

# Age-specific anti-mullerian hormone nomogram in Şanlıurfa

## Şanlıurfa'da yaşa özgü anti-müllerian hormon nomogramı

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### ABSTRACT

**Objective:** Antimullerian hormone (AMH) levels are generally considered a good predictor of ovarian reserve. Şanlıurfa, is the city with the highest fertility rate in Turkey. The aim of this study is to determine the age spesific serum AMH nomogram of the patients with infertility complaints in Şanlıurfa and its relationship with fertility.

**Methods:** The records of patients who applied to Harran University Research and Application Hospital infertility outpatient clinic and whose AMH levels were measured were reviewed retrospectively. All patients registered between February 2017 and September 2018 were included in this retrospective study. A total of 3667 patient data were included in the study. Age-specific distribution of AMH levels was determined. Serum AMH levels of patients included in the study were routinely determined in a single laboratory using the ECLIA method in a C601 autoanalyser with the same brand kits, and the results were expressed as ng/mL. Excessive values (> 30 ng/mL) were excluded from evaluation and only the first AMH value among multiple AMH measurements was included in the nomogram.

**Results:** In the histogram analysis, we found that AMH levels showed two peaks at the age of 19 and 23 and

### ÖZET

**Amaç:** Antimüllerian hormon (AMH) seviyeleri genellikle yumurtalık rezervinin iyi bir öngörücüsü olarak kabul edilmektedir. Şanlıurfa, Türkiye'nin en yüksek doğurganlık oranına sahip ilidir. Bu çalışmanın amacı Şanlıurfa'daki infertilite şikâyeti olan hastaların serum AMH düzeylerinin yaşa özgü nomogramlarını belirlemek ve doğurganlıkla ilişkisini saptamaktır.

**Yöntem:** Harran Üniversitesi Araştırma ve Uygulama Hastanesi infertilite polikliniğine müracaat eden ve AMH düzeyleri ölçülmüş olan hastaların kayıtları retrospektif olarak incelendi. Bu retrospektif çalışmaya Şubat 2017 ile Eylül 2018 arasında kayıtlı olan tüm hastalar dahil edildi. Çalışmaya toplam olarak 3667 hasta verisi dâhil edildi. AMH seviyelerinin yaşa özgü dağılımı tespit edildi. Çalışmaya dahil edilen hastaların serum AMH düzeyleri, aynı marka kitlelere sahip bir C601 otoanalizöründe ECLIA yöntemi kullanılarak tek laboratuvarında rutin olarak saptanmış ve sonuçlar ng/mL olarak ifade edildi. Aşırı değerler (>30 ng/mL) değerlendirme dışında tutuldu ve birden fazla AMH ölçümlerinden yalnızca ilk AMH değeri nomograma dahil edildi.

**Bulgular:** Histogram analizinde, AMH düzeylerinin 19 ve 23 yaşında iki tepe noktası gösterdiğini ve daha sonra azalan bir eğilim gösterdiğini tespit ettik. Serum AMH

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then entered a decreasing trend. Serum AMH levels were negatively correlated with age ( $r = -0.518$ ,  $p < 0.001$ ).

**Conclusion:** This study presents a review of age-specific AMH reference values detected in the same conditions, based on a great number of women with infertility complaints evaluated in Şanlıurfa. It is clear that, without clinical outcome data, the reference values provided for AMH cannot be used alone for counseling about a woman's successful ovulation induction or chances of having a child. Whereas, these data may be useful for clinicians to evaluate the ovarian reserve of infertile patients and individualizing the treatment for this, by providing options to evaluate the AMH value together with regional nomogram, instead of mere the AMH value.

**Key Words:** Antimüllerian hormone, in vitro fertilization, infertility, ovarian reserve

düzeyleri yaş ile negatif korelasyon gösterdi ( $r = -0.518$ ,  $p < 0.001$ ).

**Sonuç:** Bu çalışma, Şanlıurfa'da değerlendirilen infertilite şikayeti olan çok sayıda kadında, aynı koşullarda saptanan yaşa özgü AMH referans değerlerinin incelemesini sunmaktadır. Klinik sonuç verileri olmadan, AMH için sağlanan referans değerlerinin, bir kadının başarılı yumurtlama indüksiyonu veya çocuk sahibi olma şansı hakkında danışmanlık için tek başına kullanılamayacağı açıktır. Bununla birlikte, bu veriler klinisyenlerin infertil hastaların yumurtalık rezervini değerlendirmesinde ve bunun için tedavinin bireyselleştirilmesinde sadece AMH değeri yerine bölgesel nomogramla birlikte değerlendirmelerine imkân sunarak yararlı olabilir.

**Anahtar Kelimeler:** Antimüllerian hormon, yardımcı üreme teknikleri, kısırlık, over rezervi

## INTRODUCTION

Şanlıurfa is the city with the highest fertility rate in Turkey (1). It is located in southeastern Turkey, it is based on the pre-existing history of 10.000 BC. Being an important city in the Middle East, Şanlıurfa is home to many different ethnic cultures and has the characteristics of representing the Middle East ethnically.

Although the rate of fertility is the highest in Turkey, infertility rate tends to rise here as well as all over the world. In this respect, it has become an attractive subject to investigate the factors affecting fertility and infertility in Şanlıurfa.

Many tests are currently being used in the female factor research of infertility. Of these, age, antral follicle count (AFC) and antimüllerian hormone (AMH) levels are generally accepted as the best predictors of ovarian reserve (2). AMH is a common marker of

ovarian reserve (3). However, AMH is accepted as the best biochemical test for ovarian reserve (4).

AMH was first discovered in 1947 by Alfred Jost (5). AMH is also called müllerian inhibiting agent, müllerian inhibiting factor and müllerian inhibiting hormone. It is a dimeric glycoprotein hormone belonging to the "transforming growth factor-beta" family (4). In humans, the Antimüllerian Hormone is encoded by the AMH gene located on chromosome 19p13.3 (6), while the AMH receptor is encoded by the AMHR2 gene located on chromosome 12 (7).

Although the AMH assay is frequently used today, there are discrepancies in the literature regarding its direct association with pregnancy or birth rates. In general, AMH levels play an important role in determining the counseling provided for the patient and choice of infertility treatment.

Currently, many studies have shown the correlation between AMH and age. However, no data are based on large samples of AMH values with age in Turkey. AMH may have racial or environmental differences (8). Thus, the external reference values may not apply to Turkey.

The aim of our study was to determine the distribution of AMH levels, which is the ovarian reserve test, of the patients who applied to the infertility outpatient clinic of the University Hospital in Şanlıurfa due to their desire to have a child, and also to partly help determine the correct treatment strategies for the patients in the future.

## MATERIAL and METHOD

### Study population

In this study, records of patients who applied to Harran University Research and Application Hospital Obstetrics Outpatient Clinic with the complaint of child request, diagnosed with infertility and was measured AMH levels were reviewed retrospectively. All patients registered between February 2017 and September 2018 were included in our retrospective study. AMH data in the records were included regardless of the period in which the menstrual cycle occurred. Data of 3667 patients were included in the study. 7 patients' data were excluded because of abnormal results ( $> 30$  ng/mL) and among the subsequent AMH measurements only the first AMH value was included in the nomogram. As a result 3660 results were used for calculation. Demographic characteristics of the patients were also examined from the file records.

### AMH assay

All serum AMH levels were measured as routinely in same laboratory using ECLIA (Electrochemiluminescence Immunoassay Analyzer) method on an C601 autoanalyzer (Roche, Rotkreuz,

Switzerland) with same-brand kits and the results are presented as ng/mL. Within-run CV for AMH concentrations ranging between 0.7 and 3.4%; and the repeatability CV value were 1.2% for cobas e 601/ MODULAR ANALYTICS E 170 analyzers (9). The limit of detection for AMH was 0.01 ng / mL. AMH values of 125 patients whose results were below this were adjusted to 0.01 ng / mL for statistical calculation.

### Statistical analysis

Analyzes was performed using SPSS for Windows version 22 (SPSS Inc. Co., Chicago, IL, USA). The normality evaluation of AMH values was done by Kolmogorov-Smirnov test and it was found that it did not show normal distribution. Spearman's correlation analysis was used to analyze correlation between serum AMH values and age. The linear R2 value was calculated to evaluate the degree of fitting of the curve and log-transformed AMH values were used to establish the Simple linear regression model.

## RESULTS

In our histogram analysis, we found that AMH levels showed two peaks at the age of 19 and 23 and then entered a decreasing trend (Figure-1). Table-1 summarizes the mean (95% Confidence Interval), median, minimum, maximum and Interquartile Range (IQR) values of different age groups. Figure 2 illustrates the trend of change of AMH presented in Table 1.

Table 2 shows the patterns of decrease in AMH levels according to age groups. Decrease rates of AMH according to age groups increased with increasing age. However, the increased acceleration in this decline is not easily noticeable, as AMH levels decline with age. The highest decreases were observed in the age groups of 31-35, 26-30 and 21-25, respectively.

Although the rate of decrease was found to be different for each age group, when all age groups

were taken together, the correlation between AMH levels and age was investigated and regression was formulated.

The correlation of serum AMH values with patient ages was negative ( $r = -0.518$ ,  $p < 0.001$ ). The simple

linear regression model was  $\log(\text{AMH}) = 1.698 - 0.052 * \text{age}$  ( $R^2 = 0.249$ ,  $p < 0.001$ ), indicating that approximately 24.9% of serum AMH concentrations were age-related and that AMH values would decrease by an average of 5.2% per year.

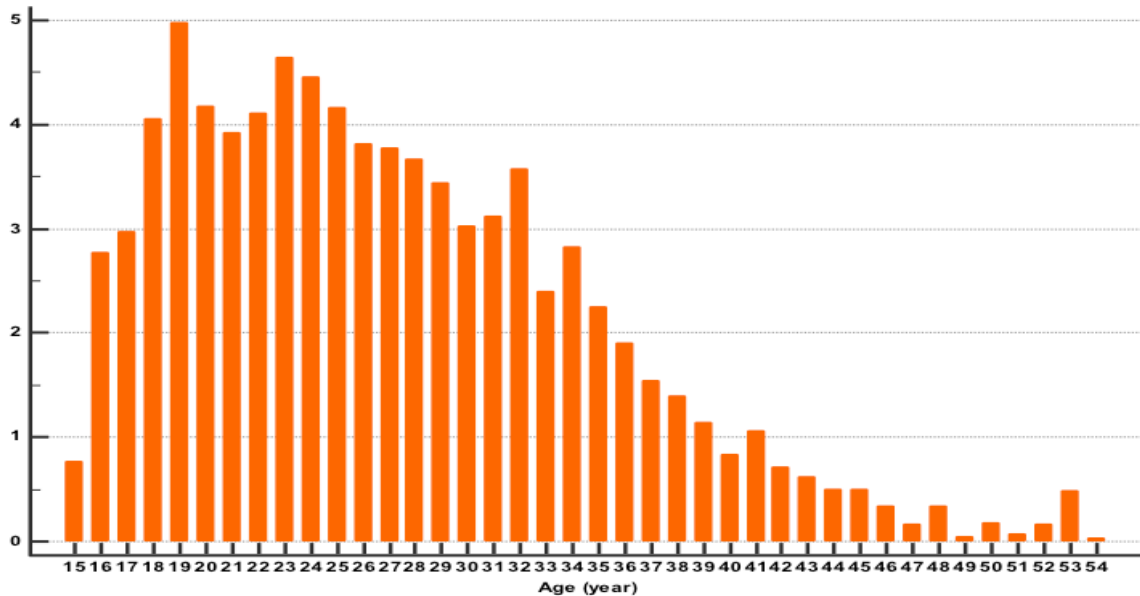
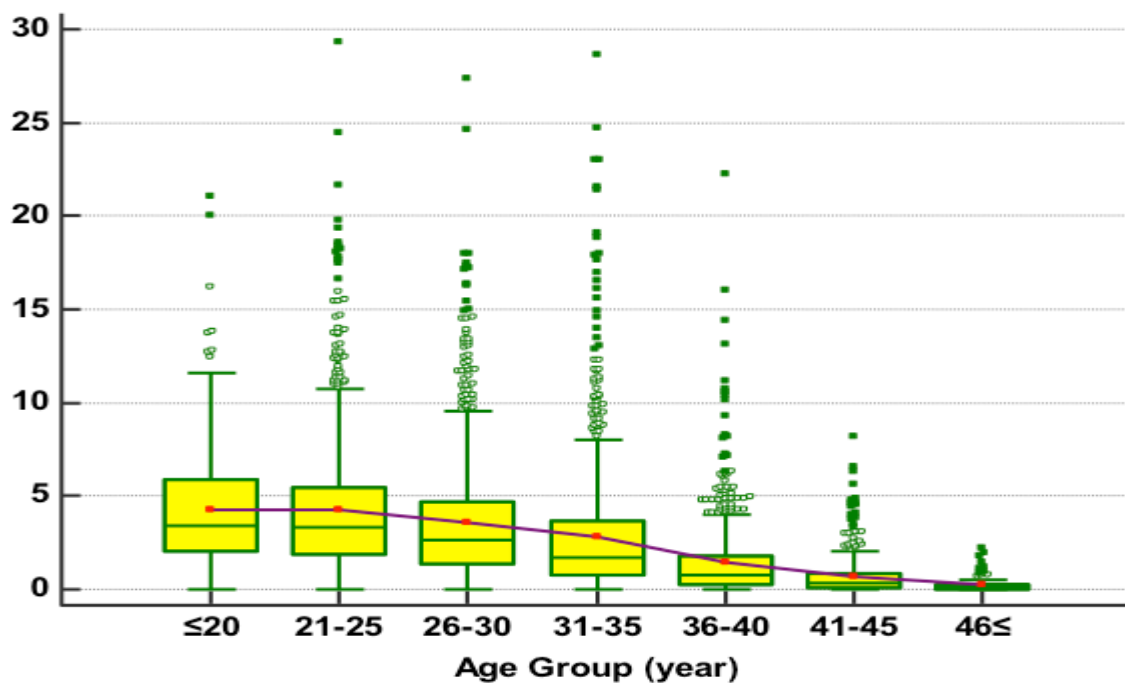


Figure 1. Age specific AMH levels

Table 1. Distribution of AMH levels (ng/mL) in different age groups

Age Group	n	Mean	SD	Median	Min	Max	Q1	Q3	IQR
≤20	150	3,89 (3,71 - 4,87) <sup>a</sup>	3,6	3,38	0,01	21,08	2,05	5,92	3,86
21-25	662	3,85 (3,99 - 4,58)	3,85	3,30	0,01	29,30	1,87	5,42	3,55
26-30	893	3,16 (3,32 - 3,76)	3,34	2,67	0,01	27,40	1,33	4,65	3,32
31-35	780	2,34 (2,60 - 3,10)	3,54	1,72	0,01	28,60	0,73	3,68	2,94
36-40	695	1,14 (1,28 - 1,58)	2,03	0,79	0,01	22,27	0,26	1,77	1,50
41-45	375	0,52 (0,58 - 0,81)	1,09	0,30	0,01	8,14	0,06	0,88	0,81
≥46	105	0,16 (0,15 - 0,31)	0,40	0,06	0,01	2,23	0,01	0,23	0,21

a : 95% Confidence Interval  
IQR : Interquartile Range



**Figure 2.** Serum AMH concentrations of different age groups. Values are median (short lines), mean (red point), 25-75th percentiles (boxes) and 95% confidence interval (whiskers).

**Table 2.** Declining tendencies of AMH levels according to different age groups

Age Group	n	Mean <sup>a</sup>	AMH Reduction Amount (ng/mL)	AMH Reduction Ratio (%)
≤20	150	3,89	0,04	1
21-25	662	3,85	0,69	18
26-30	893	3,16	0,82	26
31-35	780	2,34	1,20	51
36-40	695	1,14	0,62	54
41-45	375	0,52	0,36	69
≥46	105	0,16		

a: According to 95% Confidence Interval

## DISCUSSION

In our study, serum AMH values was oppositely proportional to rising age, and previous studies confirm this output (10-12). There are similar analysis data concerning AHM which is distributed according to age in both Turkey and the entire world. Scheffer et. al. stated that the AMH reached its peak level after the 20s and progressively decreased after this age (13). In the study by Zhu et al., the age at which serum AMH levels reached its peak was 24 years (14). In our study, the ages at which AMH reached its peaks was 19 and 23 years old.

The fact that AMH reaches its peak at the age of 19, that the decline is stable after 23 years of age and that the ovarian reserve decreases excessively after the age of 31-35 seems to explain the biological reason for the observational early child demand of the people in Şanlıurfa region. Akira Iwase recent review showed also that AMH is the most reliable marker of ovarian reserve in a wide group of clinical settings however poor for predicting menopause age for rAMH trajectories of time to menopause and the usability of AMH in the assessment of current fertility is limited and dependent on age (15). See Ling Loy et. al. study similiar results with Chinese women however their study population different from ours. Our study age starts detecting AMH values from age 16 compared to their starting age 26 which made it possible to detect an earlier decline of AMH value at an early age (16).

Such nomograms may have different local results, as we have done and we have seen in other study results. Such original studies are likely to benefit from the following: When examining a patient with such nomograms to be extracted in the future, it may be more logical to evaluate the patient's own age range in her own region rather than merely assessing the outcome of AMH as an indicator of ovarian reserve.

In this context, Barad et al. (10) demonstrated that in women reaching IVF (In Vitro Fertilization),

age-specific AMH predicts low oocyte yield when it is below the 25th percentile and higher oocyte yield if it is above the age-specific 75th percentile. Our study was retrospective and no antral follicle count was performed. However, these local nomograms can help clinicians choose the right treatment strategies at the right time, taking into account local differences. These local nomograms can help clinicians choose the right treatment strategies at the right time, taking into account local differences.

As a result, when considering the AMH value, the patient can be provided with better information and counseling compared to the average value generated by the data pool. If local differences are too small to be taken into account, perhaps in the future more accurate AMH reference ranges can be identified by combining all these data.

Although AMH is a very good ovarian reserve marker, it is seen that some issues remain inadequate in explaining infertility. One of them, AMH does not have a full predictive value in terms of pregnancy and delivery (17). So it is not a marker that guarantees pregnancy or birth.

High AMH values can only stimulate the clinician to be careful and take the necessary precautions. By gathering such data in different parts of the world in a common pool, it can be hoped that these data will contribute to a more accurate clinical prediction. It is important to include such nomograms in the literature.

Although the AMH assay is frequently used today, there are discrepancies in the literature regarding its direct association with pregnancy or birth rates (15, 16). In general, AMH levels play an important role in determining the counseling and choice of infertility treatment.

We hope that with such published studies, regional AMH nomograms can be used to increase the pregnancy rate and success rate in infertility treatments by providing more accurate counseling to patients. One of the missing points of our study is that

the AMH blood levels, which are ovarian reserve, do not include all women in Şanlıurfa region. The results here belong only to the patient population. Since AMH is still an expensive test, it is not used as a screening test in the world. In the future, as the test price decreases and accessibility increases, larger data can be obtained. Nevertheless, the data included in our study reflects the general population to a large extent because it is as high as 3600. The real weakness of this study that we do not know the patients pregnancy rates. This study consists of ovarian reserve data of patients only. Future studies may designed as ovarian reserves corelated with pregnancy rates. This study can open a horizon also for future studies. We know that AMH is not a scanning test of ovarian reserve because of it is high costs, hence this study can open a horizon also for future studies.

## CONCLUSION

This study provides a review of age-specific AMH reference values detected in the same conditions, based on a great number of women with infertility complaints evaluated in Şanlıurfa. In similar studies around the world, the presence of peaks at the age of 23-24 for serum AMH value was expressed, whereas two peaks (19 and 23 years) were detected at an earlier ages in this study may be related to the high fertility rate in Sanliurfa. Whereas, these data may be useful for clinicians to evaluate the ovarian reserve of infertile patients and individualizing the treatment for this, by providing options to evaluate the AMH value together with regional nomogram, instead of mere the AMH value.

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