

Age-related normograms of serum antimüllerian hormone levels in a population of infertile women: a multicenter study

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Objective: To produce age-related normograms for serum antimüllerian hormone (AMH) level in infertile women without polycystic ovaries (non-PCO).

Design: Retrospective cohort analysis.

Setting: Fifteen academic reproductive centers.

Patient(s): A total of 3,871 infertile women.

Intervention(s): Blood sampling for AMH level.

Main Outcome Measure(s): Serum AMH levels and correlation between age and different percentiles of AMH.

Result(s): Age-related normograms for the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles of AMH were produced. We found that the curves of AMH by age for the 3rd to 50th percentiles fit the model and appearance of linear relation, whereas the curves of >75th percentiles fit cubic relation. There were significant differences in AMH and FSH levels and in antral follicle count (AFC) among women aged 24–33 years, 34–38 years, and ≥39 years. Multivariate stepwise linear regression analysis of FSH, age, AFC, and the type of AMH kit as predictors of AMH level shows that all variables are independently associated with AMH level, in the following order: AFC, FSH, type of AMH kit, and age.

Conclusion(s): Age-related normograms in non-PCO infertile women for the 3rd to 97th percentiles were produced. These normograms could provide a reference guide for the clinician to consult women with infertility. However, future validation with longitudinal data is still needed. (Fertil Steril® 2011; ■:■–■. ©2011 by American Society for Reproductive Medicine.)

Key Words: Antimüllerian hormone, ovarian reserve, age, normogram

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TABLE 1

Correlation between the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles of serum AMH level and age.

Age (y)	No. of samples	Serum AMH level (ng/mL)						
		3rd	10th	25th	50th	75th	90th	97th
<24	78	0.38	0.72	1.28	2.24	3.67	5.49	7.90
25	40	0.35	0.70	1.25	2.22	3.69	5.56	8.05
26	32	0.33	0.67	1.23	2.21	3.71	5.63	8.20
27	77	0.31	0.64	1.20	2.20	3.73	5.70	8.35
28	83	0.29	0.62	1.18	2.18	3.73	5.75	8.47
29	128	0.27	0.59	1.15	2.16	3.73	5.79	8.57
30	136	0.25	0.56	1.11	2.12	3.71	5.80	8.64
31	172	0.23	0.53	1.07	2.08	3.67	5.77	8.64
32	187	0.21	0.50	1.02	2.01	3.58	5.68	8.55
33	199	0.19	0.46	0.96	1.92	3.47	5.53	8.37
34	259	0.17	0.42	0.90	1.83	3.33	5.34	8.13
35	260	0.15	0.39	0.84	1.73	3.19	5.16	7.88
36	225	0.13	0.36	0.79	1.65	3.07	4.99	7.67
37	195	0.12	0.33	0.74	1.57	2.95	4.84	7.47
38	192	0.10	0.30	0.70	1.49	2.84	4.68	7.25
39	189	0.09	0.27	0.64	1.40	2.69	4.47	6.96
40	120	0.08	0.24	0.58	1.29	2.51	4.18	6.55
41	77	0.06	0.21	0.51	1.16	2.26	3.80	5.97
42	51	0.05	0.17	0.44	1	1.97	3.32	5.25
43	34	0.04	0.14	0.35	0.82	1.63	2.77	4.39
44	22	0.03	0.10	0.27	0.63	1.26	2.15	3.43
>45	14	0.02	0.07	0.18	0.43	0.87	1.50	2.41

Almog. Age-related normogram for antimüllerian hormone. Fertil Steril 2011.

Antimüllerian hormone (AMH) is a member of the transforming growth factor- β superfamily (1). In females it is synthesized in the granulosa cells of preantral and small antral follicles (2–4). Antimüllerian hormone was previously thought to have a sole role in the embryonic life as a male sex differentiation factor (5). Today, it is recognized as a good indicator for ovarian reserve and potential fertility. Several studies have demonstrated the role of AMH to predict the quantitative and qualitative ovarian response in assisted reproductive technologies (ART) (6–19).

Antimüllerian hormone might also be used as a marker for ovarian failure, polycystic ovarian syndrome, ovarian hyperstimulation syndrome, and menopause (20–25). As some women tend to delay procreation and fertility declines with advancing age, ovarian reserve testing is becoming increasingly relevant. In addition, we can now preserve fertility by cryopreserving oocytes and/or embryos.

Ovarian aging is characterized by a gradual decrease in both quantity and quality of the oocytes residing within the follicles.

FIGURE 1

Correlation between the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles of serum AMH level and age.

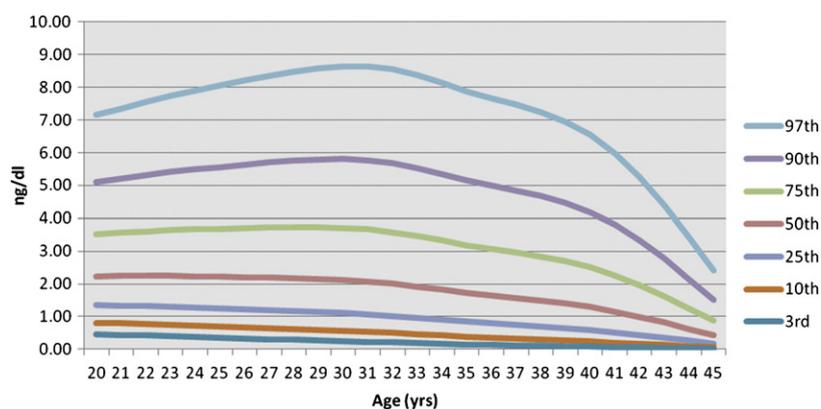
*Almog. Age-related normogram for antimüllerian hormone. Fertil Steril 2011.*

TABLE 2

Serum AMH and baseline FSH levels and AFC among women of different age groups.

Parameter	24–33 y	34–38 y	> 39 y	P value (95% CI) ^a	P value (95% CI) ^b
AMH level (ng/mL) ^c	2.1 (1.1–3.4)	1.6 (0.8–2.9)	1.1 (0.5–2.3)	<.001 (0.2–0.5)	<.001 (0.2–0.5)
FSH level (IU/L) ^c	6.9 (5.5–8.3)	7.4 (6–9.4)	7.9 (6.2–10.6)	<.01 (0.4–0.9)	<.02 (0.2–0.8)
AFC ^c	11 (8–16)	10 (6–13)	7 (4–11)	.001 (1–3)	<.001 (2–3)

Note: CI = confidence interval.

^a Age 24–33 y vs. age 34–38 y.

^b Age 34–38 y vs. age >39 y.

^c Median (interquartile range).

Almog. Age-related normogram for antimüllerian hormone. *Fertil Steril* 2011.

The availability of a test able to provide reliable information with respect to a woman's ovarian reserve within a given age category would help the clinician to provide an individually tailored treatment plan and prognosis (26). Different studies in the literature suggest that AMH is a potential marker of the ovarian follicle pool, thus reflecting the ovarian reproductive age (27, 28).

The purpose of our study was to create age-related normograms of AMH that could serve as a clinical tool for consulting infertile women without polycystic ovaries (non-PCO).

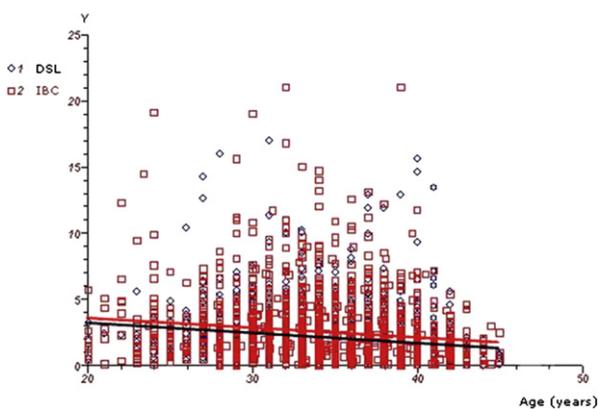
MATERIALS AND METHODS

Our study population included 3,871 infertile women from 15 academic reproductive centers mainly from Europe and North America. Blood samplings of AMH level were on day 3 of the cycle.

As we planned to study non-PCO infertile women, we excluded 1,101 cases with PCO. Exclusion criteria included women whose PCO status was unknown ($n = 885$) and women who were defined as having PCO by the Rotterdam criteria ($n = 216$) (29). The remaining 2,770 cases were available for analysis.

FIGURE 2

Grouped linear regression lines of AMH levels by age and by the type of AMH kit (IBC or DSL). Each kit creates a statistically different line parallel to the line of the other kit. The vertical difference between the lines is 0.4 ng/mL, with the IBC AMH kit higher than the DSL kit.



Almog. Age-related normogram for antimüllerian hormone. *Fertil Steril* 2011.

We created a database using Excel (Microsoft, Redmond, WA). It contained information from 15 reproductive centers on age of the patients, antral follicle count (AFC), baseline (day-3) levels of E₂, FSH, and LH, and AMH. Antimüllerian hormone was measured using either Immunotech–Beckman Coulter (Brea, CA) ELISA (IBC; $n = 1,550$) or Diagnostic System Laboratories (Webster, TX) (DSL; $n = 1,220$). We converted AMH in SI units (pmol/L) to ng/mL by the conversion factor 7.14.

To construct a model that best described the percentile curves of AMH by age, we used the LMS method, whereby L stands for skewness, M for median, and S for coefficient of variation (30–32). Using LMS software (Institute of Child Health, London, United Kingdom), we constructed the centile curves. For each age, seven empirical percentiles (3rd, 10th, 25th, 50th, 75th, 90th, and 97th) were applied. To evaluate the independent effects of age, AFC, FSH, and the type of kit used for AMH measurements, we performed multivariate stepwise regression analysis.

The normality of data distribution was evaluated using the Shapiro-Wilk test. Because the data were not normally distributed, differences of AMH level, FSH level, and AFC by age group were analyzed using the Kruskal-Wallis test and Mann-Whitney *U* test.

Data were analyzed using the SPSS software package (SPSS, Chicago, IL). The differences were considered significant at $P < .05$.

RESULTS

The correlation between the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles of AMH and age is demonstrated in Table 1 and Figure 1. The values presented were adjusted to obtain the minimal deviation for the percentile curves; in other words, the data were normalized to create the best fit to a smoothened curve. We found that the curves of AMH by age for the 3rd to 50th percentiles fit the model and appearance of linear relation, whereas the curves of >75th percentiles fit cubic relation.

Table 2 shows serum AMH, baseline FSH levels, and AFC among women of different age groups. There were significant differences in AMH, FSH levels, and AFC among women aged 24–33 years, 34–38 years, and ≥ 39 years. The correlation of AMH and age was significant ($P < .001$, $r = -0.15$), albeit with a low correlation coefficient. Higher correlation coefficients were seen between age and AFC ($r = 0.24$, $P < .01$) and FSH ($r = -0.19$, $P < .01$).

Multivariate stepwise linear regression analysis of FSH, age, AFC, and the type of AMH kit as predictors of AMH level showed that all variables were independently associated with AMH level, in the following order: AFC, FSH, the type of AMH kit, and age (Supplementary Table 1). Using group linear regression we found that the regression line of AMH results by IBC was different from

that of results by DSL. The vertical difference of the IBC AMH kit was 0.4 ng/mL higher than DSL (Fig. 2). The proportion of women with very low AMH level (<0.35 ng/mL) ranged from 2.9% at age <24 years to more than 40% at the age >44 years, suggesting decreased fertility with increasing age.

DISCUSSION

Antimüllerian hormone level is a potential predictor for menopause, poor ovarian reserve, and premature ovarian failure (14, 33–40). Unlike FSH, AMH level is independent of the time of menstrual cycle (8). In this study, we produced AMH percentiles normograms in a non-PCO infertile population. We found that the curves of AMH by age for the 3rd to 50th percentiles fit the model and appearance of linear relation, whereas the curves of >75th percentiles fit cubic relation.

The US Centers for Disease Control and Prevention suggest an approach to interpret growth normograms. One measurement is used as a screening tool and a series of measurement as a warning sign (41). This approach could be applied to our results. For example, a single measurement that places AMH level at the 3rd percentile in a 25-year-old woman suggests low ovarian response, whereas a 40-year-old woman with AMH at the 97th percentile indicates otherwise. A series of measurement in a 30-year-old woman showing AMH levels at the 90th percentile, 50th percentile, and then 25th percentile suggests rapidly declining fertility.

Our normograms may also have an application in prediction and prevention of ovarian hyperstimulation syndrome and in creating individually tailored gonadotropins dose adjustment. We excluded women with PCO because of their higher levels of AMH (three times higher [22]) and higher AFC compared with non-PCO patients. Incorporating PCO results into the analysis would have created significantly higher deviation of the percentiles result and curves.

Our study population did not include normal fertile women. It is possible that their AMH levels are not the same and follow a different normogram. A comparison with such a group could be explored in a future study. At this time we are not aware of a large database of AMH tests results for fertile women.

Antimüllerian hormone levels were independently associated with the following order of variables: AFC, FSH, the type of AMH kit, and age. Currently there are two different kits for AMH measurement, IBC and DSL. The difference between the two assays is due to their dissimilarity in the antibodies, leading to differences in the assay sensitivities. Initial studies have shown that AMH levels seem to be four- to fivefold lower with the DSL assay compared with the IBC assay (42, 43). However, recent studies have demonstrated that the correlation between the two assays is very high (44, 45). We

found the difference between the two assays to be 0.4 ng/mL (by grouped linear regression) to 0.5 ng/mL (by multivariate stepwise linear regression). In agreement with others, we found that compared with the DSL kit, the IBC kit resulted in higher levels. However, although developed independently, these assays are now both produced by a single company (Beckman Coulter), therefore suggesting that the methodologic problems mentioned by Bersinger et al. (42) should have been addressed and solved by the assay manufacturer (5).

Current tests of ovarian reserve, including AMH, are designed to predict how a woman is likely to respond to controlled ovarian stimulation and are better considered as evaluating “functional ovarian reserve” (24). It is also important to remember that although certain ovarian reserve tests, such as AMH and AFC, have been linked to the outcome of treatment and pregnancy, they do not predict the quality of oocytes in the ovarian pool, and they do not perform well enough in this respect. Thus couples should not be precluded from having ART on the basis of these tests alone. Women with ovarian reserve tests that imply a significant impairment in their follicle pool still maintain an ability to conceive both naturally and after fertility treatment, but they should be made aware that these chances seem to be reduced compared with women of similar age with more reassuring test results. We hope that the centile charts presented in this article help clinicians demonstrate this by providing patients with a graphic representation of how their results compare with these other women.

The weakness of our study is the use of cross-sectional data, making it difficult to distinguish the behavior of different cohorts over time. Thus, it is possible that the percentile curves merely represent the normal distribution of AMH levels. Accordingly, the analysis of rate of decline may be a rough estimation according to our data. Longitudinal studies are certainly needed to validate our normogram. However, longitudinal studies require a large cohort that could be followed for many years. As a result, other commonly used normograms, such as fetal growth and birth weight curves, are also based on cross-sectional data (30, 46, 47). Another weakness is that there are no data correlating the AMH levels with response to controlled ovarian stimulation, spontaneous pregnancy, and clinical outcome of ART. However, those subjects have been extensively studied (1–25, 48, 49).

We conclude that age-related normograms in non-PCO infertile women show that the curves of AMH by age for the 3rd to 50th percentiles fit a model and appearance of linear relation, whereas the curves of >75th percentiles fit cubic relation. These normograms could provide a reference guide for the clinician to consult women with infertility. However, future validation with longitudinal data is still needed.

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SUPPLEMENTARY TABLE 1

Multivariate stepwise linear regression analysis of FSH, age, type of AMH kit, and AFC as predictors of AMH level.

Model	Slope (B)	SE	Standardized coefficient	P value	95% CI
(Constant)	3.60	0.425		<.001	2.76, 4.43
AFC	0.105	0.008	0.348	<.001	-0.04, -0.007
FSH	-0.087	0.016	-0.149	<.001	0.08, 0.12
Age	-0.021	0.01	-0.069	<.008	-0.11, -0.05
Type of AMH kit	-0.515	0.088	-0.146	<.001	-0.68, -0.34

Note: CI = confidence interval.

Almog. Age-related normogram for antimüllerian hormone. *Fertil Steril* 2011.