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Age specific serum anti-Müllerian hormone levels in 1,298 Korean women with regular menstruation

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Objective: To determine the age specific serum anti-Müllerian hormone (AMH) reference values in Korean women with regular menstruation.
Methods: Between May, 2010 and January, 2011, the serum AMH levels were evaluated in a total of 1,298 women who have regular menstrual cycles aged between 20 and 50 years. Women were classified into 6 categories by age: 20-31 years, 32-34 years, 35-37 years, 38-40 years, 41-43 years, above 43 years. Measurement of serum AMH was measured by commercial enzyme-linked immunoassay.
Results: The serum AMH levels correlated negatively with age. The median AMH level of each age group was 4.20 ng/mL, 3.70 ng/mL, 2.60 ng/mL, 1.50 ng/mL, 1.30 ng/mL, and 0.60 ng/mL, respectively. The AMH values in the lower 5th percentile of each age group were 1.19 ng/mL, 0.60 ng/mL, 0.42 ng/mL, 0.27 ng/mL, 0.14 ng/mL, and 0.10 ng/mL, respectively.
Conclusion: This study determined reference values of serum AMH in Korean women with regular menstruation. These values can be applied to clinical evaluation and treatment of infertile women.

Keywords: Anti-Müllerian Hormone; Ovarian Reserve; Korean; Human

Introduction

Assessment of ovarian reserve has been an important issue in infertility and assisted reproductive technololy [1,2]. Ovarian reserve tests include serologic markers and ultrasonographic markers, such as antral follicle count (AFC) and ovarian volume. Basal FSH, estrogen, and inhibin B have been commonly measured by serologic tests, but have several limitations in that it is difficult to predict hyperresponders and poor responders and they shoud be measured during menstrual periods [1-4].

Recently, serum anti-Müllerian hormone (AMH) has emerged as a novel marker for ovarian function [5-9]. AMH is a member of the transforming growth factor-beta superfamily and is expressed in the growing preantral or small antral follicles in the ovary, but is not expressed in ovulatory, atretic follicles, and theca cells. Serum AMH concentrations show little inter- and intra-cycle variability and reflect the recruited ovarian follicular pool [7]. Currently, serum AMH level is often measured during an initial work-up for infertility at several fertility centers in Korea. However, there are no age-related reference values for AMH levels based upon a large Korean population. The aim of this study was to establish reference values for AMH in Korean women with regular menstrual cycles.

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Methods

1. Study population

All individual patients' serum AMH levels were measured between May, 2010, and January, 2011, in Cheil General Hospital and Women's Health Care Center, Seoul, Korea. This study population included a total of 1,298 women who had regular menstrual cycles (interval 21-

35 days) aged between 20 and 50 years. Exclusion criteria consisted of the following factors: 1) Polycystic ovary syndrome (PCOS), 2) Previous history of ovarian surgery (including oophorectomy and enucleation of ovarian cysts), 3) body mass index ≥ 30 kg/m^2, 4) other endocrine disease (thyroid disease, diabetes mellitus, Cushing's syndrome). This study population was divided to six age groups: 20-31 years, 32-34 years, 35-37 years, 38-40 years, 41-43 years, and over 43 years.

The study was approved by the Institutional Review Board of Cheil General Hospital.

2. AMH assay

On day 2-3 of a spontaneous menstrual cycle, the blood samples for the assay of FSH and AMH were obtained by venipuncture. The serum AMH levels were measured by enzyme immunoassay using an AMH/MIS EIA kit, which is a two-immunological step sandwich type assay (Immunotech version; Beckman Coulter, Marseille, France). First, the hormone was captured by a monoclonal antibody bound to the microtiter plate. Then, another monoclonal antibody with streptavidin-peroxidase bound to the solid phase antibody-antigen complex. After incubation, the antibody-antigen complex was detected by addition of a chromogenic substrate. The serum AMH concentration was presented by intensity of the coloration in the blood samples and then converted to ng/mL (conversion factor to pmol/L = ng/mL × 7.14). The measurement range of the assay was from 0.14 ng/mL to 21 ng/mL. Serum AMH values below the reported clinical level of measurement (0.14 ng/mL) were treated as a zero value for analysis. The intra- and interassay coefficients of variation were 12.3% and 14.2%, respectively.

3. Statistical analysis

Statistical analysis was performed using SPSS ver. 12.0 (SPSS, Chicago, IL, USA). To determine the correlation between AMH and other variables, the data was analyzed by Pearson's correlation. Regression analysis was performed to present the age-related changes in AMH and FSH. Comparisons between age groups were performed using one-way analysis of variance. Each variable was presented as mean ± SD. $p < 0.05$ was considered statistically significant.

Results

The mean age of the 1,298 women was 34.4 ± 0.1 years, and the mean AMH level was 3.6 ± 0.1 ng/mL. The mean level of serum FSH and estradiol were 8.9 ± 0.2 mIU/mL and 35.0 ± 1.4 pg/mL, respectively. Serum AMH was correlated with age ($r_p = -0.417$, $p < 0.001$) and FSH ($r_p = 0.328$, $p < 0.001$), but was not correlated with other variables. Figure 1 shows a continuous regression between AMH value and age ($R^2 = 0.183$, $p < 0.001$) and FSH value and age ($R^2 = 0.077$, $p < 0.001$). Approximately 75% of women (978/1,298) showed a serum AMH level below 5.0 ng/mL (Figure 2).

Women were classified into 3 categories by age: 20-29 years, 30-39 years, 40-50 years. There are significant differences in mean and median serum AMH (5.46 ± 2.23 vs. 3.74 ± 2.89 vs. 1.47 ± 1.59 ng/mL, $p < 0.001$) and serum FSH (6.73 ± 2.30 vs. 8.47 ± 6.45 vs. 12.58 ± 8.91 mIU/mL, $p < 0.001$)of each age groups. To present the age-specific AMH levels associated ovarian reserves, women were subdivided

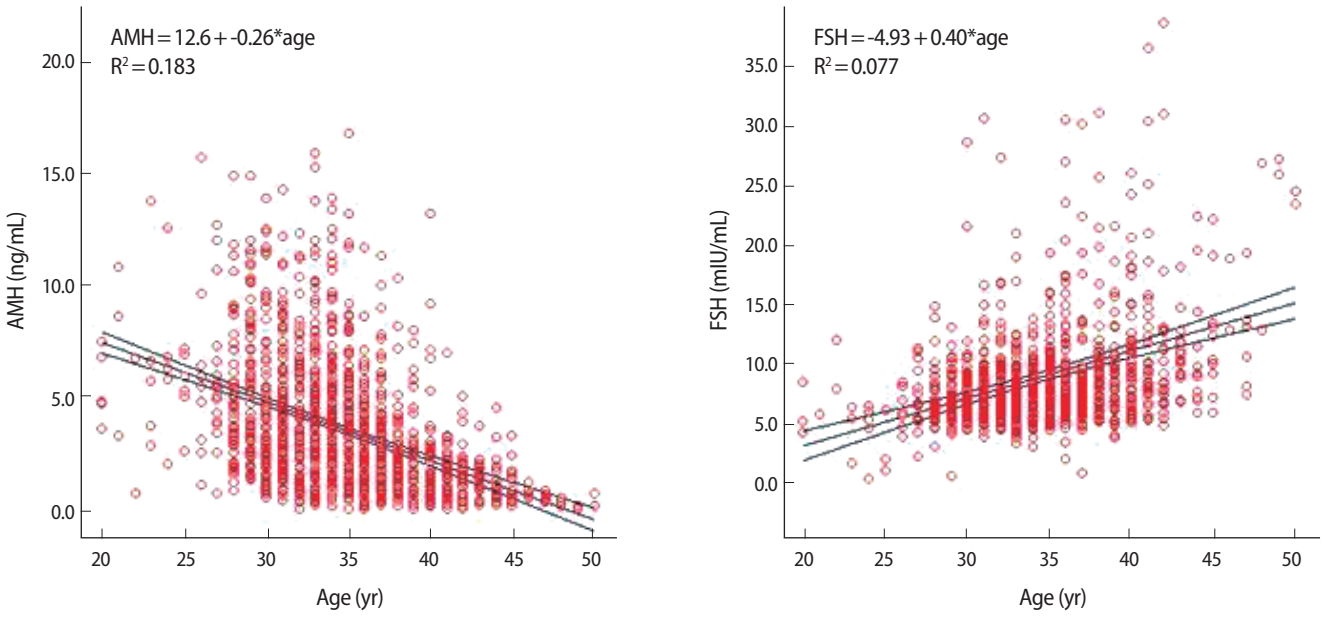

Figure 1. Age-specific anti-Müllerian hormone (AMH) and FSH concentrations.

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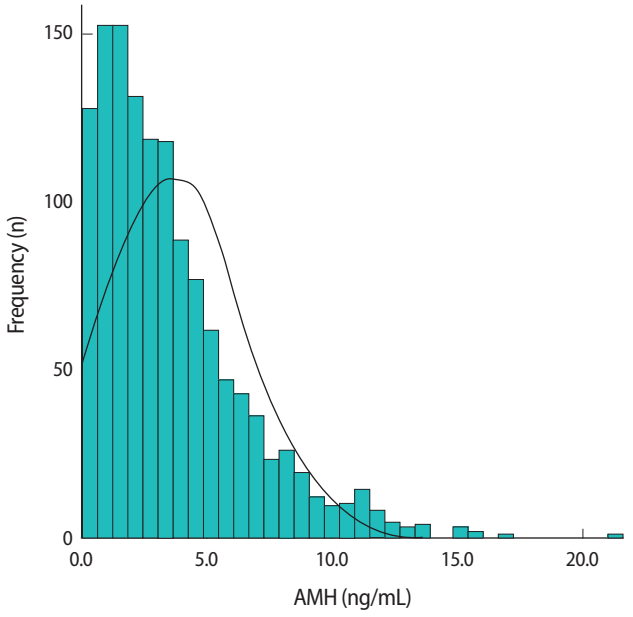

Figure 2. Distribution of serum anti-Müllerian hormone (AMH) concentrations in women with regular menstruation.

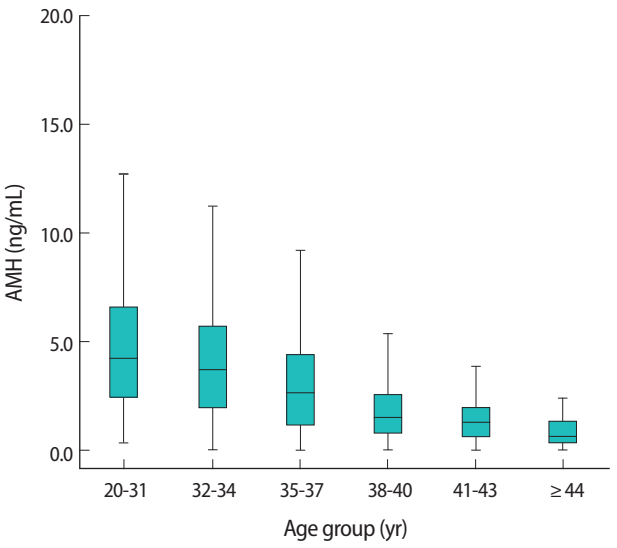

Figure 3. Serum anti-Müllerian hormone (AMH) concentration of the age group. Values are median (Lines), 25-75th percentiles (boxes) and 95% confidence interval (whiskers).

Table 1. Basal characteristics of each age group with regular menstruation

	Age (yr)					
	20-31	32-34	35-37	38-40	41-43	≥44
Cases (n)	377	331	283	173	87	47
Age (yr)	29.1±0.1	33.1±0.4	35.9±0.5	39.0±0.6	41.7±0.1	45.6±0.3
Body mass index (kg/m^2)	20.1±0.1	20.5±0.1	21.0±0.2	21.1±0.2	21.4±0.3	22.2±0.5
Basal FSH (mIU/mL)	7.3±0.2	7.7±0.2	9.4±0.7	9.9±0.4	12.1±1.0	15.9±1.8
Basal E_2 (pg/mL)	36.1±3.0	34.0±1.5	33.7±3.8	37.2±3.7	31.8±3.3	38.7±10.3
TSH (μU/mL)	2.3±0.1	2.3±0.1	2.4±0.1	2.2±0.1	2.3±0.2	2.5±0.3
Prolactin (ng/mL)	15.8±0.8	15.0±0.7	15.0±0.6	15.4±0.8	12.1±0.7	15.0±2.3

The values are expressed as means±SD.

Table 2. The serum AMH levels of the age group with regular menstruation

	Age (yr)					
	20-31	32-34	35-37	38-40	41-43	≥44
AMH (ng/mL)	4.94±0.17[a]	4.25±0.17[a]	3.22±0.15[a]	2.13±0.15[a]	1.47±0.13[a]	0.95±0.14[a]
(95% CI)	(4.61-5.26)	(3.92-4.57)	(2.92-3.51)	(1.83-2.44)	(1.21-1.71)	(0.68-1.23)
Percentile						
5th	1.19	0.60	0.42	0.27	0.14	0.10
10th	1.60	1.10	0.70	0.50	0.30	0.10
25th	2.50	2.00	1.20	0.80	0.60	0.40
50th	4.20	3.70	2.60	1.50	1.30	0.60
75th	6.65	5.70	4.40	2.60	2.00	1.30
90th	9.30	8.30	6.40	4.42	2.92	2.24
95th	11.41	10.70	8.18	6.82	3.82	3.26

AMH, anti-Müllerian hormone; CI, confidence interval.
[a]The values are expressed as means±SD.

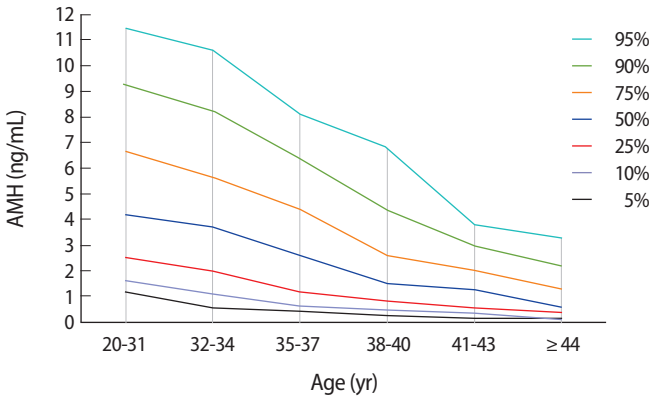

Figure 4. Age-related nomogram of serum anti-Müllerian hormone (AMH) concentrations in Korean women with regular menstruation.

into the following groups on the basis of their ages: 20-31 years (n=377); 32-34 years (n=331); 35-37 years (n=283); 38-40 years (n=173); 41-43 years (n=87); >43 years (n=47). The basal characteristics of each age group are described in Table 1. Both median and mean AMH levels of each age group decreased steadily with increasing age (Table 2). Figure 3 shows the median, 95% confidence intervals, and 25-75th percentiles calculated for each age group. The AMH values in the lower 5th percentiles of each age group were 1.19 ng/mL, 0.60 ng/mL, 0.42 ng/mL, 0.27 ng/mL, 0.14 ng/mL, and 0.10 ng/mL, in order from the youngest to oldest groups. Figure 4 shows also age-related nomogram of serum AMH concentrations.

Discussion

A large number of studies have decribed the correlation of serum AMH levels with age. This hormone appears in the 36th week of gestation and decreases continuously through puberty. It becomes undetectable when menopause occurs [10,11]. Serum AMH is produced by granulosa cells from the preantral and small antral follicles. Several studies have demonstrated that AMH inhibits primordial follicle recruitment and also decreases the responsiveness of growing follicles to FSH. Thus, serum AMH levels are considered to reflect the number of small growing follicles, and are reduced through reproductive life. Accordingly, many studies have suggested that the serum AMH could be a novel ovarian reserve test.

La Marca et al. [12] showed that serum AMH levels, unlike other ovarian reserve tests, do not change significantly throughout the menstrual cycle. Other studies have also confirmed that the intercycle and intracycle variability of serum AMH levels is very low enough, in fact, to allow random timing of AMH measurement during the menstrual cycle. Hence, it has been suggested that AMH values are more convenient and more effective than other serum ovarian reserve tests like FSH and inhibin B or estradiol.

However, few studies reported on normal reference levels for serum AMH values. Recently, Seifer et al. [8] have examined age-specific serum AMH values for 17,120 women of reproductive age from 24 to 50 years old presenting to fertility centers within the United States. The study showed that the serum AMH levels decreased steadily with increasing age. However, the results of that study derived from the serum AMH levels of women during fertility evaluation, and thus were not representative of the general population. In that study, the age-specific mean and median values were somewhat lower (both 1.0 ng/mL) than our study. Another study performed in the United States reported age-specific reference values of AMH examined in 792 infertile women within five age groups [13]. More than half in that study population had diminished ovarian reserves and premature ovarian failure. Therefore, that study presented lower median AMH values than our study. In Italy, La Marca et al. [7] evaluated a cohort of 277 women that had regular menstruation patterns: they excluded women affected by confounding factors such as PCOS or a history of ovarian surgery. Those within a given age group in that study had similar median AMH levels, like our study.

This is the largest study to present serum AMH reference values in Korean women with normal mensturation patterns between 20 and 50 years old. We excluded women who had a history of ovarian surgery and PCOS. We found that serum AMH levels decline with increasing age, and approximately 75% of women showed a serum AMH value below 5 ng/mL. To present age-specific serum AMH levels, the study population was divided to six age groups. The median and the mean AMH levels in the 32- to 34-year-old, 35- to 37-year-old, 38- to 40-year-old age groups may be especially useful in determining fertility treatment options [5]. In addition, women who have AMH levels below the 5th percentiles of their own age group may need consultation on more aggressive fertility treatment options.

In assisted reproductive technology, several studies have demonstrated that serum AMH levels accurately reflect the total developing follicular cohort, and predict ovarian response to controlled ovarian stimulation. In addition, there have been many studies demonstrating that serum AMH is a better marker in predicting the number of retrieved oocytes than the other serum markers. A prospective study comparing serum AMH with AFC in 130 controlled ovarian hyperstimulation (COH) cycles showed that the two markers performed similarly in the prediction of the number of retrieved oocytes [14]. A few studies found AMH to be superior to AFC, whereas two studies produced the opposite result. Hence, it is a still a matter of debate whether AMH is a more accurate predictive marker compared to AFC in predicting ovarian response to COH. However, AMH appears to be the best predictive marker because it can be measured independent of the menstrual cycle.

Serum AMH testing may also be performed as a preoperative and

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postoperative evaluation of ovarian surgery in younger women. A prospective study presented the impact of laparoscopic cystectomy on ovarian reserve by investigating the serial changes in serum AMH levels after operation [15]. It was suggested that serum AMH is a useful marker of ovarian reserve after ovarian surgery. Measuring serum AMH after ovarian surgery could be helpful in considering fertility treatment options. Also, serum AMH as preoperative evaluation of ovarian surgery may allow for clinical use to predict iatrogenic premature ovarian failure and consider the range of operation.

In conclusion, this is the largest study to document age-specific AMH values for Korean women. Our results will be helpful to perform the evaluation of a women's ovarian reserve, prediction of menopause and iatrogenic premature ovarian failure. Also, the AMH levels presented in the current study may be applied to performing clinical evaluation of fertility and considering treatment options. Furthermore, these results will be helpful in discussions of treatment options for infertility between infertile couples and clinicians. In assisted reproductive technology, having this data will be useful in predicting the number of retrieved oocytes for COH and ovarian hyperstimulation syndrome, which is a complication of COH. More studies with regards the serum AMH should be performed to extend its application in variable fields of female reproductive health.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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