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Age-specific serum anti-Mullerian hormone reference values for infertile Filipino women in a tertiary *in vitro* fertilization center in the Philippines

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Abstract:

BACKGROUND: Anti-Mullerian hormone (AMH) levels have been used as an invaluable tool in reproductive medicine for over a decade, especially in predicting ovarian reserve and follicular response during *in vitro* fertilization (IVF) cycles. Age-specific reference values of AMH levels have been derived from mostly Western and few Asian population groups but none from the Philippines. In this study, we attempted to determine the first age-specific AMH reference values from infertile Filipino women to be able to provide local infertility centers a guide in treating Filipinos and those with similar racial and lifestyle characteristics.

MATERIALS AND METHODS: This was a retrospective cross-sectional study that was conducted at the Center for Advanced Reproductive Medicine and Infertility at St. Luke's Medical Center Global City. Age, serum AMH levels, body mass index (BMI), and cause of infertility of Filipino women who underwent IVF from August 2015 to March 2020 were taken. AMH was assayed using the automated Access AMH Immunoassay (Beckman Coulter).

RESULTS: A total of 1463 women who underwent IVF and with valid AMH results were initially found but only 1233 were included in the study. Mean age was 36.67 + 4.35 years and mean BMI was 24.43 + 4.14 kg/m^2. There was minimal effect of BMI on AMH levels and increasing age (R^2 = 0.0068), but there were significant differences of mean AMH levels among the general causes of infertility. The mean and median AMH values decreased with advancing age (R^2 = 0.1391) although the mean values were consistently higher than the median values. The mean level of the AMH was 2.32 ± 1.90 ng/mL with a 0.16 ng/ml (confidence interval: 0.14–0.19 ng/ml) level decrease per year of increase in age. By age category, the following were the derived AMH values (ng/ml) at the 25th to 75th percentiles: 25–29 = 1.52–4.92; 30–34 = 1.60–4.10; 35–39 = 0.95–3.13; 40–44 = 0.44–1.99; 45 = 0.47–1.08. The mean AMH in this study appears to be similar to several but lower than most other reported AMH nomograms from other population groups.

CONCLUSION: The first age-specific AMH reference values for infertile Filipino women are presented and may serve as a useful diagnostic marker in local infertility centers, especially those treating Filipino patients or others with similar characteristics.

Keywords:

Anti-Mullerian hormone level, Filipino, *in vitro* fertilization, infertile, reference range

Introduction

As a woman ages, her fertility potential decreases in part due to the decline in the number of primordial follicles that are proportional to the decline in anti-Mullerian hormone (AMH) levels.[1] AMH is a glycoprotein produced by granulosa cells

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of small and large preantral and small antral follicles that belong to the transforming growth factor-β superfamily.

A number of studies have reported the strong correlation of serum AMH levels with antral follicle count and are more accurate than other serum biomarkers such as follicle-stimulating hormone, serum estradiol, or inhibin B in predicting ovarian reserve and response to controlled ovarian stimulation.[2-4] It can be tested on any day of the cycle because it is independent of the circulating gonadotropins and is reliable with high sensitivity and specificity.[2] One of the limitations of AMH, however, is the lack of an internationally standardized assay.[5]

It is critical to mention the type of AMH immunoassay that is utilized. The first and second generations of commercial AMH immunoassays including the Gen II Immunoassay (Beckman Coulter) were in use until 2013 when problems about reproducibility were reported.[6] Since then, third-generation automated AMH immunoassays such as the Access (Beckman Coulter) have been introduced. These third-generation systems used a far more sensitive measuring method and have significantly simplified the AMH process with improved uniformity and reliability of results plus a dramatic reduction in measurement time.[7] Historically, St. Luke's Medical Center Global City was the first facility in the Philippines to offer serum AMH determination and is believed to have the largest number of AMH results in its databank because of its *in vitro* fertilization (IVF) unit. The Gen II Immunoassay was introduced in 2011 but was replaced by Access in 2015.

Serum AMH is often requested during the initial fertility evaluation and has a variety of uses in the management of infertile couples. It can guide clinicians in identifying women who are more likely to respond poorly or hyper-respond to gonadotropin stimulation. The other proposed uses of AMH are as a biomarker forpolycystic ovary syndrome (PCOS), as a predictor of menopause, and as a predictor of time to menses in women receiving gonadotoxic medications.[8]

Considering the wide application of AMH levels in clinical practice, it is important to arrive at a local reference range. Although studies on normal AMH reference values have been conducted in a number of Western and some Asian studies,[9-17] no AMH nomogram has been established for Filipino women. Knowing the age-specific reference values of serum AMH for Filipino women would provide a framework for those presenting at their initial fertility assessment.[18] These values would guide clinicians in counseling and managing these patients in the local setting.

Materials and Methods

This is a retrospective cross-sectional study that primarily aimed to establish the age-specific AMH reference values of infertile Filipino women undergoing IVF from August 2015 to March 2020. This was conducted at the Center for Advanced Reproductive Medicine and Infertility (CARMI) at St Luke's Medical Center Global City.

The absence of local data on the prevalence of infertility, the limited number of women who took the serum AMH test, and the unknown utility of the biomarker made sample size computation to be inefficient. Thus, all eligible patients within the study period were included.

The secondary objectives were to determine if body mass index (BMI) affected AMH levels and to determine differences of AMH levels, if any, among the causes of infertility.

Upon approval of the hospital review board (RPC-078-04-20; SL-20109), data on patient's age, serum AMH levels, BMI, and cause of infertility were extracted from the center's database and entered into the datasheet.

Patients and Anti-Müllerian hormone measurement

Filipino women, 25–45 years of age, that underwent IVF treatment at CARMI from August 2015 to March 2020 with serum AMH results that were processed at the hospital's Pathology laboratory were included. Women diagnosed with polycystic ovary syndrome (PCOS), non-Filipinos, and those with previous ovarian surgery such as oophorectomy or oophorocystectomy were excluded.

The machine for measuring AMH levels was the Access AMH immunoassay (Beckman Coulter) that was utilized in the hospital from August 2015. Values were reported in ng/ml. Assay kit performance was validated by in-house assessment of reproducibility and linearity.

Statistical analysis

After the data were extracted from the patient charts, all the information were manually entered into an electronic spreadsheet, with subsequent data processing and analysis carried out using the software, Stata 13 (StataCorp.2013. Stata Statistical Software: Release 13. College Station, USA: Texas: StataCorp LP).

Descriptive statistics using mean, standard deviation, and median for continuous data variables and frequency, and percentage for the categorical data variables were computed to provide an overview of the study population.

The study participants were then stratified according to the following age categories: 25–29, 30–34, 35–39, 40–44, and ≥45 years old. The mean (and their 95% confidence interval [CI]), standard deviation, median, range, and percentiles (5th, 10th, 25th, 50th, 75th, 90th, and 95th) of the AMH levels were tabulated and presented in a series of graphs and charts.

The correlation between age and the levels of AMH was also estimated using Pearson's correlation coefficient including its interval estimate.

A series of one-way analysis of variance and Kruskal–Wallis tests was performed to determine differences in the AMH levels across the age range and body mass index. Analysis of covariance was also performed with the actual age in years, BMI classifications, and causes of infertility as the covariates for the different levels of the AMH in the sample population.

The level of significance for all sets of analysis was set at a $P < 0.05$ using two-tailed comparisons. The said levels were adjusted for the multiple comparison procedure performed using the Fisher-Hayter method.

Results

A total of 1463 women were initially found but only 1233 were eligible and included in the study. Those excluded were women with PCOS (113), non-Filipinos (70), those <25 or >45 years old (43), and those with a history of ovarian surgery (4).

Baseline clinicodemographic characteristics

The baseline demographic and clinical characteristics of the study population are summarized in Table 1 where mean age, mean BMI, general causes of infertility, and mean AMH levels are presented. It is interesting to note that almost half of the women are classified as being obese (n: 530, 42.98%, CI: 40.20%–45.80%) based on the BMI categories for Asian women.[19] Furthermore, half of the women have infertility attributed to a female factor only, followed by more than a quarter of the subjects reported to have both male and female factors at 30.33% (95% CI: 27.78%–32.98%) both representing significant proportions of the study population. Finally, with mean levels of AMH at 2.32 ± 1.90 ng/mL, it is noted that half of the sample population have the biomarker levels below 1.89 (IQR: 2.36) ng/ml.

Table 1: Baseline clinicodemographic characteristics of the study population

Variables	Summary measures, frequency (%)
Mean age (years)	36.67±4.35
Mean BMI (kg/m^2), range	24.43±4.14 (14.6-51.1)
Categories of BMI[19]	
Underweight	28 (2.27)
Normal	468 (37.96)
Overweight	207 (16.79)
Obese I	424 (34.39)
Obese II	106 (8.60)
Causes of infertility	
Male factor only	81 (6.57)
Female factor only	625 (50.69)
Both male and female factors	374 (30.33)
Unknown/unexplained	153 (12.41)
Serum AMH levels (ng/ml)	
Mean±SD	2.32±1.90
Range	0.01-13.39

BMI: Body mass index, AMH: Anti-Müllerian hormone, SD: Standard deviation

Anti-Mullerian hormone and age

The AMH values of women who were classified into five age-based categories or their actual age are presented according to mean, median, and percentile in Table 2. The results consistently show decreasing AMH levels with increasing age in terms of mean, median, or percentile of AMH levels. This is better illustrated in Figure 1 where box plot levels of mean AMH across the age categories show the progressive decrease with increasing age. The 25th to the 75th percentile values across the different age categories may be used as a guide in managing Filipino women seeking infertility treatment.

The mean and median values of AMH across the five age categories may also be compared [Table 2]. The mean levels of AMH are significantly different across age categories ($F[4, 1228] = 43.56$, $P < 0.01$). Based on the Fisher-Hayter multiple comparisons procedure, the levels of AMH among women at the 35–39, 40–44, and 45-year-old groups were significantly lower than the mean AMH levels of women between 25 and 34 years old. Similarly, median AMH values across age categories decreased with age. It is interesting to note that mean

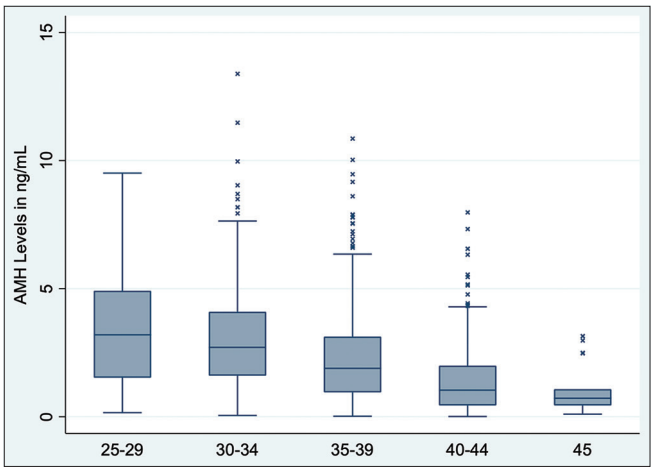

Figure 1: Box plot of AMH levels and age categories

Table 2: Correlation of mean, median, and percentiles of serum anti-Mullerian hormone level with actual age and age categories

Age	*n*	Frequency (%)	Mean AMH±SD	Median AMH (IQR)	Percentiles of AMH level (ng/mL)													
					5th		10th		25th		50th		75th		90th		95th	
25	5	82 (6.65)	3.43±2.27	3.20 (3.40)	2.38	0.71	2.38	1.08	3.39	1.52	3.63	3.20	7.56	4.92	9.51	6.64	9.51	7.54
26	9				1.54		1.54		2.92		3.57		6.36		7.54		7.54	
27	11				1.14		1.32		2.35		3.67		6.64		7.13		8.83	
28	26				0.24		0.61		1.37		3.65		5.89		6.59		6.65	
29	31				0.71		1.00		1.12		1.86		3.28		4.29		5.63	
30	43	288 (23.36)	3.08±1.99	2.71 (2.50)	0.16	0.49	0.48	0.93	1.64	1.60	2.59	2.71	4.23	4.10	5.29	5.60	8.50	6.55
31	48				0.85		1.23		2.04		2.54		3.27		5.06		5.61	
32	43				1.01		1.19		1.60		2.79		4.91		6.67		8.70	
33	66				0.61		0.72		1.48		2.81		3.90		5.25		5.83	
34	88				0.64		1.04		1.56		2.79		4.10		5.63		6.21	
35	99	513 (41.61)	2.32±1.82	1.89 (2.18)	0.41	0.34	0.61	0.49	1.16	0.95	2.25	1.89	3.77	3.13	5.76	4.86	7.13	6.12
36	94				0.43		0.66		1.07		2.03		3.70		5.66		6.78	
37	111				0.34		0.67		1.00		2.09		2.98		4.24		5.79	
38	108				0.17		0.34		0.64		1.55		2.44		4.37		5.34	
39	101				0.39		0.51		0.91		1.84		2.66		4.06		4.47	
40	96	328 (26.60)	1.44±1.35	1.04 (1.55)	0.22	0.11	0.27	0.22	0.63	0.44	1.45	1.04	2.45	1.99	3.90	3.30	4.37	4.19
41	85				0.14		0.31		0.63		1.36		1.99		3.52		5.13	
42	60				0.10		0.13		0.36		0.72		1.35		2.82		3.19	
43	55				0.08		0.14		0.27		0.70		1.21		2.07		3.18	
44	32				0.05		0.20		0.33		0.75		2.22		3.16		3.80	
45	22	22 (1.78)	1.08±1.03	0.72 (0.64)	0.12	0.12	0.14	0.14	0.44	0.47	0.72	0.71	1.08	1.08	2.98	2.98	3.15	3.15
Overall	1233	100%	2.32±1.90	1.89	0.24		0.39		0.89		1.89		3.25		4.97		6.12	

AMH: Anti-Müllerian hormone, SD: Standard deviation, IQR: Interquartile range

AMH values were consistently higher than median AMH levels across all age categories.

The Kruskal–Wallis test was also performed which showed that for the same age groups (i.e., 35–39, 40–44, ≥45 years old), more than half of the women in their categories have AMH levels lower than median value for the age group ($\chi^{2,4} = 126.70$, $P < 0.01$). Moreover, the levels of the serum AMH was negatively correlated with age ($\rho = -0.37$, 95% CI = −0.32 to − 0.42, $P < 0.01$), and it was noted that a single year of increase in the age of a woman can result to about 0.16 ng/ml reduction (95% CI: −0.14 to − 0.19) in the levels of the biomarker ($R^2 = 13.91\%$).

The trend of decreasing AMH levels with increasing actual age is illustrated in a nomogram [Figure 2] where the decline appears greater in the younger ages and becomes less with increasing age. The scattergram presented [Figure 3] with an $R^2 = 0.1391$ suggests that the average rate of decline of AMH level is 13.91 ng/ml per year.

Effect of BMI on Anti-Müllerian hormone

Based on Table 3, the mean levels of AMH appear to be significantly different across the categories of BMI (F[4, 1228] =2.43, $P = 0.04$). The Fisher-Hayter multiple comparison procedure has shown that the levels of AMH among women in the Obese II groups are significantly lower than the average levels of women in the normal and overweight groups.

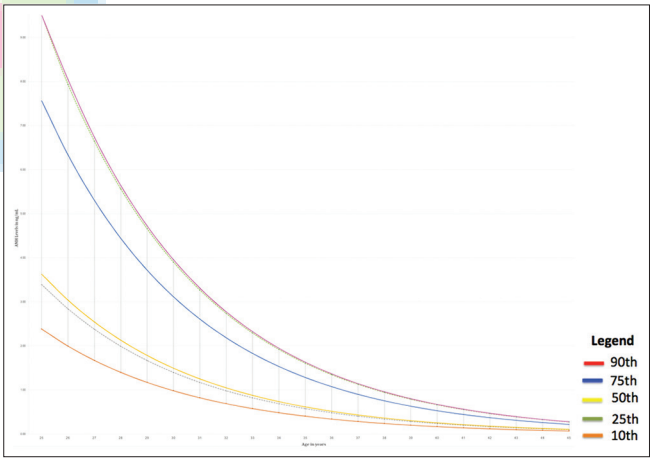

Figure 2: Distribution of the various AMH percentiles and actual age

The levels of the serum AMH was correlated with BMI ($\rho = -0.08$, 95% CI = −0.03 to −0.14, $P = 0.04$), and it was found that a single kilogram per square meter of increase in a woman is associated with 0.04 unit reduction (95% CI: −0.01 to −0.06) in the level of the AMH ($R^2 = 0.68\%$). This suggests that increasing BMI decreases AMH levels quite minimally.

The estimated marginal means were also computed and presented in Table 4, which shows the "adjusted" average AMH levels along the five age categories after statistically removing the effect of BMI. This further

Table 3: Serum anti-Mullerian hormone levels across body mass index categories

BMI category[19]	AMH levels (ng/ml)				
	Mean±SD	*P*	Median (IQR)	*P*	Range (minimum-maximum)
Underweight	2.53±1.80	0.04	2.03 (2.57)	<0.01*	0.26-5.96
Normal	2.38±1.87		2.04 (2.42)		0.02-11.48
Overweight	2.43±1.98		1.98 (2.64)		0.05-9.97
Obese I	2.31±1.93		1.86 (2.27)		0.01-13.39
Obese II	1.79±1.66		1.25 (1.90)		0.04-7.56

AMH: Anti-Mullerian hormone, SD: Standard deviation, IQR: Interquartile range, BMI: Body mass index

Table 4: Estimated marginal means of anti-Mullerian hormone levels after controlling for body mass index

Age category (years)	Adjusted mean AMH level (ng/ml)	SE	95% CI
25-29	3.40	0.20	3.02-3.79
30-34	3.07	0.10	2.86-3.27
35-39	2.33	0.08	2.17-2.48
40-44	1.45	0.10	1.26-1.65
45	1.11	0.38	0.37-1.86

AMH: Anti-Mullerian hormone, SE: Standard error, CI: Confidence interval

confirms the earlier trends of decreasing AMH levels with increasing age.

Effect of cause of infertility on Anti-Müllerian hormone

Summarized in Table 5 are the mean and median AMH levels along four major categories of causes of infertility. There is a significant difference in the AMH levels across the different causes of infertility ($F[3, 1229] = 4.55$, $P < 0.01$), which could be attributed to multiple clinical factors such as the significantly younger women among those couples with male factors ($F[3, 1229] = 33.33$, $P < 0.01$).

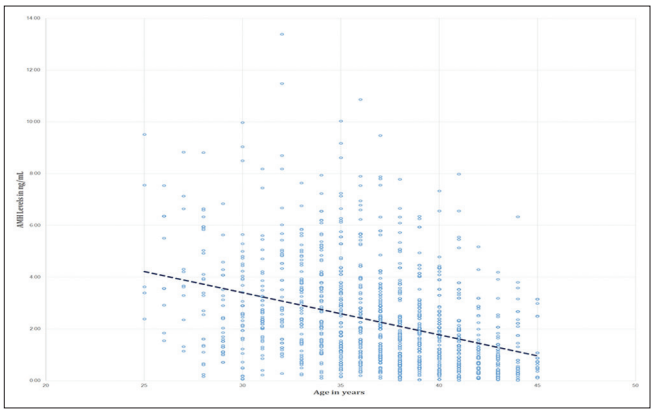

Figure 3: Scattergram of AMH values with age in years ($R^2 = 0.1391$ or 13.91%)

Discussion

Main findings

The mean age of 36.67 ± 4.35 years old by the study participants is similar to the average ages of other AMH reference range studies.[14,16] That a significant portion of the cause of infertility is female in origin is similar to the results of a Japanese study where majority of the of the causes were due to purely female reasons.[12]

However, the mean level of AMH was 2.32 ± 1.90 ng/mL, a result which is relatively low compared to published studies reporting higher mean AMH levels of 2.42–2.51 ng/ml among Japanese,[12] Lebanese,[17] and British[20] and Southeast Asian[21] women [Figure 4]. Furthermore, the average decrease in AMH level per year of increase in age is 0.16 ng/ml, a figure comparatively lower than in other studies.[9,15,17] These lower values that were found in the current study are attributed to three main reasons: (1) the exclusion of PCOS women from the study where AMH levels are at least two to three times higher than healthy controls;[22] (2) the study population has more female factor causes than male factor causes where the latter groups tend to have younger women and thus higher AMH levels. Most other studies included women diagnosed with PCOS in their study population;[12,15,17,20] (3) the utilization of Access, the third generation AMH immunoassay by Beckman Coulter. Most of the other cited studies used the generation II ELISA AMH immunoassay. This third-generation AMH immunoassay is automated, has a higher sensitivity,[7] and the derived values are substantially lower than those obtained from preexisting assays.[23]

The finding that the mean AMH is consistently higher than its median levels in the different age categories is a similar trend found in a recent Japanese study.[12] These findings suggest that frequency of low values of AMH in the same group for all categories is far more than the frequency of high AMH values.

The finding of decreasing AMH with increasing age supports the same trend reported in published nomograms.[12,15,17,20] This trend is apparent in Table 2 and illustrated in the downward slope in the box plot in Figure 1 and the nomogram in Figure 2. The values within the 25th to 75th percentile of each age group may best represent their reference range (25–29 years = 1.52–4.92; 30–34 years = 1.60–4.10; 35–39 years = 0.95–3.13; 40–44 years = 0.44–1.99; 45 years = 0.47–1.08). The values beyond these ranges need to be studied further. There are several papers on AMH levels that predict poor response during IVF ovarian stimulation in the range from 0.3 to

Table 5: Effect of causes of infertility on age and anti-Müllerian hormone levels

Cause of infertility	Age (years)		AMH level (ng/ml)		*P*
	Mean	Median (range)	Mean	Median (range)	
Male factors only	32.41±3.54	32 (25-44)	3.03±1.63	2.70 (0.70-7.54)	<0.01
Female factors only	37.22±4.40	38 (25-45)	2.21±1.96	1.73 (0.01-13.39)	
Both male and female factors	36.95±4.00	37 (25-45)	2.34±1.96	1.78 (0.01-10.86)	
Unexplained infertility	36.01±4.05	36 (26-45)	2.33±1.54	2.08 (0.08-7.64)	

AMH: Anti-Mullerian hormone

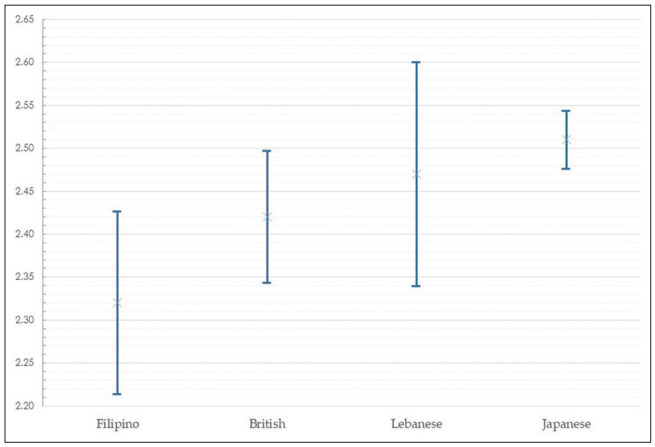

Figure 4: Comparison of mean AMH levels among British,[20] Lebanese,[17] and Japanese[12] population groups with the current study

0.75 ng/ml.[2,24-27] Based on these, the lower limit of each age-specific reference range may be used as the threshold level to predict poor response. Similarly, the upper limits of the derived nomogram values may be used to predict hyper response among regularly cycling women, but their utility in predicting the presence of PCOS is another totally different issue that needs to be verified.

The pattern of AMH decline with increasing age was similar to the pattern observed in the AMH nomograms of Indonesian and Korean women.[14,16] The pattern described is a general decline below the 50th percentile but variable in the 75th, 90th, and 95th percentiles. Other studies have mentioned a biphasic pattern among older women,[28] but the current findings do not support this observation. Instead, a moderate, negative relationship exists between the levels of AMH and age as illustrated in the correlation, regression, and the analysis of covariance.

There are conflicting reports on the effect of BMI on AMH levels. Multiple studies have shown that BMI does not affect AMH levels,[29,30] although Moy *et al*. found the same finding in African-American, Hispanic, and Asian but not Caucasian women.[31] Several studies report a negative correlation of BMI with AMH levels among those more than or equal to 35 years old.[32-34] A lower AMH concentration in obese compared to age-matched women of normal weight despite similar AFC suggests that decrease in AMH in obese women may be due to physiological issues related to obesity and not indicative of ovarian reserve.[34] Our study found a marginal negative correlation between BMI and age (R^2 = 0.68%) in the subpopulation of infertile Filipino women.

The general cause of infertility may also have an effect on the mean levels of AMH and age. These may imply that the differences among the various causes of infertility may be a reflection of the treatment-seeking behavior of patients and the duration of infertility management, knowing that the study population represents those who are undergoing *in vitro* fertilization treatment that is known as the last step in infertility management. For instance, apparent in our study is that in couples with pure male factor infertility, the female partner tends to undergo IVF treatment at a younger age where mean AMH levels tend to be higher [Table 5].

Limitations

A restriction of the current study is its cross-sectional design. To address this concern, a longitudinal prospective study will verify the inverse relationship of AMH levels with increasing age, validate our nomograms, and help improve our understanding of reproductive aging with its implications for planning pregnancies and decision-making in infertility treatment. Furthermore, the dataset is restricted only to infertile Filipino women who underwent IVF treatment and should not be used for other population groups. Additional factors that can potentially influence AMH levels such as a genetic and environmental background, socioeconomic status, history of smoking, and the presence of other diseases were not characterized and could not be assessed. Finally, a comparative study of the current results with other published nomograms and a separate nomogram that includes the subset of PCOS women may be useful information in understanding racial differences.

Conclusion

The results of this study confirm that among Filipino infertile women undergoing IVF, there is a pattern of decline in AMH levels with increasing age. The computed percentile and age-specific values produced in the current study could serve as a reference guide for the local practitioner as it is the first study on AMH reference

values in the country. These results will be useful for an individualized ovarian stimulation approach in IVF by identifying possible low, normal, and hyper responders. The same could be useful in predicting ovarian reserve or in calculating the onset of menopause. It is recommended that similar studies on fertile Filipino women and longitudinal studies among various subpopulations should be explored to better understand this pattern of AMH decline.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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