



# Relationship Between Anti-Mullerian Hormone and Demographic or Obstetric Features in Infertile Women, in Rasht, North of Iran

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## ABSTRACT

**Background & Objective:** Anti Mullerian hormone (AMH) level is a practical index of ovarian reserve and an indicator of ovarian response in infertile women. The aim of this study was to evaluate the relationship between AMH levels and demographic factors.

**Materials & Methods:** In this cross-sectional study, conducted in Al-Zahra hospital Rasht in the north of Iran, 234 patients, 18-45 years old, were enrolled. The demographic parameters (e.g. age, education, habitat and etc.) and AMH levels were recorded for each patient. The patients were divided into two groups based on their AMH levels [group 1: less than 1.1 (ng/ml) vs. group 2: more than (1.1 ng/ml)]. The data was analyzed by SPSS-21 software.

**Results:** The mean AMH level was 2.66 ng/ml. Age, education status and habitat showed significant differences among subjects with AMH levels, so that with aging the AMH levels decreased. Patients living in villages or the ones with low educational degrees and those with higher parities also revealed lower levels of AMH ( $p < 0.05$ ).

**Conclusion:** There was a significant relationship between age, educational status and habitat with AMH values. Similar to other studies, we believe that with aging the AMH levels decrease. Furthermore, the other two aforementioned demographic features could affect a woman's ovarian reserve and fertility status, too.

**Keywords:** Infertility, Anti Mullerian Hormone, Demography



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## Introduction

In recent years, infertility has become a leading medical issue, which spiked as high as 50% from 1955 up to today (1). According to the references, infertility rates are about 3.5-16.7% and 6.9-9.3% in the developing and developed countries, respectively (2). Among these, about 30-40% of etiologies are related to ovarian disorders, leaving the other figures for uterine abnormalities, immune factors or other systemic diseases (3).

Despite numerous achievements in medical and surgical approaches to the infertility, still, the number of oocytes following ovarian stimulation plays an essential role in the process of treatment. For instance, failures in Assisted Reproductive Technology (ART) are noticed in 10-15% of cases, because of poor ovarian responses to hormonal stimulation (4-6).

Factors reflecting the ovarian reserve, as suggestive indexes of quality and quantity of oocytes, have been investigated in different study settings. Anti Mullerian

Hormone (AMH) has recently been introduced as a promising definitive factor, indicating both as a proper marker for ovarian reserve and also fertility (7, 8). This hormone is secreted from granulosa cells of the ovaries, with its amounts in the blood stream decreasing gradually from early twenties until 3-5 years before menopause, which becomes unidentifiable in the serum by then (9, 10).

Hormones are usually affected by many factors, including demographic, social or geographical triggers, therefore, like some other studies (11, 12), we aimed to evaluate such variables on changing the serum levels of AMH among an Iranian population with infertility complaints, to report any additional outcomes, as infertility seems to be progressively showing an upward trend in Guilan province, north of Iran.

## Methods

In this cross-sectional study, during 2019-20, 234 infertile women in reproductive age who have been referred to the infertility clinic of Al-Zahra university hospital of Rasht were enrolled. The inclusion criteria were informed consent and women aged between 18-45 years old willing to participate in the study; while exclusion criteria were as follows: menopause, underlying co-morbidities (e.g. cancers, endocrine diseases), history of hormonal medications, polycystic ovarian syndrome (PCOS) based on Rotterdam criteria, any history of ovarian or uterine surgeries, and history of endometriosis.

To have a comprehensive record of demographic features, checklists were designed and filled out for all individuals. The variables include: age, age at menarche, menstrual patterns (regular or irregular), patients' income, age of mother's menopause, Body Mass Index (BMI), smoking or addiction history, history of taking oral contraceptive pills (OCPs), parity status, history of lactation, history of stillbirths or abortions, season of obtained sample, habitat, occupation, and air-conditioning system of the current living house. AMH < 1.1 ng/ml was defined as a low AMH level according to Bologna criteria, and according to NICE consensus AMH > 3.5 ng/ml was defined as a high responder. Also, regular menstrual cycles were defined as 3 to 10 days of menstruation periods, every 25 to 35 days, while, other patterns were considered as irregular cycles.

To measure the BMI, height and weight of patients were recorded. They were weighed by a Seca scale (Seca, Deutschland) with the accuracy of 0.1 kg, while their heights were measured by wall-mounted tape-measure with the accuracy of 0.5 centimeters. To measure the AMH levels, 5 ml blood samples were obtained from all women. The blood serums were separated after a centrifuge with 3500 rounds/minutes for 10 minutes. The samples were stored in -20°C. AMH levels were measured by ELISA test (Beckman co. kits, France).

## Statistical analysis

All the data was analyzed by SPSS software (Version 21, IBM, SPSS Inc., USA). To evaluate the relationship between variables, Kruskal-Wallis one-way analysis of variance, t-test, chi-square test or Fischer's exact test and Multiple Logistic model of Regression were applied. P-values less than 0.05 were considered as significant cut-off values.

## Results

In this cross-sectional study, 234 patients referred to our infertility clinic with the age range of 18-45 years old were evaluated for study variables.

The mean amount of AMH was 2.66 ng/ml. 99 women (42.3%) had AMH levels less than or equal to 1.1 ng/ml (group1), while the others had more than 1.1 ng/ml of AMH (group2). The demographic and obstetric features of the subjects are shown in [Table 1](#).

Among all evaluated variables; age, educational status and habitat revealed significant differences among the two groups, so that with aging AMH levels showed a downward trend (mostly in women over 40 years old or older).

Although in the present study, the education of the majority of people in both AMH groups was a diploma. Most of the patients in the group with AMH levels above 1.10 ng/ml lived in cities (in contrast to the ones living in villages), showing a significant difference in the hormone levels ( $P=0.02$ ). Smokers were not analyzed due to small numbers ( $n=6$ ), 51 women (44.6%) were passive smokers. Parity status, history of stillbirth, history of abortion, history of lactation, history of OCP consumption of our study subjects are shown in [Table 1](#).

**Table 1.** Comparison of social demographic and midwifery variables between the two groups

Variables	AMH ≤ 1.10 (n=99)	AMH > 1.11 (n=135)	P-value
Age (year)			
18-30	9(9.1%)	37(27.4%)	0.0001*
30-40	37(37.4%)	85(63%)	
>40	53(53.3%)	13(9.6%)	

Variables	AMH $\leq$ 1.10 (n=99)	AMH>1.11 (n=135)	P-value
<b>Education</b>			
High school	90(90.9%)	101(74.8%)	0.002*
University	9(9.1%)	34(25.2%)	
<b>Habitat</b>			*0.020
City	65(65.7%)	107(79.3%)	
Village	34(34.3%)	28(20.7%)	
<b>Income</b>			
Low	12(12.1%)	14(10.4%)	*0.510
Moderate	82(82.8%)	109(80.7%)	
High	5(5.1%)	12(8.9%)	
<b>Occupation</b>			*0.348
housewife	54(56.3%)	83(62.4%)	
Employed	42(43.8%)	50(37.6%)	
<b>BMI (kg/m<sup>2</sup>)</b>			
<25	33(33.3%)	42(31.1%)	*0.371
25-30	44(44.4%)	52(38.5%)	
$\geq$ 30	22(22.2%)	41(30.4%)	
<b>Breastfeeding history</b>			
No	90(90.9%)	120(88.9%)	*0.615
Yes	9(9.1%)	15(11.1%)	
<b>OCP consumption history</b>			*0.200
No	83(83.3%)	104(77%)	
Yes	16(16.2%)	31(23%)	
<b>Heating system</b>			
Ovens	91(91.9%)	129(95.6%)	*0.247
Oil heater	8(8.1%)	6(4.4%)	
<b>Season</b>			
Spring	18(18.2%)	28(20.7%)	
Summer	36(36.4%)	34(25.2%)	0.07
Fall	21(21.2%)	47(34.8%)	chi
winter	24(24.2%)	26(19.3%)	
<b>Menstrual pattern</b>			*0.429
Regular	75(75.8%)	96(71.1%)	
Irregular	24(24.2%)	39(28.9%)	
<b>Gravidity</b>			*0.918
0	72(72.2%)	99(73.3%)	
1=>	27(27.3%)	36(26.7%)	
<b>Abortion</b>			*0.115
0	79(79.8%)	118(87.4%)	
1=>	20(20.2%)	17(12.6%)	
<b>Live birth</b>			*0.295
	92(92.9%)	120(88.9%)	

Variables	AMH≤1.10 (n=99)	AMH>1.11 (n=135)	P-value
0	7(7.1%)	15(11.1%)	
1=>			
<b>Still birth</b>			
0	96(97%)	132(97.8%)	**0.700
1=>	3(3%)	3(2.2%)	
<b>Age of menarche</b>	12.86±1.31	12.93±1.33	***0.70
<b>Menopausal age in the mother</b>	50.54±2.47	50.85±2.86	0.38

\* Chi-Square, \*\*Fisher test, \*\*\* t-test

The results of multiple logistic regression analysis ([table2](#)) showed that there was a significant relationship between patients' age and AMH levels ( $P < 0.001$ ). Accordingly, the odds ratio of AMH levels > 1.1 among women aged 18-30 years compared to the women over 40 years was more than 16 times; and in the patients aged 30-40 years compared to people over 40 years was more than 9 times.

According to the results of logistic regression analysis, the probability (odds ratio) of increasing of AMH levels in women with a history of at least one pregnancy is about three times higher than women with higher pregnancy, (OR=3.2,  $P$ -value<0.05).([Table2](#))

**Table 2.** Relationship between AMH with social demographic and obstetric characteristics based on multiple logistic regression

Predictive variables		Effect size	Standard Error	Odds ratio	P-value
Age (year)	18-30	2.824	.526	16.846	.000
	30-40	2.243	.393	9.420	.000
	>40				
Education	High school	-1.045	.799	.352	.191
	Diploma	-.727	.519	.483	.162
	University				
Occupation	Housewife	.741	.385	2.099	.054
	Others				
Habitat	City	.208	.421	1.231	.621
	Village				
Income	Low	.532	.888	1.702	.549
	Moderate	-.295	.741	.745	.691
	High				
Menopausal age in the mother		.259	.304	1.296	.393
Menstrual pattern	Regular				
	Irregular	-.327	.316	.721	.300
Breastfeeding history	Yes	.043	.584	1.044	.941
	No				
OCP consumption history	Yes	.579	.382	1.785	.129
	No				

Gravidity (No)	0	1.014	.558	2.757	.069
	1	1.180	0.587	3.253	.044
	>1				
Constant		-1.191	.826	.304	.149

\*P-value<0.05

## Discussion

In the present study, evaluating various demographic features in patients with infertility, we found that there was a significant relationship between age, educational status and habitat with AMH values. The [Table 1](#) shows that patients with higher educational degrees or the ones living in the cities had higher levels of serum AMH. We also confirm a downward trend in hormone levels with aging. Similar outcomes were recorded by Shaw *et al.* in 2011 in Columbia (13, 14).

Consistent with Jung *et al.* study, evaluating 671 cases, BMI, smoking, age at menarche, irregular menstruations and occupation were not related to AMH values (15).

In 2016, Bernardi *et al.*, in Africa, found a significant decline in the AMH levels in women with higher BMI scores, among study subjects, with the age range of 23-35 years old (16). Likewise, similar patterns were reported by Steiner *et al.* (11). Our findings were in contrast with the findings of these two researchers. We believe that excluding patients with PCOs could be the underlying reason for such results. Moreover, age, race or lifestyle differences could also affect the final outcomes.

It has been proven that various environmental factors, by means of complicated mechanisms, may induce follicular atrophy within the follicle growth cycles by means of inducing oxidative stress and apoptosis (17). These harmful factors include smoking, marijuana use, heat from cooking or heating devices, as they produce aromatic, polycyclic hydrocarbons in the air (18). The four factors, for instance, were studied by White *et al.*, in 2016, in 913 participants, for their adverse effects on AMH serum levels. Smoker women with 20 cigarettes a day, compared to non-smokers, showed lower levels of AMH. This pattern was even more significant among women with 10 years or more history of smoking, however, no such relationship was reported for marijuana use (19). These findings are in contrast with the results of our study. These differences could be due to the small number of women consuming alcohol / cigarettes, or the smaller sample sizes in the present study.

In the study of White *et al.*, women who lived in houses with wood interior heating system showed lower values of AMH, compared to the ones using ovens or living in places with fire paces (19).

Menstrual cycle and OCP consumption were the subject of Do'lleman *et al.* study in 2013, which

revealed, lower serum levels of AMH among 311 Caucasian women with irregular cycles taking OCP, compared with the ones with normal menstrual cycles (20). Racial differences and higher numbers of women taking OCPs could be a possible reason for the inverse outcomes of the study with ours.

Kristensen *et al.*, in 2012, reported that there was a significant relationship between AMH levels with OCP consumption and age at menarche in Denmark, so that the levels declined in patients who used OCPs (21). Similar pattern of relationship between AMH levels and OCP was also reported from Schuh-Huerta *et al.*, study, in 2012, evaluating 232 and 200 women from Kazakhstan and the USA, respectively. Besides, smoking was also significantly related in the latter study (12). This different outcomes from ours, is probably because of the higher rates of OCP consumers or smokers in those studies (e.g. OCP consumers: 180 out of 256 vs. 47 of 234 cases in Kristensen *et al.* vs. our study, respectively).

Hagen *et al.* found no association between AMH levels and decreased fertility in young healthy women of reproductive ages (22-24). However, in another study, researchers categorized the patients in three groups based on their AMH values [low: <1.3 ng/ml, moderate: 1.3-4.6 ng/ml, high: >4.6 ng/ml], confirming that high AMH serum levels were significantly related to increased rates of pregnancy, compared to low or moderate values (25). We assumed that a higher number of parities leads to increased levels of AMH, might be due to increased progesterone secretions during pregnancy, lower functioning rates of follicles and decreased numbers in follicular reserve. Although, the final outcome was only consistent with only one study (26).

## Conclusion

AMH level is considered as a valuable index of ovarian reserve in women of reproductive ages. Our study revealed that not only age, but other demographic or obstetric features could be responsible for infertility and possible changes in AMH levels. Therefore, by modifying or changing the affecting factors, better results with infertility treatments could be achieved. Although, future studies with larger sample sizes are required for more accurate outcomes.

## Ethical consideration

The study was approved by the Ethics Committee of Guilan University of medical sciences, Rasht, Iran (Ethical code: IR.GUMS.REC.1398.375). All stages of this research have been performed according to the Helsinki declaration. All procedures of the study were explained clearly to the participants who met the eligible inclusion criterion. Moreover, all participants voluntarily filled out the written informed consent form before they joined the study and they were free to decide whether to attend or withdraw the study at any time and for any reason without changing the medical care.

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### Conflict of Interest

There are no conflicts of interest.

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