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Age-Related Changes in Antimülerian Hormone Levels: A Study of Ovarian Reserve in Pollog Region Women

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Abstract

The level of anti-Müllerian hormone (AMH) has become an important element in the initial investigation of female patients. Reproductive aging in women is primarily based on the decline in oocyte numbers over time. In principle, oocytes are non-reproductive; some oocytes mature and ovulate. Thus, AMH is clinically useful as a screening tool for diminished ovarian reserve. This study aims to evaluate the influence of age on serum AMH levels in women in the Polog region. The study is retrospective. The patients included in the study were classified into 3 groups according to age. Patient samples were analyzed using the ELFA technique on the Vidas analyzer using the sandwich immunological method. From statistical analysis of the data, it is observed that there is a significant statistical difference between group 1 (<20 years) and group 3 (>41 years) where the p value is 0.0005. A significant difference was also observed between group 2 (21 - 40 years) and group 3 (>41 years), with a p value < 0.0001. With a p value of p = 0.33, no significant statistical difference was found between group 1 (<20 years) and group 2 (21 - 40 years). Increasing age has a significant impact on decreasing levels of AMH, which reflects a woman's ovarian reserve. After age 35, the decline in AMH is more rapid, highlighting the importance of early fertility consultations and possible treatment options.

Keywords

AMH, Age, Ovarian Reserve

1. Introduction

Infertility is of great medical importance and has a significant socio-economic impact, affecting more than 186 million people worldwide. Factors directly associated with this condition include a woman's age, prior illnesses, and the undesired period of inability to conceive. The level of anti-Müllerian hormone (AMH) has become an important element in the initial evaluation of female patients [1] [2]. AMH is a dimeric glycoprotein composed of two identical 70 kDa subunits and constitutes the transforming growth factor-beta (TGF-β) superfamily along with inhibins, activins, bone morphogenetic proteins (BMPs), and growth differentiation factors (GDFs). Members of the TGF-β family play important roles in regulating cell proliferation and apoptosis in many biological processes, including folliculogenesis and spermatogenesis [3]. Anti-Müllerian hormone (AMH) is produced exclusively by granulosa cells (GC) of developing preantral and antral follicles, and AMH is increasingly used to assess ovarian function [4]-[8]. Reproductive aging in women is primarily attributed to the decline in oocyte numbers over time. In principle, oocytes are non-reproductive; only some oocytes mature and ovulate [9]. Anti-Müllerian hormone (AMH) is known to cause the regression of the Müllerian duct in male fetuses and is a member of the transforming growth factor-β superfamily. Although AMH levels do not directly correlate with the number of follicles in the ovary, serum AMH levels reflect a growing population of follicles and are related to the number of remaining primordial follicles. AMH levels exhibit a linear correlation with age; therefore, it is considered a more sensitive indicator of the decline in oocyte numbers [10]-[12].

In females, AMH is produced by small antral follicles in the ovary and acts as an autocrine and paracrine regulator of follicular maturation. Since the size of the remaining follicular pool depends on the number of small antral follicles and decreases over time, serum AMH levels in women follow a characteristic trajectory: a gradual decline during the reproductive years and a sharp decrease at menopause, becoming undetectable shortly thereafter. Therefore, AMH is clinically useful as a screening tool for diminished ovarian reserve [13]-[16]. The interindividual variability of AMH is high, primarily due to the highly variable number of follicles within groups of subjects of similar age.

Since there are no serum markers for the direct determination of primordial follicle number, markers that indicate follicular growth have emerged as optimal alternatives for measuring ovarian reserve. AMH levels are closely related to the number of growing follicles, as these follicles release AMH, and AMH expression continues from the secondary follicle to the antral follicle stage. Recently, AMH has gained attention in clinical practice as a reliable indicator for assessing ovarian reserve due to its potential value in predicting pregnancy outcomes. This is attributed to its consistent levels throughout the menstrual cycle, with minimal diurnal fluctuations and high stability, making it a valuable tool for research [17]-[20].

Reference values for AMH have been established through several studies across the lifespan of women. The interindividual variability of AMH is high, primarily

due to the highly variable number of follicles within groups of subjects of similar ages. Several features raise concerns about its clinical use: first, AMH levels, after peaking around the second decade of life, decrease with age and are strongly correlated with ovarian reserve; second, while intra-menstrual cycle variability exists, it is not clinically significant [21] [22]. In a meta-analysis of individual patient data from 4786 women, the prognostic power for predicting ovarian hyperresponse using serum AMH levels, AFC (antral follicle count), and age was suggested. According to their model, serum AMH levels, AFC, and the patient's age showed an area under the receiver operating characteristic curve (AUC) of 0.85. These findings indicate that serum AMH levels and AFC may provide good predictive accuracy for ovarian hyperresponse by adding value to the woman's age [23]. Alterations in serum AMH are associated with a range of pathological conditions, such as polycystic ovary syndrome (PCOS), where the pathophysiological link is the excessive follicles in this syndrome that produce increased amounts of AMH [24]. AMH is also elevated in several ovarian tumors, such as adult granulosa cell tumors, and can be used as a tumor marker to assess therapy response and monitor recurrence. Within the field of assisted reproductive technology (ART), serum AMH analyses are widely used to extract prognostic information, such as the chances of successful ovarian stimulation, subsequent embryo quality, and even pregnancy rates. Recently, in the rapidly evolving field of oncofertility, serum AMH holds great promise as a predictor of ovarian reserve after the completion of cancer therapy [13].

2. Aim of the Study

The main aim of this study is to evaluate the serum concentration of anti-Müllerian hormone (AMH) as an indicator of ovarian reserve in women of different ages in the Pollog region. The study also aims to address the lack of specific data for this biomarker in this region, thus contributing to the creation of a local database that can be used in clinical practice for fertility assessment and reproductive planning.

3. Material and Method

The study was retrospective and conducted during the period from January 2022 to March 2024. A total of 728 female patients, aged 16 to 56 years, who presented for hormonal analyses at the Diagnostic Laboratory of the Tetova Clinical Hospital, were included in this study. Inclusion criteria were: female patients within the specified age group, with a documented request for hormonal analyses. Patients with a history of known endocrine diseases, active hormonal treatments, or incomplete laboratory data were excluded from the study.

Patient recruitment was carried out continuously during the study period, based on existing records in the laboratory database. Patient samples were analyzed using the ELFA (Enzyme Linked Fluorescent Assay) technique on the Vidas analyzer using the sandwich immunological method, with a detection limit of 0.01 ng/ml, a measurement range of 0.02 - 9 ng/ml.

The patients included in the study were classified into 3 groups according to age, namely:

- Group I: <20 years with mean age 17.29 ± 1.113
- Group II: 21 - 40 years with mean age 32.13 ± 4.900
- Group III: >41 years with mean age 44.63 ± 3.005

The data were analyzed using the statistical program IBM SPSS Statistics, version 20. Descriptive statistics, such as minimum and maximum values, mean, standard deviation, frequencies, and percentages, were used to describe the data, depending on the nature of the data. A p-value < 0.05 was considered statistically significant for evaluating differences between groups.

4. Results

From the statistical processing of the data obtained from the subjects included in the study classified into 3 different age groups and from the measurement of AMH levels, it results that (Table 1):

Table 1. Classification of patients based on age and AMH level.

	N %	Average age	Min	Max	Mean	SD
<20 years	8 (1.09%)	17.29 years	0.01	7.09	2.65	2.71
21 - 40 years	508 (69.78%)	32.13 years	0.01	9.00	3.66	2.95
>41 years	212 (29.12%)	44.63 years	0.01	9.00	0.85	1.35

The study population was divided into three age groups: under 20 years, 21 - 40 years, and over 41 years. The largest proportion of participants belonged to the 21 - 40-year age group, comprising 508 patients (69.78%) with a mean age of 32.13 years. This group showed a mean value of 3.66 (SD = 2.95), with a minimum value of 0.01 and a maximum of 9.00. The group over 41 years included 212 individuals (29.12%), with a mean age of 44.63 years. This group demonstrated the lowest mean value of 0.85 (SD = 1.35), also ranging from 0.01 to 9.00. The youngest group, comprising participants under 20 years of age, included only 8 patients (1.09%), with an average age of 17.29 years. The mean value for this group was 2.65 (SD = 2.71), with a minimum value of 0.01 and a maximum of 7.09. Overall, the 21 - 40-year group was the most represented in the study, while the under-20 age group was notably underrepresented. Additionally, the >41 age group exhibited the lowest mean value among all groups.

Statistical comparisons between the three age groups (Table 2) revealed the following findings: The comparison between the <20 and 21 - 40 age groups showed no statistically significant difference in mean values (2.65 ± 2.71 vs. 3.66 ± 2.95; **p = 0.33**), indicating a similar distribution of the measured parameter between these two groups. In contrast, a statistically significant difference was observed between the <20 and >41 age groups (**p = 0.0005**), with the <20 group having a notably higher mean value (2.65 ± 2.71) compared to the >41 group (0.85 ± 1.35). The most significant difference was found between the 21 - 40 and >41 age groups,

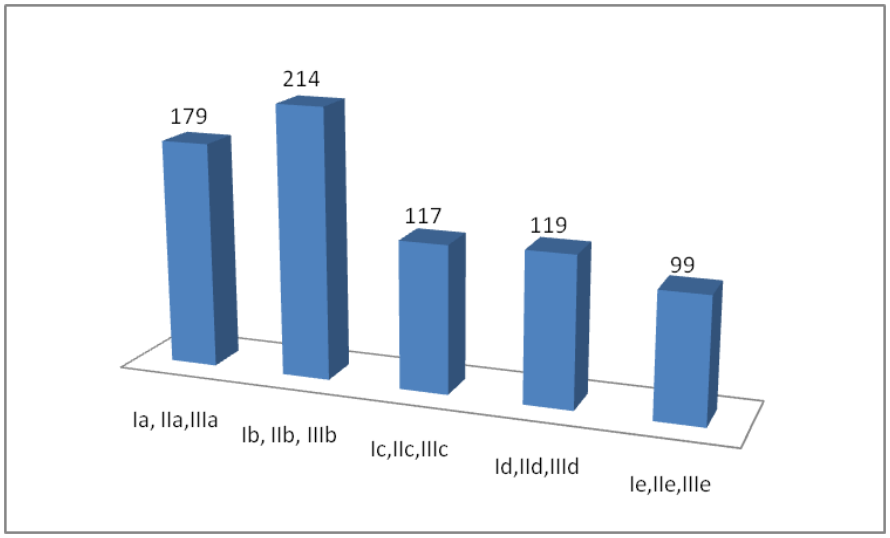
with the 21 - 40 group demonstrating a substantially higher mean (3.66 ± 2.95) compared to the >41 group (0.85 ± 1.35), and this difference was highly statistically significant (**$p < 0.0001$**).

Table 2. Intergroup comparison of patients from the different age groups.

	Mean	P value
<20 vs. 21 - 40	2.65 ± 2.71 vs. 3.66 ± 2.95	0.33
<20 vs. >41	2.65 ± 2.71 vs. 0.85 ± 1.35	0.0005
21 - 40 vs. >41	3.66 ± 2.95 vs. 0.85 ± 1.35	<0.0001

These results suggest that the measured parameter significantly decreases in individuals over 41 years of age when compared to the younger age groups.

As shown in Figure 1, the distribution of patients in a study is shown according to age and levels of anti-Müllerian hormone (AMH), divided into five groups: Ia, IIa, IIIa; Ib, IIb, IIIb; Ic, IIc, IIIc; Id, IId, IIId; and Ie, IIe, IIIe. Each group represents a specific category of AMH level and possibly age, although this is not clearly specified in the figure. The first group (Ia, IIa, IIIa) includes 179 patients, while the second group (Ib, IIb, IIIb) contains the highest number of patients, a total of 214. Furthermore, the group Ic, IIc, IIIc includes 117 patients, and the group Id, IId, IIId consists of 119 patients. Finally, the group Ie, IIe, IIIe includes the smallest number of patients, totaling 99. This distribution suggests that the majority of patients are concentrated in groups with higher or medium levels of AMH, while the number of patients decreases in groups with lower levels of this hormone, possibly reflecting a trend related to age or ovarian reserve.



Ia, IIa, IIIa: <0.3 ng/ml; Ib, IIb, IIIb: 0.3 - 2.2 ng/ml; Ic, IIc, IIIc: 2.2 - 4.0 ng/ml; Id, IId, IIId: 4.0 - 6.8 ng/ml; Ie, IIe, IIIe: 6.8 - 9.0 ng/ml; If, IIf, IIIf: >9.0 ng/ml.

Figure 1. Patient distribution according to age and Anti-Müllerian Hormone (AMH) categories.

From the processing of the data obtained from measuring the AMH level of the subjects included in the study (Table 3), it results that in the group < 21 years of age, a high AMH value was found in one patient ($\overline{x}$ = 7.09), a normal value in two patients ($\overline{x}$ = 4.89 ± 0.58), a low normal value and a low value were obtained in two cases ($\overline{x}$ = 2.32; $\overline{x}$ = 1.97), while a very low AMH value in 3 cases ($\overline{x}$ = 0.01). In the second age group (21 - 40 years), which includes the largest number of patients (69.78%), we obtained the following results: high values were found in 96 patients ($\overline{x}$ = 7.73 ± 0.63; $\overline{x}$ = 9.00), in 109 patients normal values ($\overline{x}$ = 5.20 ± 0.78), 103 patients have low normal values ($\overline{x}$ = 3.05 ± 0.49), in 135 patients the AMH value is low in the range of 0.30 - 2.2 ng/ml ($\overline{x}$ = 1.14 ± 0.09) and 65 cases a very low AMH value was obtained ($\overline{x}$ = 0.11 ± 0.09). In the third age group, a total of 212 patients were analyzed: 2 patients with high AMH values ($\overline{x}$ = 9.00; $\overline{x}$ = 7.48), 8 patients with normal values ($\overline{x}$ = 4.59 ± 0.62), 13 patients with low normal values ($\overline{x}$ = 3.27 ± 0.60), 78 patients with low AMH values ($\overline{x}$ = 0.96 ± 0.47) and 111 patients with very low AMH values in the range < 0.3 ng/ml ($\overline{x}$ = 0.08 ± 0.88).

Table 3. Distribution into groups according to serum AMH level.

	N	Minimum	Maximum	Mean	Std. Error	Std. deviation
Ia	3	0.01	0.01	0.0100	0.00000	0.00000
Ib	1	1.97	1.97	1.9700		
Ic	1	2.32	2.32	2.3200		
Id	2	4.48	5.31	4.8950	0.41500	0.58690
Ie	1	7.09	7.09	7.0900		
If	0					
IIa	65	0.01	0.30	0.1122	0.01185	0.09550
IIb	135	0.31	2.16	1.1404	0.04775	0.55483
IIc	103	2.03	3.99	3.0584	0.04891	0.49638
IId	109	4.02	6.79	5.2040	0.07483	0.78130
IIe	37	6.83	8.95	7.7384	0.10520	0.63988
IIf	59	9.00	9.00	9.0000	0.00000	0.00000
IIIa	111	0.01	0.29	0.0861	0.00844	0.08892
IIIb	78	0.31	2.12	0.9673	0.05386	0.47568
IIIc	13	2.25	3.90	3.2277	0.16815	0.60629
IIId	8	4.07	5.73	4.5950	0.22006	0.62241
IIIe	1	7.48	7.48	7.4800		
IIIf	1	9.00	9.00	9.0000		

Legend: Ia, IIa, IIIa: <0.3 ng/ml; Ib, IIb, IIIb: 0.3 - 2.2 ng/ml; Ic, IIc, IIIc: 2.2 - 4.0 ng/ml; Id, IId, IIId: 4.0 - 6.8 ng/ml; Ie, IIe, IIIe: 6.8 - 9.0 ng/ml; If, IIf, IIIf: >9.0 ng/ml.

5. Discussion

Numerous studies on the evaluation of the ratio of AMH and oocyte quality have been conducted in many countries such as England, USA, France, Japan, Turkey, Kuwait and Iran. These studies show a positive relationship between AMH levels and oocyte quality [25].

This study evaluated age-related changes in Anti-Müllerian Hormone (AMH) levels among 728 women aged 16 to 56 years in the Pollog region. The findings demonstrate a clear decline in AMH levels with increasing age, which is consistent with its established role as a marker of ovarian reserve.

Women aged 21 - 40 years represented the largest portion of the sample (69.78%) and exhibited the highest mean AMH levels (3.66 ± 2.95). In contrast, women over 41 years of age had significantly lower AMH levels (mean: 0.85 ± 1.35), and the difference between these two groups was highly statistically significant ($p < 0.0001$). Interestingly, although the <20 years group had a small sample size ($n = 8$), their mean AMH levels (2.65 ± 2.71) were lower than the 21 - 40 age group but notably higher than those over 41. The difference between <20 and >41 was also statistically significant ($p = 0.0005$), while the difference between <20 and 21 - 40 was not ($p = 0.33$).

These results align with previous studies that have consistently demonstrated a progressive decline in AMH with age, particularly after the age of 35.

Low serum AMH levels are reported in women over 41 years of age, but can also be found at younger ages, data that also correlate with the results found in the study conducted by Jirge [26].

Gunasheela *et al.*, (2021) in their study report that 50.5% of women over 35 years of age have low AMH values (<1.1 ng/ml) [27], as well as the studies of Lee *et al.*, (2012), confirm that AMH levels decline progressively with age and verify this in a different population with alternative statistical methods, data that are also close to the results obtained in our study where the average AMH value in women over 41 years of age is 0.85 ng/ml [28].

When considering ovarian reserve and fertility, it is essential to evaluate the decline in AMH with age. In this study, a progressive decline in serum AMH levels was observed at the age of >41 years, where low levels were found in 52.35% of the patients from the total number ($N = 212$) of patients included in the third group, compared to the second group (20 - 40 years) where values < 0.3 ng/ml were found in 12.79% of the women from the total number ($N = 508$). Regarding these data, the study by Seifer *et al.*, (2011) in a retrospective study involving over 17,000 infertile women observed a decrease in the average AMH value from 0.2 to 0.1 ng/ml per year after the age of 35 [29].

Study conducted by Oh *et al.* (2019) reports that there is consensus of AMH as a good marker of ovarian reserve, but there is no current agreement on its use as a fertility screening test in fertile women [30]. Meczekalski *et al.* (2016) also showed in their study that AMH is the best endocrine biomarker for predicting age-related decline in ovarian reserve in healthy women, results which correlate

with the findings in our study [31].

Because AMH is a marker for predicting the onset of menopause, measurement of this parameter may be useful in counseling women of reproductive age about family planning. It may also be useful to compare a serum AMH parameter with an age-matched group to assess whether the parameter is within a normal range for the patient's age [32] [33].

The results of our study align with the findings reported by Martin Vladimirov. In their study, which included a sample of 28,016 Bulgarian women, it was confirmed that median levels of anti-Müllerian hormone (AMH) decrease progressively with increasing age, reflecting the gradual decline of ovarian reserve [34]. Similarly, in our data, a comparable distribution is observed, where the groups with the highest number of patients are those with higher AMH levels, whereas the number of patients significantly decreases in the groups with lower AMH levels—typically associated with more advanced age. This correspondence confirms the validity of the relationship between age and AMH levels as a strong indicator of ovarian reserve. Therefore, regular monitoring of AMH represents an important tool for assessing fertility in women, especially those planning pregnancy at an older age. These findings emphasize the need for an individualized and early approach to fertility planning and the use of assisted reproductive technologies.

6. Conclusions

This study provides a foundational understanding of age-related AMH variation in women from the Polog region. However, further research is essential to deepen our knowledge and enhance the clinical applicability of these findings. Firstly, longitudinal studies tracking AMH levels in the same individuals over time would provide more precise insights into the dynamics of ovarian reserve decline and help identify critical points of hormonal change throughout the reproductive lifespan. Secondly, expanding the sample population to include women from other geographic areas within the country or neighboring regions would allow for meaningful regional comparisons. Such studies could reveal whether environmental, genetic, or lifestyle differences influence AMH levels and reproductive aging patterns. Finally, it would be valuable to conduct clinical studies that correlate AMH levels with fertility treatment outcomes in the Pollog region.

These future directions would not only contribute to the growing body of literature on ovarian reserve but also offer practical tools for improving reproductive health management in the local context.

Availability of Data and Material

Materials related to this study can be obtained from the author with a reasonable request.

Authors' Contributions

Mimoza Bafqari-Bakiji—Conceptualization, Methodology, Formal analysis,

Writing—original draft, Writing—review & editing. **Luzana Shabani**—Conceptualization, Resources, Formal analysis, Writing—original draft, Writing—review & editing. **Mije Reçi**—Conceptualization, Writing—review & editing.

Conflicts of Interest

The authors declare no competing interests.

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