INTEGRATED EVALUATION OF OVARIAN RESERVE MARKERS IN INFERTILE WOMEN: THE ROLE OF AGE, ANTI-MULLERIAN HORMONE, FOLLICLE-STIMULATING HORMONE AND ANTRAL FOLLICLE COUNT

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Abstract

Introduction: Ovarian reserve is a pivotal indicator of reproductive potential, assessed through biochemical and ultrasonographic markers, with age playing a dominant role in its decline.

Objectives: The study aimed to evaluate the correlations between anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH) and antral follicle count (AFC), and to assess the impact of age on these markers in infertile women.

Materials and methods: A cross-sectional study was conducted in 36 women aged 20–42 years. Serum AMH and FSH levels were measured, and transvaginal ultrasound was used for AFC. Data were analyzed using Spearman's correlation and linear regression.

Results: Age proved to be a negative predictor for AMH and AFC ($p < 0.05$) and positively associated with FSH. A strong positive correlation was found between AMH and AFC ($\rho = 0.866$; $p < 0.001$), while FSH showed significant negative correlations with both parameters. Regression analysis demonstrated that AMH decreases by approximately 14% per year ($B = –0.152$; $p = 0.020$).

Conclusion: AMH emerges as the most sensitive and stable marker of ovarian reserve, and combined assessment of AMH and AFC offers the most reliable detection of diminished ovarian function in infertile women.

Keywords: *anti-Müllerian hormone; antral follicle count; follicle-stimulating hormone; infertility; ovarian reserve.*

Introduction

Ovarian reserve (OR) is a complex clinical phenomenon primarily influenced by age, genetics, and environmental variables. (1) Although OR declines with age, considerable variability exists among women of similar age. (2) Diminished ovarian reserve (DOR) most commonly refers to the process of follicular depletion and a reduction in oocyte quality and describes women of reproductive age who menstruate and ovulate but whose fecundity and/or

ovarian response to stimulation is reduced compared with women of similar age. (3,4) In recent years, with modern societal trends toward delayed childbearing, DOR has become one of the most frequent challenges in clinical reproductive medicine, particularly among women over 35 years of age. In large cohort and randomized studies, the prevalence of DOR ranges from 10% to 30% and largely depends on age, ethnicity, and the diagnostic criteria used for its definition.

The most commonly used markers for the assessment of ovarian reserve serve primarily as quantitative rather than qualitative indicators; that is, they have limited predictive value for oocyte quality. Age remains the main predictor of oocyte quality. (5)

An ideal ovarian reserve test should be affordable, non-invasive, and easy to interpret. It should also be easily reproducible, yield consistent results upon repetition, and demonstrate minimal variability throughout the menstrual cycle and/or between cycles. The test should detect a decline in ovarian reserve early enough to allow timely therapeutic intervention. Ultimately, it should possess good specificity and sensitivity for the detection of ovarian hypofunction. (6)

Today, the most commonly used markers of ovarian reserve include basal follicle-stimulating hormone (FSH) testing, cycle-independent measurement of anti-Müllerian hormone (AMH), and ultrasonographic determination of antral follicle count (AFC).These parameters currently represent fundamental tools for the assessment of ovarian reserve and are crucial in planning infertility treatment.

Modern reproductive medicine has reached a level of standardization through the use of AMH and AFC as more stable and predictable biomarkers. The most commonly used cut-off values for defining DOR—AMH < 1.2 ng/mL and AFC < 5—are derived from extensive studies and meta-analyses linking these marker values to a high predictive value for poor ovarian response and lower pregnancy rates in IVF treatment. (8)

FSH is primarily used to assess gonadal function. An increase in serum FSH indicates insufficient ovarian hormone production, follicular depletion, and consequently diminished ovarian reserve. Serum FSH levels exhibit daily, intra-cycle, and inter-cycle variability. Limitations of this test include daily and cycle-related variations, the absence of clearly defined cut-off values, and the use of different assays across laboratories. (9) Basal FSH measurement is performed between days 2 and 5 of the menstrual cycle, most commonly on day 3. Its values may be analyzed as a single parameter, but it is more often evaluated in combination and correlation with other hormones and ovarian reserve tests to improve diagnostic sensitivity and specificity for this condition and other reproductive disorders. For clinical and physiological assessment, immunoassay methods are used, which are sensitive, rapid, widely available, and cost-effective. FSH does not have a universally accepted cut-off value and a normal serum concentration does not necessarily indicate adequate ovarian reserve. According to the literature, the clinical usefulness of FSH measurement in the general subfertile population of regularly menstruating women remains unclear. (10)

AMH is a glycoprotein produced by granulosa cells of preantral and small antral follicles. Within the ovary, AMH expression increases in follicles up to 8 mm in diameter and is absent in follicles larger than 8 mm, showing a marked decline, as demonstrated by its measurement in follicular fluid. Therefore, it may be more appropriate to describe AMH as an indicator of functional ovarian reserve (FOR), representing the cohort of primordial follicles measuring 2–6 mm, from which one follicle is selected by FSH to grow and ovulate. The ovary begins AMH production during fetal life, approximately around the 36th gestational week. Serum AMH levels increase in young women with the onset of puberty, reaching a plateau around 25 years of age, when a positive correlation is observed due to the high number of primordial follicles. After this age, AMH levels decline to undetectable values before, during, and after menopause. (11) Because AMH is secreted during the early stages of folliculogenesis—by small growing follicles up to approximately 6 mm in diameter—its levels are relatively independent of circulating gonadotropin concentrations, allowing testing at any phase of the menstrual cycle. One of the main limitations in comparing results obtained by different methods is the lack of an international standard, even after 25 years of AMH testing. (12) Measurement of antral follicle count (AFC) in the ovary using transvaginal ultrasound represents an acceptable surrogate marker and a valuable complement to biochemical markers in assessing ovarian reserve. AFC determination is a non-invasive and well-tolerated procedure. AFC is defined as the total number of ovarian follicles measuring 2–10 mm in diameter. The average AFC varies with age, and according to multiple studies, an antral follicle count ≤5 is considered low and indicative of diminished ovarian reserve. (13,14,15) In numerous studies, age-specific nomograms for AFC values have been established. According to these nomograms, at a mean age of 38 years, AFC is approximately 8, while at the average age of menopause onset (51 years), AFC is approximately 2, demonstrating a clear correlation between antral follicle count and age, with an average annual decline of 2.4%. (16)

Objectives

The aim of this study was to analyze the biochemical and ultrasonographic markers of ovarian reserve in infertile women of reproductive age. The study sought to examine the correlation between AMH, FSH, and AFC, as well as the impact of age on these parameters.

The specific objectives of the study were as follows: to determine the mean values of AMH, FSH, and AFC in infertile patients; to investigate the correlation between AMH and FSH, as well as between AMH and AFC across different age groups; to analyze the age dependency of these markers; and to evaluate the clinical value of AMH, FSH, and AFC as indicators of ovarian reserve.

Materials and methods

This cross-sectional study was conducted at the Department of Infertility and Assisted Reproduction of the University Clinic of Gynecology and Obstetrics, Faculty of Medicine, Skopje. The study population consisted of 36 women of reproductive age, specifically aged 20

to 42 years, who were evaluated at the subspecialist outpatient clinic for infertility and assisted reproduction for infertility assessment and treatment over a three-month period.
All patients were evaluated using a standard infertility diagnostic protocol. For the purposes of this study, informed consent was obtained, and participants completed a brief questionnaire regarding demographic characteristics and menstrual history, as well as for the application of the exclusion criteria required for the study. All study participants were referred for laboratory evaluation of basal hormonal status and AMH levels. During the baseline gynecological ultrasound examination, antral follicle count was assessed in all participants.
Patients excluded from the study included those with existing ovarian pathology (benign or malignant ovarian lesions), endometriosis, previous ovarian surgery, prior oncological treatment (chemotherapy, radiotherapy, or surgery), use of hormonal contraceptive therapy within the three months preceding study inclusion, polycystic ovary syndrome (PCOS), and hyperprolactinemia.
Serum samples were obtained for assessment of basal hormonal status on the third day of the menstrual cycle, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), and testosterone (T). Commercial chemiluminescence assay kits were used, with analysis performed on a Centaur analyzer (Siemens, Germany). Using the same serum samples, quantitative AMH levels were determined using Elecsys technology. Reference values for all parameters were applied according to the manufacturer's instructions.
Evaluation of the size and number of antral follicles was performed during the gynecological ultrasound examination in the follicular phase of the menstrual cycle. A Voluson Expert S6 ultrasound system (GE Medical Systems) equipped with a transvaginal probe (RIC-9-D) operating at 7.5 MHz was used. Both ovaries were scanned from the lateral to the medial margin, and all antral follicles measuring 2–10 mm in diameter were counted.
The data were digitized and processed using the statistical software IBM SPSS Statistics, version 21.Descriptive analysis was performed for both categorical and continuous variables. Categorical data are presented as frequencies and percentages, while continuous variables are presented as medians and interquartile ranges (IQR), due to their asymmetric distribution. Associations between categorical variables were assessed using the chi-square test (χ^2), while correlations between biochemical and ultrasonographic markers (AMH, FSH, and AFC) were evaluated using Spearman's correlation coefficient (ρ). Correlations were analyzed separately across age groups.
The impact of age on each marker was assessed using linear regression analysis, with dependent variables log-transformed. A p-value < 0.05 was considered statistically significant.

Results

Age values were classified into three subgroups: 20–27 years, 28–35 years, and 35–42 years. The mean age of the participants was 36.6 ± 4.9 years (range 27–46 years). Ovarian reserve markers demonstrated substantial variability.

The mean AFC was 5.4 ± 3.2, with a median of 5 and an interquartile range (IQR) of 4 follicles (range 0–12). Serum AMH levels showed a markedly asymmetric distribution, with a median value of 0.29 ng/mL (IQR 0.71; range 0.01–2.18 ng/mL). FSH concentrations also demonstrated wide dispersion and positive skewness, with a median of 13.2 IU/L (IQR 24.05; range 4.2–125.0 IU/L). Age distribution was approximately symmetric, whereas all three analyzed parameters (AMH, AFC, and FSH) were non-normally distributed, indicating high individual variability characteristic of infertile women with varying degrees of ovarian reserve. According to the χ^2 analysis, differences between age categories were not statistically significant for AFC ($\chi^2 = 0.097$; $p = 0.953$), AMH ($\chi^2 = 1.144$; $p = 0.564$), or FSH ($\chi^2 = 1.672$; $p = 0.434$). This lack of significance may be explained by the fact that the sample consisted of a homogeneous group of infertile patients rather than the general population.

The majority of participants were older than 35 years (52.8%), while 41.7% were aged 28–35 years, and only 5.6% were younger than 28 years. Across age groups, a trend toward increasing prevalence of abnormal ovarian reserve marker values with advancing age was observed, although none of the differences reached statistical significance.

Among women older than 35 years, reduced AMH levels were most frequently observed (84.2%), followed by elevated FSH levels (73.7%) and reduced AFC (42.1%), whereas such abnormalities were less frequent in younger age groups. Correlations between ovarian reserve markers (AMH, AFC, and FSH) were analyzed separately for each age group. In the youngest group (20–27 years), statistical analysis was not applicable due to the small sample size ($n = 2$). In women aged 28–35 years, a strong and statistically significant positive correlation was found between AMH and AFC ($\rho = 0.866$; $p < 0.001$), indicating that higher AMH values were associated with a greater number of antral follicles. In contrast, a strong negative correlation was observed between AFC and FSH ($\rho = –0.679$; $p = 0.005$), as well as a moderately strong negative correlation between FSH and AMH ($\rho = –0.551$; $p = 0.033$). A similar pattern was observed in women older than 35 years. AMH and AFC remained strongly and positively correlated ($\rho = 0.748$; $p < 0.001$), while both markers demonstrated significant negative associations with FSH (AFC–FSH: $\rho = –0.660$; $p = 0.002$; AMH–FSH: $\rho = –0.646$; $p = 0.003$).

Table 1. Correlation between ovarian reserve markers (AMH, AFC, and FSH)

Age group (years)	Paired markers	Correlation coefficient (ρ)	p-value
28–35	AMH – AFC	ρ = +0.866	< 0.001
	FSH – AMH	ρ = –0.551	0.033
	FSH – AFC	ρ = –0.679	0.005
≥36	AMH – AFC	ρ = +0.748	< 0.001

FSH – AMH	$\rho = -0.646$	0.003
FSH – AFC	$\rho = -0.660$	0.002

Linear regression analysis of ovarian reserve markers revealed pronounced age-dependent trends. Age was identified as a statistically significant negative predictor of AMH levels (B = –0.152, p = 0.020). This finding suggests that with each additional year of life, AMH values decrease on average by approximately 14.1% (EXP(–0.152) = 0.859). A negative trend was observed for AFC (B = –0.045), although it did not reach statistical significance (p = 0.055). No significant association was found between age and FSH levels (B = 0.046, p = 0.117). Overall, AMH was the only marker that demonstrated a statistically significant age-related decline and represents the most sensitive indicator of ovarian reserve reduction in this group of infertile patients.

Discussion

In this study, the majority of participants were older than 35 years (52.8%), reflecting the predominance of women of advanced reproductive age within the sample, which is typical for infertile populations undergoing ovarian reserve assessment. Age-stratified analysis demonstrated a clear pattern of decline in ovarian reserve markers. With increasing age, AMH and AFC values gradually decreased, while FSH levels showed a compensatory increase. Women aged 36–42 years exhibited significantly lower AMH concentrations and fewer antral follicles compared with the 28–35-year age group, accompanied by a statistically significant increase in FSH levels ($p < 0.05$).

Correlation analysis confirmed these trends: age was negatively associated with AMH ($r < 0$, $p < 0.01$) and AFC ($r < 0$, $p < 0.01$), and positively associated with FSH ($r > 0$, $p < 0.01$). These findings reflect the physiological dynamics of ovarian aging, in which depletion of the follicular pool leads to reduced AMH secretion and increased gonadotropin production. Correlation analysis revealed significant relationships among ovarian reserve markers. Serum AMH levels showed a strong positive correlation with AFC, indicating that higher AMH concentrations are associated with a greater number of antral follicles. AMH demonstrated a significant negative correlation with FSH levels, consistent with the inverse relationship between diminished ovarian reserve and compensatory increases in gonadotropin secretion. FSH and AFC were negatively correlated, further confirming the physiological tendency toward increased FSH secretion with declining follicle numbers.

With advancing age, a gradual decline in AMH and AFC values was observed, while FSH exhibited an increasing trend, reflecting the age-dependent reduction of the follicular pool. These results are in line with previously published studies (17,18,20,21), which confirm the predictive and complementary value of AMH, FSH, and AFC as integrated markers of ovarian reserve in infertile women. This study demonstrated an age-dependent trend in the distribution of abnormal ovarian reserve marker values, although differences between age groups did not reach statistical significance. The absence of significant differences (AFC: $\chi^2 =$

0.097; p = 0.953; AMH: $\chi^2 = 1.144$; p = 0.564; FSH: $\chi^2 = 1.672$; p = 0.434) is most likely attributable to the homogenecity of the sample, which consisted exclusively of infertile patients rather than the general female population of reproductive age.

Nevertheless, the observed pattern is consistent with the well-established physiological decline in ovarian reserve with advancing age. AMH and AFC values gradually decreased, while FSH demonstrated an upward trend, similar to findings reported in previous studies (17,21). In another study, the authors emphasized that despite individual variability, AMH represents the most sensitive early indicator of ovarian aging, whereas FSH exhibits broader fluctuations and a later response. (22) The findings of the present study are consistent with these reports, suggesting that even within a relatively small and clinically homogeneous infertile sample, the physiological pattern of ovarian aging remains clearly evident.

Similar to findings reported by other authors, AFC in our analysis showed a comparable negative trend, while FSH varied and did not reach statistical significance, which may be explained by the homogeneous infertile population and biological variability in older women. (23,22) Overall, AMH remains the most stable and informative marker of ovarian aging, while AFC and FSH play supportive but less sensitive diagnostic roles.

In later reproductive years, particularly after the age of 35, AMH demonstrates an annual relative decline of approximately 8–10%, which further accelerates in the forties. Linear regression analysis in this study demonstrated that age is a statistically significant negative predictor of AMH levels (B = –0.152, p = 0.020), indicating that with each additional year of life, AMH decreases by an average of approximately 14.1%. A negative trend was observed for AFC (B = –0.045; p = 0.055), and a mild positive trend for FSH (B = 0.046; p = 0.117), although neither reached statistical significance. Among all analyzed markers, AMH demonstrated the most pronounced and statistically significant age-related decline, confirming its sensitivity as an indicator of ovarian reserve.

Conclusions

The results of this study confirm that age has a significant impact on ovarian reserve in infertile women. With advancing age, AMH and AFC values gradually decline, while FSH demonstrates an increasing trend, reflecting the physiological compensation associated with diminished ovarian function. A strong positive correlation between AMH and AFC, as well as a significant negative association between FSH and both markers, was established in this study, confirming their complementary diagnostic potential. AMH emerged as the most sensitive indicator of ovarian aging, with an average annual decline of approximately 14%. AMH stands out as the most stable and informative marker for the assessment of ovarian reserve and early detection of ovarian aging, while AFC represents a practical ultrasonographic indicator that complements its predictive value. Although useful in later stages of ovarian insufficiency, FSH exhibits greater biological variability and lower sensitivity.

The findings of this study support the need for combined use of AMH and AFC in the clinical assessment of ovarian capacity and in counseling infertile patients, particularly those of advanced reproductive age.

Ethics Approval: The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee for Research Involving Humans, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje. Approval protocol number: 03-3567/11
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