AFC ($r = 0.328$, $p < 0.001$). For separate age groups, this correlation was significant ($r = 0.175$, $p = 0.006$) only for group IV (35–40 years). The stronger correlation observed by de Vet et al. ($r = 0.66$, $p < 0.001$ at visit 1 and $r = 0.71$, $p < 0.001$ at visit 2) than ours might be attributed to the smaller sample size in their study ($n = 41$).[6] In our study, only 10.8% variation of AMH was explained by AFC. This finding differed considerably from that of Bentzen et al.[17] who noted that AFC accounted for 74% variation of AMH. Thus, AMH was affected not only by the quantity of antral follicles but also by their quality.[17] It had already been shown in infertile women that the discordance between AMH and AFC existed not only due to the technical limitations in counting of the follicles and analytical variability of the AMH assay used but also due to patient-specific factors such as body mass index, PCOS, socioeconomic status, environmental/nutritional factors like vitamin D status.[8] The reason for similar discordance observed in women with normal reproductive performance remains to be explained.

The limitations of this study need to be mentioned. First, its cross-sectional nature was the major drawback. Second, it was a single-center, tertiary hospital-based study and so the results could not be generalized at the community level. Future multicentric population-based studies having longitudinal study design and larger sample size are needed for validation of our results. Third, the sample size for different age groups was relatively small.

Conclusion

AMH showed better correlation with age than AFC and, therefore, could be a better marker of ovarian aging. The weak correlation obtained between AMH and AFC indicated that both the markers reflected different aspects of ovarian reserve and their results needed to be interpreted in combination. Thus, none of the two markers might be sufficient as a single marker for the counselling of a woman regarding her current or future reproductive capacity.

Clinical Significance

The counselling of a woman about her reproductive potential can be done on the basis of both AFC and AMH taken together apart from chronological aging to avoid false sense of security or unnecessary alarm. This would also help them in planning their family and assist the infertility specialists in the management of infertility by Assisted Reproductive Technology.

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