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Age-Specific Normal Reference Range for Serum Anti-Müllerian Hormone in Healthy Chinese Han Women: A nationwide Population-Based Study

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Abstract

Background: The increasing use of anti-Müllerian hormone (AMH) in clinic has raised concerns regarding the reliable reference range for this test. However, the reference range for AMH in normal Chinese female population has not been established. Furthermore, relationship between AMH and other clinical markers such as body mass index (BMI) and antral follicle counts (AFCs) and other sex-related hormones have not been examined in normal population-based women. **Objective:** We aimed to determine the age-specific reference range for serum AMH in healthy Chinese women throughout reproductive age to menopause and to estimate relationship between AMH and other clinical markers in healthy women. **Study Design:** In this multicenter and nationwide study, advertisements were used to recruit 2055 women, aged 20 to 55 years, from 6 different regions in China; 1590 (77.37%) women met the inclusion criteria for the reference range population. We measured the baseline serum AMH levels using new Beckman Coulter Gen II assay. Serum concentration of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), testosterone (T), prolactin (PRL), progesterone (PRG), and AFCs were also determined in the follicular phase. **Main Outcome Measures:** The AMH-Age nomogram and AMH levels of different age-groups and the relationship between AMH and other clinical markers. **Results:** Serum AMH concentrations declined progressively with age. A quadratic model defined as $\log(\text{AMH}) = (-1.970 + 0.296 \times \text{Age} - 0.006 \times \text{Age}^2)$ fitted best the decline of AMH with age. The median AMH levels were 6.23, 5.65, 4.55, 3.74, 2.78, and 1.09 ng/mL for the $20 \leq \text{age} < 25$, $25 \leq \text{age} < 30$, $30 \leq \text{age} < 33$, $33 \leq \text{age} < 37$, $37 \leq \text{age} < 40$, and $40 \leq \text{age} < 55$ groups, respectively. The 5th to 95th percentiles of the AMH levels, as the reference range, were 2.06 to 12.66, 1.77 to 13.83, 1.48 to 11.45, 0.87 to 9.76, 0.56 to 9.49, and 0.08 to 5.70 ng/mL for each age-group. The AMH levels were positively correlated with AFCs and T, LH, PRL and PRG levels and negatively correlated with BMI and FSH levels and were not significantly correlated with E_2 levels. The relationship between AMH and other variables remain unchanged except for PRL, which was not

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significantly correlated with AMH levels after controlling for both age and BMI. **Conclusions:** This study determined the normal reference ranges for serum AMH levels in a large population-based sample of healthy Chinese women.

Keywords
AMH, reference range, Chinese women, nationwide population-based study

Introduction

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein which is a member of the transforming growth factor beta (TGF-β) superfamily, is produced by granulosa cells of primordial follicles that have undergone initial recruitment, and is thought to reflect the size and quality of the ovarian reserve.[1] In addition, AMH inhibits follicular sensitivity to follicle-stimulating hormone (FSH) and plays a role in the process of dominant follicle selection.[2] Anti-Müllerian hormone is undetectable in cord blood samples, and its level increases between birth and 3 months. From the age of 8 to 25 years, the AMH level is stable,[3] but it begins to decrease at the age of approximately 30 years[4] and reaches undetectable levels approximately 5 years before the final menstrual period.[5] The AMH level reflects subtle changes in the menstrual cycle[6,7] and appears to be stable during pregnancy,[8] gonadotropin-releasing hormone (GnRH) agonist treatment,[9] and short-term oral contraceptive administration.[10,11] Currently, AMH is thought to be the optimal biomarker to reflect ovarian reserve, and its detection is more convenient and more effective than other biomarkers, such as FSH and estradiol (E_2). The AMH measurements have been increasingly used in the clinic to assess ovarian function and diagnose ovarian diseases now.[12-14] For example, AMH measurements are often performed during an initial fertility workup to assess the response to ovarian stimulation and predict pregnancy outcomes from in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI), and they have also been rapidly adopted by clinicians to assess the ovarian reserve and predict menopause age. Therefore, the reference range of AMH in normal healthy women is needed to provide useful information in the clinical diagnosis of ovary function.

Anti-Müllerian hormone is usually measured using enzyme-linked immunosorbent assays (ELISAs). Previous studies have mostly measured AMH levels using 2 ELISAs, the Diagnostics Systems Laboratory (DSL 10-14400) assay[15-17] or the Immunotech (IOT, A11893 IVD, EU) assay.[18-20] These assays utilize 2 different primary antibodies against AMH and different standards, and consequently, the crude values reported differ substantially.[21] With the consolidation of these 2 companies by Beckman Coulter in 2011, and their sole ownership of the patent to measure mammalian AMH, there is finally a single commercially available assay: the AMH Gen II assay (A79765), which has fully replaced the DSL and IOT assays.

Additionally, because these assays differ both in their pairing of monoclonal antibodies and in their standardization protocols, they lack a uniform quantitative reference value for the AMH level.[21] The AMH Gen II assay is widely used now and has been accepted as the standard for AMH measurement in laboratories worldwide.[21] Therefore, using the Beckman Coulter Gen II assay to estimate the reference range for AMH is more practical. However, only a few studies established the reference range for AMH using the new Gen II assay, especially for Chinese women.

Several AMH–age nomograms have been reported before[15-19,22]; however, most of the studies used hospital inpatient samples, particularly samples from infertile women instead of healthy females. The reference range for AMH levels obtained from a large, population-based sample of healthy women was believed to be more practical, reliable, and accurate in representing the normal population, therefore, additional studies were warranted to determine that.

We conducted a 3-year (October 2011 to December 2014), multicenter, and nationwide population-based study to determine the levels of serum AMH in a large sample of healthy Chinese women using the Beckman Coulter Gen II assay. Additionally, we evaluated the relationship between AMH and other clinical markers for normal healthy female population.

Materials and Methods

Healthy Women and Sample Preparation

A total of 2055 women, with ages range from 20 to 55 years, were recruited, through advertisements, from 6 different regions of China, including the city of Shenyang (northern China), Foshan (southern China), Chengdu (western China), Zhengzhou, and Yichang and Wuhan (central China). More information on the recruitment method is shown in Supplementary Table 1. Of the initial recruits, 1590 (77.37%) women met the following strict inclusion criteria for the reference range population: (1) for women <40 years old having regular menstrual cycles and for women >40 not required to have regular menstrual cycles considering that they may be in normal perimenopause or menopause; (2) no hormone therapy in the past 6 months; (3) no history of radiotherapy or chemotherapy; (4) no history of hysterectomy, oophorectomy, or any other type of ovarian surgery; (5) no ovarian cysts or ovarian tumors; and (6) no known chronic, systemic, metabolic, or endocrine diseases such as hyperandrogenism or hyperprolactinemia. The reasons for patient exclusion are represented in Supplementary Figure 1.

All volunteers were interviewed one-on-one using prepared questionnaires that included questions about their demographic, geographic, and reproductive characteristics.

This study was approved by the ethics committee of Tongji Medical College, and all participants have provided written

Table 1. Characteristics of 1590 Study Population.[a]

Age, years							
Characteristics	$20 \leq$ age <25	$25 \leq$ age <30	$30 \leq$ age <33	$33 \leq$ age <37	$37 \leq$ age <40	$40 \leq$ age <55	*P* Value of ANOVA
Cases	253	531	284	247	126	149	/
Age, y	23.39 ± 1.21	27.48 ± 1.38	31.45 ± 0.88	34.67 ± 1.15	38.48 ± 0.86	43.21 ± 2.69	.000
BMI, kg/m^2	20.37 ± 3.08	20.99 ± 3.31	21.39 ± 2.92	22.24 ± 3.08	22.61 ± 2.80	23.20 ± 2.68	.000
Gravidity	0.37 ± 0.67	0.65 ± 1.00	0.99 ± 1.19	1.04 ± 1.33	2.06 ± 1.83	2.13 ± 1.46	.000
Parity	0.13 ± 0.17	0.16 ± 0.28	0.34 ± 0.72	0.32 ± 0.52	0.63 ± 0.63	0.74 ± 0.59	.000
AFC	14.65 ± 4.86	13.62 ± 5.19	12.20 ± 4.19	11.24 ± 4.83	9.22 ± 4.51	5.60 ± 4.16	.000
Basal FSH, mIu/mL	6.30 ± 1.95	6.96 ± 2.25	7.10 ± 1.75	7.19 ± 2.39	7.53 ± 2.21	11.79 ± 12.43	0.000
Basal LH, mIu/mL	5.05 ± 2.74	5.00 ± 3.31	4.48 ± 2.25	4.42 ± 2.63	4.17 ± 2.07	5.83 ± 5.76	.000
Basal E_2, pg/mL	43.39 ± 17.65	44.47 ± 21.94	43.97 ± 20.96	47.69 ± 25.77	45.74 ± 22.58	47.60 ± 31.61	.213
T, ng/dL	33.50 ± 15.24	32.49 ± 16.20	29.27 ± 15.48	27.72 ± 14.05	24.57 ± 13.34	18.97 ± 13.72	.000
PRL, ng/mL	16.27 ± 10.57	15.80 ± 8.72	15.09 ± 8.14	14.07 ± 7.30	12.77 ± 8.27	12.39 ± 6.70	.000
PRG, ng/mL	0.62 ± 0.36	0.78 ± 1.66	0.60 ± 0.76	0.82 ± 1.99	0.52 ± 0.27	0.66 ± 1.19	.220

Abbreviations: AFC, antral follicle count ANOVA, analysis of variance; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; PRG, progesterone; SD, standard deviation; E_2, estradiol; T, testosterone.
[a]The values are expressed as means ± SD.

informed consent. The participants were physically examined and received free hormone and ultrasound testing. All blood samples were taken from the participants' antecubital vein between 7:00 AM and 11:00 AM, after a 12-hour overnight fast, on days 2 to 5 of a spontaneous menstrual cycle or during the follicular phase for nonmenstruating women (defined by checking endometrial thickness with ultrasonic monitoring) to test the baseline hormone levels. The samples were then centrifuged using standard conditions within 2 hours of venipuncture. After centrifugation, serums were obtained, aliquoted, transported to the central laboratory, and were stored at -80°C for no more than 2 weeks until the assays were performed. To avoid the potential bias produced by differences between laboratory test results, we chose the gynecologic endocrine laboratory of Tongji hospital as the central laboratory; all serums were transported to the central laboratory using dry ice within 48 hours of collection, and all serum hormones were tested in the central laboratory.

Anti-Müllerian Hormone Assays

Serum concentrations of AMH at the time of recruitment were measured using the AMH Gen II ELISA kit (Beckman Coulter, Inc, California), and all serum AMH measurements were performed in the same laboratory using the same kit. The AMH Gen II control A79766 was used at 2 concentrations to monitor the accuracy of the assay. The intra- and interassay coefficients of variation (CVs) were 3.6% and 4.5%, respectively. The lowest amount of AMH that could be detected with a 95% probability in a sample was 0.08 ng/mL; therefore, we replaced all values recorded as <min (undetectable) with a value of 0.08 ng/mL for the purposes of this analysis.

Other Hormone Assays

Serum FSH, luteinizing hormone (LH), estradiol (E_2), testosterone (T), prolactin (PRL), and progesterone (PRG) levels were measured using a chemoluminescence-based immunometric assay on an ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics Inc, Tarrytown, New York). All these serum hormones levels were measured in the same laboratory using the same kit. The intra- and interassay coefficients of variation were all <15%.

Ultrasound Examination

A transvaginal ultrasound scan of the ovaries was performed to determine the antral follicle count (AFC). This ultrasound examination was performed at the multicenters. All participating research institutes were modernized large comprehensive hospitals and had our regular supervision and verification. We formulated the unified standard for this examination in the beginning, and all ultrasound doctors were strictly trained and tested AFCs according to the same standard. In this study, the AFC was defined as the total number of visible round or oval structures with diameters 2 to 10 mm in both ovaries. All ultrasound examinations were performed on days 2 to 5 of a spontaneous menstrual cycle or in the follicular phase for nonmenstruating women. None of the eligible participants had follicles larger than 10 mm. We didn't find significant differences between each center. The intra-analysis coefficient of variation for the follicle diameter measurements was <5%, and the lower limit of detection was 0.1 mm.

Statistical Analysis

Data entry and statistical analyses were performed using EpiData software version 3.1 (www.epidata.dk/download.php) and SPSS version 16.0 (IBM, Armank, New York).

The Kolmogorov-Smirnov test was used to assess whether the investigated parameters were distributed normally. The data are expressed as the means ± standard deviations (for normally distributed data) or as medians with 25th to 75th percentiles (for skewed-distribution data).

Because the level of AMH had a significant negative correlation with age, we established the reference ranges in terms of age-groups, and the reference range parameters for AMH were determined using standard descriptive statistics. To determine the correlation between AMH and the other variables, the data were analyzed using Pearson and partial correlations, whereas relationships between AMH and the other variables were determined using linear regression analysis. Comparisons between age-groups were performed using 1-way analysis of variance (ANOVA).

Results

The Basal Clinical Characteristics

The mean age of the selected 1590 women was 30.97 $\pm$ 5.59 years, and the mean AMH level was 5.25 $\pm$ 3.57 ng/mL. The mean levels of FSH and E_2 were 7.38 $\pm$ 4.42 mIu/mL and 45.07 $\pm$ 22.89 pg/mL, respectively. The mean AFC was 12.14 $\pm$ 5.37.

The 1590 women were classified into 6 groups by age: 20 $\leq$ age $<$ 25 years (n = 253), 25 $\leq$ age $<$ 30 years (n = 531), 30 $\leq$ age $<$ 33 years (n = 284), 33 $\leq$ age $<$ 37 years (n = 247), 37 $\leq$ age $<$ 40 years (n = 126), and 40 $\leq$ age $<$ 55 years (n = 149). The demographic, reproductive, and endocrine characteristics of the 1590 healthy women included in our study are shown in Table 1. Variance analyses indicated that there were significant differences among the age-groups in terms of BMI, gravidity, parity, AFC, and basal FSH, LH, T and PRL levels ($P < .05$). The mean BMI of each age-group increased steadily with increasing age, and there were significant differences in BMI between the age-groups. Gravidity and parity also increased with age: The small numbers of gravidity and parity in 20- to 30-year-old women may be mainly because of China's family planning program and the advocacy of late childbirth. Serum FSH levels correlated positively with age and were especially high in 40- to 55-year-olds. The AFC decreased with age and was particularly low in 40- to 55-year-olds. The level of T tended to decrease with age, with significant differences among the groups. The level of LH revealed no significant differences in the first 5 age-groups but rose suddenly in the 40- to 55-year-olds. There were significant differences in PRL levels in the 25- to 30-year-olds, the 37- to 40-year-olds, and the 40- to 55-year-olds; the PRL level in the 30- to 33-year-olds also differed significantly compared with the 40- to 55-year-olds. There were no significant differences among the age groups for the E_2 and PRG levels ($P > .05$).

The Optimal Model Fitted the Decline in AMH With Age

We observed that female serum AMH concentrations declined progressively with age. We first constructed and evaluated 6 candidate regression models (linear, quadratic, cubic, logarithmic, exponential, and power models) to describe the decline of AMH with age. For each model, the response variable was the value of AMH, and the predictor variable was age in years. We found the exponential model provided the best fit. The summaries of the goodness of fit for each model are shown in Supplementary Table 2.

The AMH values were skewed-distributed, so these values were log-transformed and analyzed further. After performing logarithmic transformation of AMH values to normalize data, we further built 3 candidate regression models (linear, quadratic, and cubic models) for the log AMH values as functions of age. We found this quadratic model: $\log(\text{AMH}) = (-1.970 + 0.296 \times \text{Age} - 0.006 \times \text{Age}^2)$ was the most appropriate model, given its comparable R^2 values ($R^2 = .390$) and ease of interpretation. F test of this quadratic equation is statistically significant ($F = 382.044$, $P = .000$). The regression coefficients are also distinguished by significance testing (linear effect coefficient = 0.296, $t = 10.216$, $P = .000$; quadratic effect coefficient = -0.006, $t = -13.060$, $P = 0.000$). The summaries of the goodness of fit for each model are shown in Supplementary Table 3.

The AMH–Age Nomogram Building

The AMH–age nomogram with the 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles is shown in Figure 1. We first performed a logarithmic transformation of AMH values and then calculated age-specific 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles of Log AMH at each age. For each percentile at all ages, we fitted a quadratic model for which Y = log (AMH) at each percentile and X = age (years) to estimate the model parameters. Then, we took antilog for the obtained equations and calculated functions of AMH values with age. Based on the obtained model, percentile curves were produced by plotting the model predicted AMH level against age. The AMH–age nomogram and the age-specific AMH and their corresponding percentiles we developed can give guidelines as to the relative position of an individual's AMH level, given her age.

Serum AMH Levels of Different Age-Groups

The serum AMH levels of the different age-groups are displayed in Table 2. The median AMH levels were 6.23 ng/mL for the 20 $\leq$ age $<$ 25-year-old group, 5.65 ng/mL for the 25 $\leq$ age $<$ 30-year-old group, 4.55 ng/mL for the 30 $\leq$ age $<$ 33-year-old group, 3.74 ng/mL for the 33 $\leq$ age $<$ 37-year-old group, 2.78 ng/mL for the 37 $\leq$ age $<$ 40-year-old group, and 1.09 ng/mL for the 40 $\leq$ age $<$ 55-year-old group. The AMH values of the lower 5th percentile of each age-group were 2.06 ng/mL, 1.77 ng/mL, 1.48 ng/mL, 0.87 ng/mL, 0.56 ng/mL and 0.08 ng/mL, respectively. Based on the accuracy of the chosen application, we recommend to clinicians that the 5th to 95th percentile reference range for AMH are 2.06 to 12.66 ng/ mL for 20 $\leq$ age $<$ 25 years, 1.77 to 13.83 ng/mL for 25 $\leq$ age $<$ 30 years, 1.48 to 11.45 ng/mL for 30 $\leq$ age $<$ 33 years, 0.87 to 9.76 ng/mL for 33 $\leq$ age $<$ 37 years, 0.56 to 9.49 ng/mL for 37 $\leq$ age $<$ 40 years, and 0.08 to 5.70 ng/mL for 40 $\leq$ age $<$ 55 years.

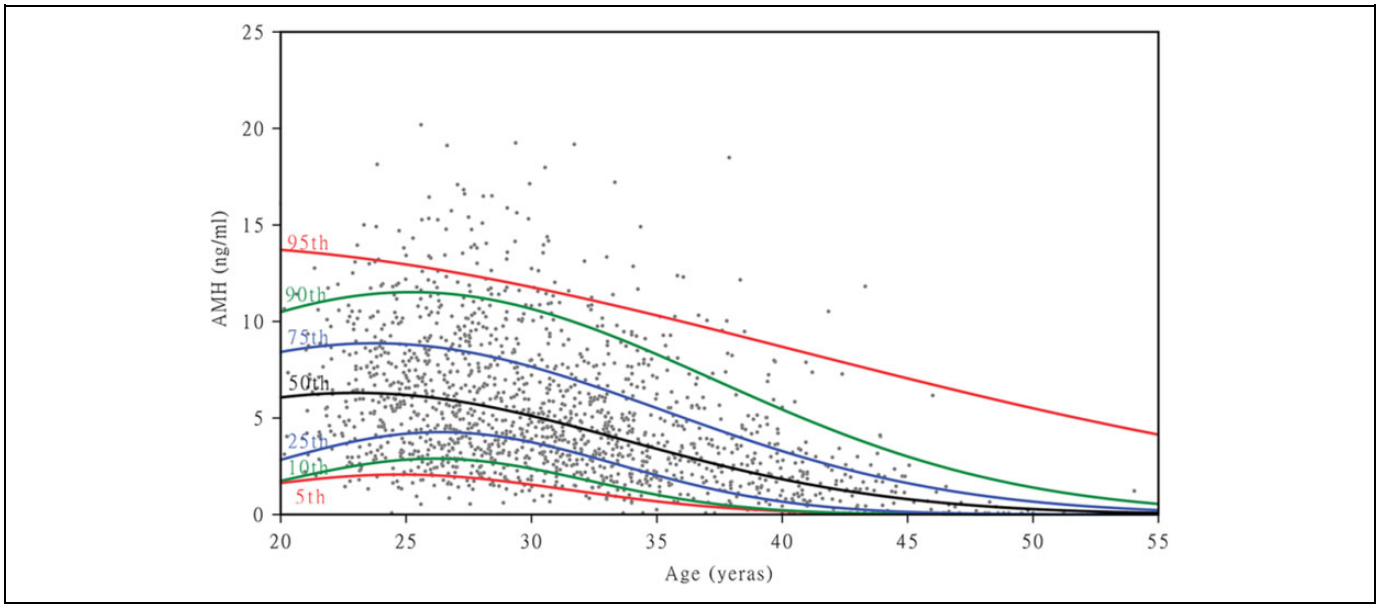

Figure 1. It shows the anti-Müllerian hormone (AMH)-Age nomogram (age-specific centiles). Gray dots represent the observations. AMH values are a function of age, and the estimated values of AMH for the 5th, 10th, 25th, 50th, 75th, 90th and 95th centiles in 1590 study participants was obtained based on the most appropriate model.

Table 2. Serum AMH Levels of the Age-Groups.

Age, years						
	20 ≤ age < 25	25 ≤ age < 30	30 ≤ age < 33	33 ≤ age < 37	37 ≤ age < 40	40 ≤ age <55
AMH, ng/mL (95%CI)	6.65 ± 3.26 (6.24-7.06)[a]	6.41 ± 3.72 (6.07-6.72)[a]	5.23 ± 3.11 (4.88-5.58)[a]	4.45 ± 2.93 (4.07-4.84)[a]	3.50 ± 2.82 (2.98-4.06)[a]	1.52 ± 1.88 (1.22-1.83)[a]
Percentile						
5th	2.06	1.77	1.48	0.87	0.56	0.08
10th	2.80	2.26	2.00	1.50	0.82	0.08
25th	4.07	3.49	3.09	2.20	1.53	0.17
50th	6.23	5.65	4.55	3.74	2.78	1.09
75th	8.66	8.64	6.56	6.03	4.44	2.06
90th	11.21	11.57	10.05	8.68	7.35	3.22
95th	12.66	13.83	11.45	9.76	9.49	5.70

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

Relationship Between AMH and Other Clinical Variables

The correlations of AMH and age with other clinical variables were analyzed using the Pearson correlation analyses and partial correlation analyses after controlling for potential confounders. Because AMH has relationship with BMI and BMI also has relationship with age, and BMI is statistically different in our population, so BMI may be a confounder for the separate analyses. To determine the relationship of AMH and other variables, we first performed Pearson correlation analyses and then also performed partial correlation analyses by employing variables of Age, BMI, and AMH as control variables in turn, as shown in Supplementary Table 4. Pearson correlation analyses showed that AMH levels were positively correlated with AFC ($r = .632$; $P < .001$), T ($r = .300$; $P < .001$), LH ($r = .251$; $P < .001$), PRL ($r = .088$, $P < .001$) and PRG ($r = .070$, $P < .05$) levels; negatively correlated with FSH levels ($r = -.214$; $P < .001$) and BMI ($r = -.145$; $P < .001$); and not significantly correlated with E_2 levels. The results of partial correlation analyses after controlling for confounder (BMI) were consistent with that of Pearson correlation analyses. After controlling for both age and BMI, the relationship between AMH and other variables remain unchanged except for PRL, which was not significantly correlated with AMH levels afterward. And the results of controlling for variable age were consistent with that of controlling for both age and BMI as shown in Supplementary Table 4. In addition, we also assessed the correlation of AMH and age according to the women with normal BMI. We followed the BMI standard criteria for China set forth by the Chinese Obesity Working Group: BMI < 18.5: underweight;

$18.5 \leq$ BMI < 24: normal; $24 \leq$ BMI < 28: overweight; and BMI ≥ 28: general obesity.[23] Of the 1590 women for reference range population, 1056 (66.42%) women were with normal BMI (18.5-24 kg/m^2), and in these women, the AMH levels also showed a negative correlation with age ($r = -.427$, $P = .000$).

Because regression equation can quantitatively describe the interdependent relationship between variables, we then performed linear regression to further test the nature of the relationships identified. Figure 2 shows the results of continuous regression analyses between AMH and some related variables such as age, AFC and FSH, BMI, LH, T, and PRL levels.

Discussion

We conducted a 3-year, multicenter, cross-sectional, population-based study, wherein 2055 women aged between 20 and 55 years were recruited from 6 different geographic regions of China. A total of 1590 (77.37%) women met the inclusion criteria for the reference range population. We determined the age-specific reference range for serum AMH in healthy Chinese women and estimated relationship between AMH and other clinical markers. To our knowledge, this is the first study to establish a reference range for AMH levels in healthy Chinese women who were recruited systematically from randomly selected communities in different districts of China. We used strict inclusion criteria to ensure that the participants did not have diseases that may have influenced the level of AMH. Therefore, our results are trustworthy and applicable to women of reproductive age with normal ovarian function and women of menopause. The median and mean AMH levels in the $30 \leq$ age $<$ 33-year, $33 \leq$ age $<$ 37-year, and $37 \leq$ age $<$ 40-year groups may be especially useful in determining fertility treatment options.

We found AMH levels vary significantly in women of the same chronological age, so the reference ranges at various ages are a little too wide. Based on the accuracy of the chosen assay, we recommend to clinicians that the reference ranges of AMH be 2.06 to 12.66 ng/mL for $20 \leq$ age < 25 years, 1.77 to 13.83 ng/mL for $25 \leq$ age < 30 years, 1.48 to 11.45 ng/mL for $30 \leq$ age < 33 years, 0.87 to 9.76 ng/mL for $33 \leq$ age < 37 years, 0.56 to 9.49 ng/mL for $37 \leq$ age < 40 years, and 0.08 to 5.70 ng/mL for $40 \leq$ age < 55 years. The AMH is a useful predictor of ovarian reserve, so women with AMH levels below the 5th percentiles of their own age-group may signal poorer ovarian reserve. We hope this study could give clinicians a more reliable reference for correctly interpreting AMH levels and facilitate the adoption of AMH as a diagnostic tool in clinical practice in China.

Ovarian aging is related to the decline in the quantity and quality of the ovarian follicle pool with increasing age.[24] Follicle numbers decline nonlinearly with age.[25] In this study, we demonstrate that circulating AMH, a product of predominantly preantral and small antral follicles, exhibits a similar nonlinear decline with age, which is best expressed by the following equation: $\log(\text{AMH}) = (-1.970 + 0.296 \times \text{Age} - 0.006 \times \text{Age}^2)$, ($R^2 = .390$). This model was found to be the most appropriate model given its comparable R^2 values and ease of interpretation.

Results from some studies have suggested that ovarian aging or AMH levels may be influenced by race and ethnicity,[26-28] and racial and ethnic disparities in reproductive endocrinology and infertility have been reported.[28] Our study is the first to report age-specific AMH values among a normal healthy Chinese population. In this study, we found that the median AMH level in our population was lower than that reported in women from infertility clinics in the United Kingdom by Scott M. Nelson et al in 2013 who also measured AMH levels using the Beckman Coulter Gen II assay.[22] However, this may mainly be because that the reference populations in our study were in good health condition, while their populations were women primarily seeking IVF treatment. In addition, we found that the AMH values of each percentile calculated in our study were a little higher than those reported among general Iranian population.[29] However, this may be because the stricter selection criteria applied in our study. It still needs to be assessed in further research whether there are disparities between Chinese women and women of other races. The study based on racial and ethnic variation may help to explain, at least in part, why the age of natural menopause is considerably earlier in Chinese women than in Western women (the average age at natural menopause of Chinese women is 48.9 years,[30] while this age is 51 years in the Netherlands and many other Western countries[24]).

Yoo et al estimated the reference range for AMH among 1298 Korean women of reproductive age[31] who were visiting hospitals and had regular menstrual cycles. Women in their study were classified into 6 categories by age: 20 to 31 years, 32 to 34 years, 35 to 37 years, 38 to 40 years, 41 to 43 years, and over 43 years. However, in our study, the studied women were divided into the following 6 groups: $20 \leq$ age < 25 years, $25 \leq$ age < 30 years, $30 \leq$ age < 33 years, $33 \leq$ age < 37 years, $37 \leq$ age < 40 years, and $40 \leq$ age < 55 years. The grouping method was chosen because previous studies have demonstrated that AMH levels are stable between 8 and 25 years of age and[3] decrease after approximately 30 years of age,[4] while other studies demonstrated that AMH levels begin to decrease at 33 years of age.[32] There were also some studies, which concluded that the fecundity of women decreases gradually but significantly beginning at approximately 32 years and decreasing more rapidly after 37 years.[33] So we chose 25, 30, 33, and 37 years as age cutoffs. As women aged 25 to 35 are in exuberant fertility period, we focused more on these women and recruited more women in this age phase. Additionally, ANOVA revealed that there were significant differences in the levels of AMH among the age-groups: $20 \leq$ age < 30 years, $30 \leq$ age < 33 years, $33 \leq$ age < 40 years, and ≥ 40 years ($P < .05$), this showed that the cutoffs were clinically and statistically appropriate. We found that the AMH values of each percentile of each age-group that were calculated in our study were higher than those reported by Korean researchers (data not shown). However, Korean researchers measured AMH concentrations using the IOT assay while we used the

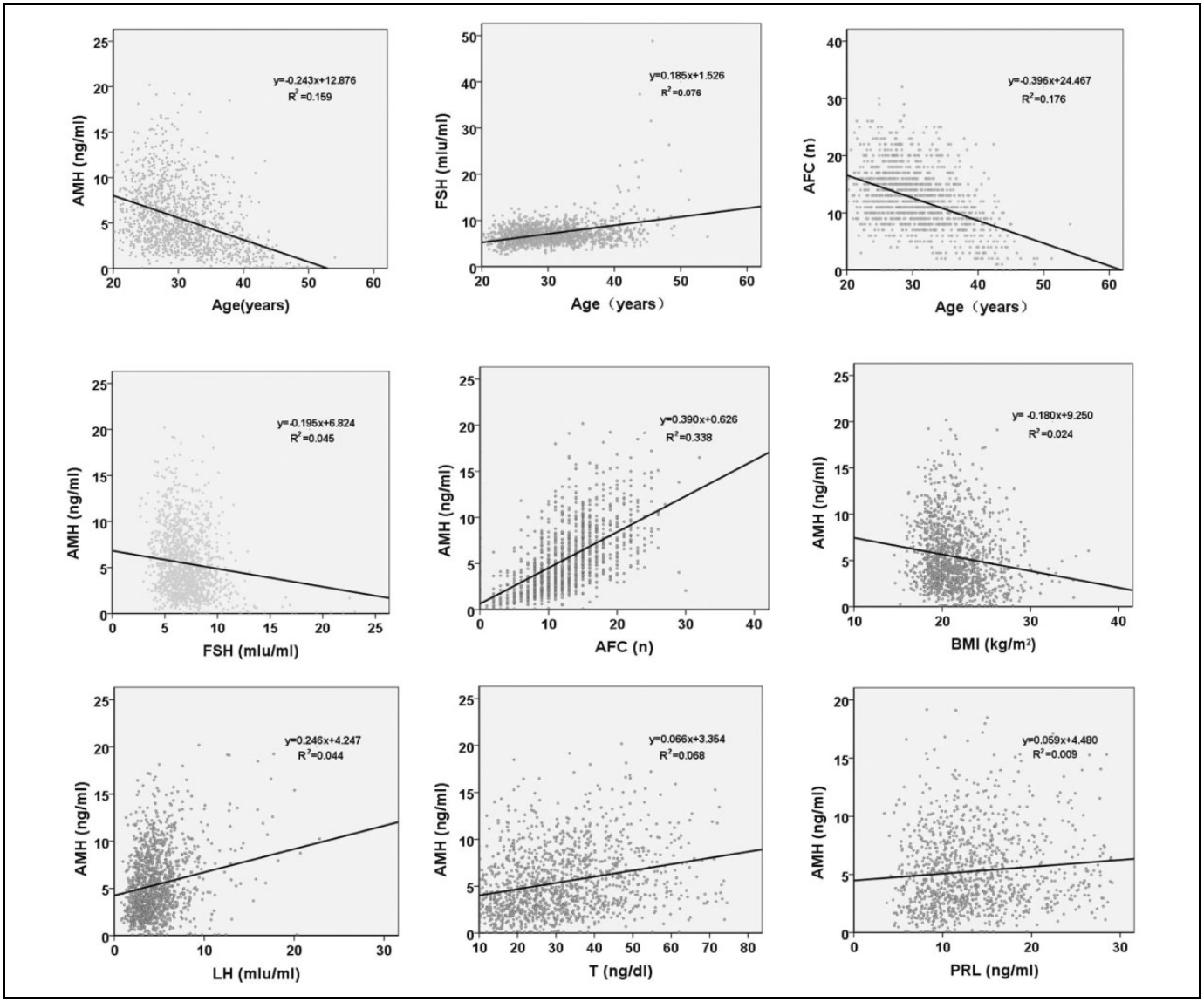

Figure 2. It shows the results of continuous regression analyses between anti-Müllerian hormone (AMH) value and age ($R^2 = .159$, B = −0.243, $P < .001$), follicle-stimulating hormone (FSH) value and age ($R^2 = .076$, B = .185, $P < .001$), antral follicle count (AFC) and age ($R^2 = .176$, B = −0.396, $P < .001$). The AMH value had good and positive relationship with AFC ($R^2 = .338$, B = .390, $P < .001$). The AMH levels showed negative correlations with FSH ($R^2 = .045$, B = −.195, $P < .001$) and body mass index (BMI; $R^2 = .024$, B = −0.180, $P < .001$). The AMH levels showed positive correlations with luteinizing hormone (LH) levels ($R^2 = .044$, B = 0.246, $P < .001$), T ($R^2 = .068$, B = 0.066, $P < .001$) and prolactin (PRL; $R^2 = .009$, B = 0.059, $P = .002$).

Beckman Coulter Gen II assay. Studies have suggested that AMH values measured using the Gen II assay are higher than those measured using the IOT[34] and DSL assays.[35] Therefore, the differences in AMH levels in the 2 studies may be due to the use of different assays rather than to differences in the study populations.

Although the AMH level reflects subtle changes during the menstrual cycle,[7] we still chose a sample collection time between days 2 and 5 of a spontaneous menstrual cycle or during the follicular phase for nonmenstruating women, mainly because some previous studies reported that the AMH level varies across the menstrual cycle based on the relative ovarian age. For example, the "younger ovary" pattern is associated with higher AMH levels that vary significantly between days 2 and 7.[36] Furthermore, the levels of FSH, E_2, and other hormones are related to the menstrual cycle,[37] and one of our objectives was to evaluate the associations of AMH with other indicators. In this study, we found that the AMH level increased with increased AFC and T, LH, PRL and PRG levels, decreased with increasing age, BMI, and FSH and was not significantly associated with E_2. Cui et al[20] also found that the AMH level was positively correlated with the AFC and LH levels, negatively associated with BMI and the FSH level, while they found that AMH was not significantly associated with T, in contrast to the present study, where a positive correlation between AMH and T was observed. This

discrepancy may be because our study sampled a healthy community population, while samples in their study were from infertile women.

Until now, most established reference ranges for AMH levels have been based on hospital inpatient samples, especially samples from infertile women.[15-17,22] The results may have been influenced by the AMH levels of women with endocrine disease or other occult fertility problems. It is, therefore, possible that the results obtained from hospital inpatient samples were not accurate in representing the normal population. Strict inclusion criteria ensuring that samples are obtained from healthy individuals may be necessary. Therefore, we excluded women who were younger than 40 years but have irregular menstrual cycles. While for women aged >40, we did not require regular menstrual cycles considering that they may be in normal perimenopause or menopause.

Furthermore, most of previous studies obtained AMH nomograms using either the DSL[15-17] or the Immunotech ELISA assays,[18-20] both of which have been replaced by the Beckman Coulter Gen II assay. In addition, conversion factors among these assays vary.[14,19] Because the DSL and IOT assays are now outdated, reference ranges for AMH that are established by the widely used Gen II assay may have more practical value. In this study, we used the new Beckman Coulter Gen II assay to measure serum AMH levels.

The present study has a few limitations that need to be addressed. First, the reference populations were volunteers recruited by advertisements rather than randomly selected participants, which may introduce bias and limited the generalizability of the results. Second, we provide reference values that can be used as normative data, while we did not have a replication sample to test the proposed values. Additional studies of larger healthy populations that genuine random sampling and including replication samples are still needed.

Conclusions

This is the first study to determine serum AMH reference ranges for Chinese women throughout reproductive age to menopause using a multicenter, nationwide population-based survey method. The results in this study may give Chinese clinicians a more reliable reference for correct interpretation of AMH levels and facilitate the adoption of AMH as a diagnostic tool in China.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

Approval for this study was obtained from the Ethical Committee of Tongji Medical College, Huazhong University of Science and Technology (2011S464). Clinical Trial Registration Number: NCT02294500

Supplemental Material

The online appendices/data supplements/etc are available at http://rs.sagepub.com/supplemental.

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