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Research Article

Distribution of Anti-Müllerian Hormone Levels According to Age in a Large Cohort of Mexican Women

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Abstract

Objective: To characterize the distribution of serum Anti-Müllerian Hormone (AMH) concentrations according to age in a large cohort of Mexican women and to generate clinically useful reference values for ovarian reserve assessment.

Materials and Methods: A retrospective observational study was conducted using hormonal records obtained from a clinical database. Serum AMH determinations with documented age were included for women between 20 and 50 years of age. AMH concentrations were analyzed according to age, and descriptive statistics together with age-specific percentiles were calculated for each age group.

Results: A total of 8,989 AMH determinations corresponding to 8,539 women with documented age were included in the analysis. Serum AMH concentrations showed a progressive decline with increasing age. In younger women, the median AMH concentration was approximately 3.0 ng/mL, whereas in older women the median decreased to approximately 0.6 ng/mL. Percentile analysis demonstrated substantial variability in AMH concentrations within each age group, with higher upper percentiles observed among younger women. These findings allowed the construction of age-specific percentile curves describing ovarian reserve patterns in the studied population.

Conclusions: Serum AMH concentrations demonstrate a clear age-dependent decline in this large cohort of Mexican women. The reference values generated in this study may serve as a useful clinical tool for ovarian reserve assessment and individualized reproductive counseling.

Keywords: Anti-Müllerian Hormone; AMH; Ovarian Reserve; Age-Specific Reference Values; Fertility; Mexican Women

Introduction

Assessment of ovarian reserve is a fundamental component of contemporary reproductive medicine. Several hormonal and ultrasonographic markers have been used to estimate female reproductive potential; however, Anti-Müllerian Hormone (AMH) has emerged as one of the most reliable and clinically useful biomarkers for this purpose [1,2].

AMH is a glycoprotein belonging to the transforming growth factor-beta (TGF-β) superfamily and is secreted by the granulosa cells of preantral and small antral follicles. Serum AMH concentrations indirectly reflect the size of the growing follicular pool, allowing a relatively accurate estimation of ovarian reserve [3].

Unlike other endocrine markers, such as follicle-stimulating hormone (FSH) and estradiol (E2), AMH exhibits minimal variation throughout the menstrual cycle, making it suitable for measurement at any point during the cycle [4,5]. This characteristic has facilitated its routine use in infertility evaluation, prediction of ovarian response during assisted reproductive technology (ART) [6] and fertility preservation counseling [7].

Several studies have demonstrated that serum AMH concentrations decline progressively with advancing age, reflecting the physiological process of ovarian aging. Nevertheless, reference values may differ across populations because of genetic, environmental, and methodological factors, highlighting the importance of establishing population-specific reference data [8,9].

Population-based AMH reference curves have been developed in several countries to facilitate the clinical interpretation of this biomarker. However, information regarding Mexican women remains limited, particularly from large-scale studies capable of accurately characterizing the distribution of AMH across different age groups [10-12].

Therefore, the objective of the present study was to characterize the age-related distribution of serum AMH concentrations in a large cohort of Mexican women and to generate age-specific reference values that may improve the clinical interpretation of ovarian reserve in routine reproductive medicine practice.

Materials and Methods

Study design and population

A retrospective observational study was conducted using hormonal records retrieved from a clinical database. The database included endocrine evaluations performed between 2020 and 2025 in women attending a reproductive medicine center.

Initially, 20,532 hormonal records were identified. For the present analysis, only records containing both documented age and serum AMH measurements were considered eligible. Women between 20 and 50 years of age were included. Following data cleaning and exclusion of incomplete records, 8,989 AMH determinations corresponding to 8,539 individual women were included in the final analysis.

Sample collection and processing

Biological specimens consisted of human serum obtained by venipuncture and collected in standard blood collection tubes, with or without separator gel, according to institutional laboratory procedures. Lithium heparin plasma samples were also considered acceptable when available, whereas EDTA plasma samples were excluded because of their potential analytical interference with the assay.

Following collection, samples were centrifuged to separate serum and remove particulate material when necessary, thereby minimizing analytical interference. Samples subjected to heat inactivation or preserved with sodium azide were excluded, in accordance with the manufacturer's recommendations.

Sample stability was maintained under controlled storage conditions as specified by the assay manufacturer: up to 3 days at room temperature (20-25°C), 5 days under refrigeration (2-8°C), and up to 6 months at −20°C (±5°C). Repeated freeze-thaw cycles were avoided, with no more than one freeze-thaw cycle permitted before analysis.

Before testing, all samples were allowed to reach room temperature (20-25°C) and were analyzed within 2 hours after loading onto the analyzer to minimize evaporation and analyte degradation.

Sample validation

Equivalence between serum and lithium heparin plasma was evaluated according to the manufacturer's analytical recovery

criteria. Recovery was considered acceptable when values remained within:

- ±30% of the serum concentration for AMH values ≥0.5 ng/mL
- ±0.2 ng/mL for AMH values <0.5 ng/mL

In addition, analytical agreement was verified by assessing assay linearity, acceptable slope values (0.90-1.10), deviations ≤10% at clinically relevant concentrations, and correlation coefficients ≥0.95, ensuring comparability between specimen types.

AMH measurement

Serum AMH concentrations were measured using the Elecsys® AMH Plus electrochemiluminescence immunoassay (ECLIA) on automated cobas® analyzers (Roche Diagnostics).

The assay is based on a sandwich immunoassay principle with an analytical runtime of approximately 18 minutes. During the first incubation step, 30 µL of sample was incubated with a biotinylated monoclonal anti-AMH antibody and a ruthenium-labeled monoclonal anti-AMH antibody, resulting in the formation of antigen-antibody immune complexes.

During the second incubation step, streptavidin-coated microparticles were added, allowing immobilization of the immune complexes through biotin-streptavidin interaction. The reaction mixture was subsequently transferred to the measuring cell, where the microparticles were magnetically captured onto the electrode surface. After removal of unbound material, application of an electrical current induced electrochemiluminescence, which was detected by a photomultiplier. AMH concentrations were calculated using a two-point calibration procedure together with the manufacturer's predefined master calibration curve.

Analytical measuring range

The assay has an analytical measuring interval ranging from 0.01 to 23 ng/mL.

Samples exceeding the upper limit of quantification were analyzed after automatic 1:2 dilution or manual dilution (up to 1:10) using the manufacturer's universal diluent, and final concentrations were corrected according to the corresponding dilution factor.

Quality control

Internal quality control was performed using PreciControl AMH Plus commercial control materials. Quality control samples were analyzed at least once every 24 hours, with each new reagent lot, and after every calibration procedure.

Analytical performance was considered acceptable according to predefined laboratory criteria, including:

- Deviation within ±12% of target values;
- Coefficient of variation (CV) ≤8%;
- Total analytical error ≤25%.

Analytical considerations

Potential pre-analytical, analytical, and biological sources of variability were considered during interpretation of AMH results.

No clinically significant analytical interference (defined as deviation ≤10%) was observed in the presence of bilirubin, hemoglobin, lipids, biotin, immunoglobulins, or rheumatoid factor within the evaluated concentration ranges. Likewise, commonly prescribed medications did not demonstrate clinically relevant interference.

Because the assay relies on biotin-streptavidin interaction, extremely high circulating biotin concentrations may interfere with the measurement and should be considered when interpreting results in selected clinical settings.

Although strong agreement exists between serum and lithium heparin plasma, variations in collection tubes, separator gels, or sample processing procedures may influence analytical performance. Consequently, standardized pre-analytical handling is recommended.

AMH is characterized by low intra- and inter-cycle biological variability compared with other reproductive hormones, supporting its role as a robust biomarker of ovarian reserve. Nevertheless, modest biological variation may occur in very young women or in women receiving hormonal contraceptives.

Finally, the assay provides excellent analytical sensitivity without evidence of a high-dose hook effect across the validated measuring range, supporting the reliability of AMH quantification over a broad spectrum of concentrations.

Statistical analysis

A dedicated database was created and analyzed using IBM SPSS Statistics version 30.

Descriptive analyses were performed to characterize serum AMH concentrations according to age. Empirical percentiles (2.5th, 10th, 25th, 50th, 75th, 90th, and 97.5th) were calculated for each age group to describe the distribution of AMH concentrations.

Age-specific percentile curves were subsequently generated to characterize the distribution of serum AMH across reproductive age groups and to establish clinically useful reference values for ovarian reserve assessment.

Results

Study population

A total of 20,532 hormonal records were initially identified in the clinical database. After data cleaning and exclusion of records with incomplete information, 8,989 serum AMH determinations corresponding to 8,539 women aged 20-50 years were included in the final analysis (Table 1).

Variable	Value
Total records	8,989
Mean age ± SD (years)	36.01 ± 5.47
Age range (years)	20-50
Mean serum AMH (ng/mL)	2.17
Median serum AMH (ng/mL)	1.49
Serum AMH range (ng/mL)	0.009-28.26

Table 1: Characteristics of the study population.

Demographic and biochemical characteristics of the women included in the study.

The mean age of the study population was 36.01 ± 5.47 years. Mean serum AMH concentration was 2.17 ng/mL, with a median of 1.49 ng/mL and an overall range of 0.009-28.26 ng/mL (Table 1).

Age-specific distribution of AMH

Age-specific AMH percentiles are presented in Table 2.

A progressive decline in serum AMH concentrations was observed with advancing age. Women in the youngest age group (20-25 years) exhibited a median AMH concentration of approximately 3.0 ng/mL, whereas women aged 41-50 yearshad a median value of approximately 0.65 ng/mL.

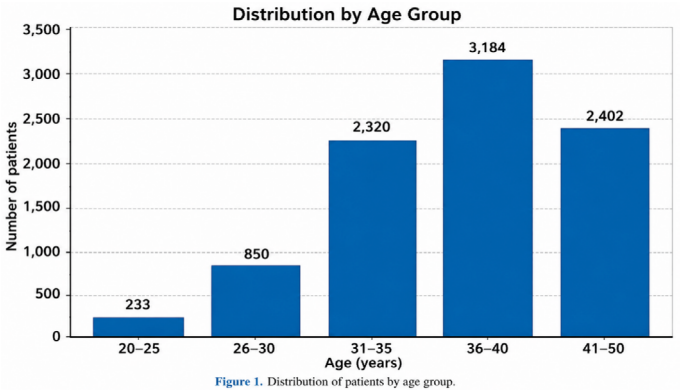

Figure 1. Distribution of patients by age group.

Table 2: Age categories.

Distribution of the study population according to age categories.

Similarly, both lower and upper percentile values progressively decreased across successive age groups. The 97.5th percentile declined from approximately 10.7 ng/mL among women aged 20-25 years to approximately 2.6 ng/mL in the oldest age group, illustrating the marked reduction in ovarian reserve associated with aging.

Distribution of AMH percentiles

Figure 1 illustrates the age-specific percentile curves for serum AMH concentrations. A consistent downward shift of all percentile curves with increasing age was observed, reflecting the physiological decline in ovarian reserve throughout the reproductive lifespan.

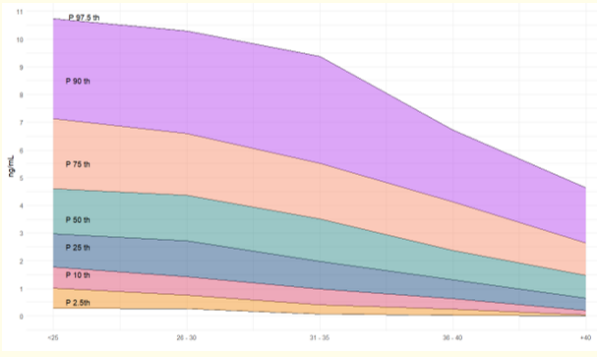

Figure 1: Distribution of Percentiles by Age Group.

Although the overall trend was similar across all percentiles, substantial interindividual variability was evident within each age group. Younger women demonstrated a wider distribution of AMH concentrations, whereas older women exhibited progressively narrower distributions concentrated at lower AMH levels.

Variability within age groups

Figure 2 presents the distribution of AMH concentrations using percentile area plots, providing a graphical representation of the variability observed within each age category.

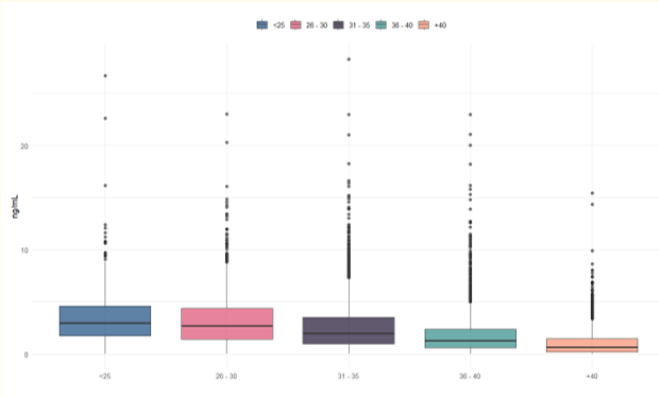

Figure 2: Percentile Distribution by Age Group.

The widest dispersion of AMH values was found among younger women, particularly between the 75th and 97.5th percentiles. In contrast, variability gradually decreased with age as the overall distribution shifted toward lower AMH concentrations.

These findings indicate that although serum AMH declines predictably with chronological age, considerable biological variability exists among women of similar age, particularly during the earlier reproductive years.

Overall findings

Taken together, the results demonstrate a clear age-dependent decline in serum AMH concentrations across this large cohort of Mexican women. The construction of age-specific percentile distributions provides clinically relevant reference values that may facilitate interpretation of individual AMH measurements and improve ovarian reserve assessment in routine reproductive medicine practice.

Discussion

The present study evaluated the age-related distribution of serum AMH concentrations in a large cohort of 8,539 Mexican women, providing one of the most comprehensive population-based descriptions of AMH reference values currently available in Mexico. Our findings demonstrate a clear age-dependent decline in serum AMH concentrations, accompanied by substantial interindividual variability within each age group. These results reinforce the role of AMH as a robust biomarker of ovarian reserve and provide clinically relevant reference values for reproductive medicine practice.

AMH is produced by granulosa cells of preantral and small antral follicles, and its circulating concentration reflects the size of the growing follicular pool. Consequently, the progressive decline in AMH observed with advancing age is consistent with the physiological depletion of ovarian follicles throughout reproductive life. This biological relationship has been extensively documented and supports the widespread use of AMH as a marker of ovarian reserve.

Our findings are consistent with previous studies reporting a gradual reduction in AMH concentrations beginning during the third decade of life, with a more pronounced decline after the age of 35 years. La Marca., *et al*. demonstrated that AMH closely reflects ovarian aging and is a reliable predictor of ovarian response in assisted reproductive technology [13]. Similarly, Seifer., *et al*. reported a strong inverse association between serum AMH concentrations and chronological age, supporting its clinical value as one of the most stable endocrine markers of ovarian reserve [14].

An important finding of the present study is the considerable variability in AMH concentrations among women of the same age. Although chronological age remains the principal determinant of ovarian reserve, the wide distribution of AMH values observed within each age group highlights the substantial biological heterogeneity that exists among individuals. Similar observations have been reported by Kelsey., *et al*. whose population-based model demonstrated that women of identical age may exhibit markedly different AMH concentrations while remaining within the expected physiological range [15].

Previous studies conducted in Mexican women, including those by Rodríguez-Purata and colleagues, have described age-related AMH values using large clinical datasets. Our results are consistent with these reports, confirming the progressive decline in AMH across reproductive life. However, the present study expands existing evidence by incorporating a larger cohort and emphasizing an age-specific percentile approach rather than relying exclusively on measures of central tendency [16,17].

The use of percentile-based reference values represents one of the principal strengths of this study. Because serum AMH concentrations exhibit a markedly non-normal distribution, reporting only mean or median values may not adequately reflect the variability observed within the population. Age-specific percentile curves provide a more comprehensive framework for interpreting individual AMH measurements by placing each patient's value within the expected distribution for her age group. This approach may facilitate more individualized clinical counseling and improve decision-making in reproductive medicine.

The graphical representation of percentile curves further illustrates the progressive downward displacement of AMH concentrations throughout reproductive life. Similar graphical models have been adopted in several population-based studies because they provide clinicians with an intuitive tool for interpreting ovarian reserve in relation to chronological age.

Several limitations should be acknowledged. First, the retrospective nature of the study precluded access to detailed clinical information, including reproductive history, body mass index, ovarian pathology (particularly polycystic ovary syndrome), previous ovarian surgery, hormonal contraceptive use, and other factors known to influence serum AMH concentrations. Second, although the cohort is large, it was derived from women attending reproductive medicine centers rather than from a randomly selected population sample, which may limit the generalizability of the findings.

Nevertheless, the study also has important strengths. The large sample size, standardized laboratory methodology, and use of a single analytical platform minimize methodological variability and enhance the reliability of the generated reference values. Furthermore, the percentile-based analysis provides clinically applicable reference intervals that may improve interpretation of AMH measurements in routine practice.

Overall, the present study contributes valuable reference data for the Mexican population and supports the incorporation of age-specific AMH percentile curves into clinical assessment of ovarian reserve. Future prospective multicenter studies including detailed clinical information and long-term reproductive outcomes will be important to further validate these findings and refine population-specific reference standards.

Conclusions

The present study characterizes the age-related distribution of serum Anti-Müllerian Hormone (AMH) concentrations in a large cohort of 8,539 Mexican women, providing one of the largest datasets currently available for this population.

Serum AMH concentrations demonstrated a progressive decline with advancing age, consistent with the physiological process of ovarian aging. In addition, substantial interindividual variability was observed within each age group, emphasizing the importance of interpreting AMH values in the context of age-specific reference distributions rather than relying solely on chronological age or measures of central tendency.

The age-specific percentile curves generated in this study provide clinically relevant reference values that may facilitate the interpretation of AMH measurements, improve ovarian reserve assessment, and support individualized reproductive counseling in women of reproductive age.

Furthermore, these findings contribute to the development of population-specific reference standards for Mexican women and may serve as a valuable resource for both clinical practice and future research in reproductive medicine.

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Conflict of Interest

The authors declare no conflicts of interest related to this study.

Data Availability Statement

The datasets generated and analyzed during the current study are not publicly available because they contain confidential patient information but are available from the corresponding author upon reasonable request and subject to institutional approval.

Ethics Statement

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

The study protocol was reviewed and approved by the Institutional Research and Ethics Committee of Fertility and Reproductive Medicine (Approval No. 1).

Because of the retrospective nature of the study and the use of anonymized clinical data, the requirement for informed consent was waived by the Ethics Committee.

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