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Association of Age and Serum Anti-Mullerian Hormone level with Serum FSH, LH, and Estradiol

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Abstract
Introduction: *Infertility affects approximately 10% to 15% of reproductive-aged couples. The number of good-quality oocytes remaining in the ovaries is referred to as ovarian reserve. As a woman ages, her ovarian reserve decreases, owing primarily to the apoptotic death of primordial follicles. Serum AMH levels are being investigated as a potential testing method for determining ovarian reserve. Other defining biomarkers of fertility include serum FSH, LH, and estradiol. The current study was designed to look for any possible link between patients' ages and these biomarkers.*
Aim of the study: *The aim of the study was to determine the possible association between patients' age and Serum AMH with serum FSH, LH, Estradiol*
Methods: *This cross-sectional observational study was conducted at the Department of Obstetrics and Gynaecology, Infertility Unit, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. The study duration was 14 months, from October 2011 to December 2012. The present study was conducted with 86 women of the reproductive age group with subfertility.*
Result: *With increasing age, there was a gradual linear decline in AMH. The mean value of FSH and LH increased more gradually in the older age groups of 40-45 years than in the younger age groups of 21-30 years. The mean BMI levels were nearly identical across all age groups, with no discernible differences. The difference in AMH levels was statistically significant across age groups. There was a statistically significant negative correlation between age and serum AMH, but a significant positive correlation between FSH and age. A significant negative correlation was found between the basal level of serum AMH and serum FSH, as well as a non-significant positive correlation between AMH and LH and Estradiol.*
Conclusion: *Serum AMH level decreases with age. Serum AMH was found to be negatively correlated with age, while serum FSH was found to be positively correlated with age. Age had a weak positive correlation with LH and a very weak negative correlation with estradiol, neither of which was statistically significant. Serum AMH and FSH had a significant inverse relationship. There was also a significant difference in mean serum AMH levels between age groups.*
Keywords: *Fertility, Follicle, Ovarian, Diminishing, Subfertility, Serum AMH, Serum FSH*

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I. Introduction

Childlessness is a major life event. According to population-based studies, 10-15% of couples in the Western world are affected by infertility.[1],[2] Due to women's delayed marriage and childbearing for their carrier, infertility is emerging as a larger conjugal problem. According to recent studies, infertility affects approximately 15-20% of reproductive-aged couples.[3] Childlessness can be a tragedy for a married couple, particularly for the women, and can cause marital discord as well as personal unhappiness and illness.[4] The main etiological factor in a series of infertile marriages is found among the female population in approximately 40% of cases, and the absence of ovulation or infrequent ovulation is seen in one-fifth of all women with infertility.[4],[5] The number and quality of oocytes available to produce a dominant follicle late in the follicular phase of the menstrual cycle at any given age are referred to as ovarian reserve.[6] Diminished ovarian reserve describes a group of patients of any age whose ovaries and the eggs contained within have a significantly reduced ability to produce pregnancies. Infertility is frequently caused by a decrease in ovarian reserve (DOR) or function. DOR has been linked to a poor response to ovarian stimulation, a lower number of oocyte retrieval

during the IVF cycle, lower pregnancy rates after ART cycles, a higher likelihood of cycle cancellation, as well as higher miscarriage and aneuploidy rates.[7] Age >35 years, previous ovarian surgery, single ovary, unexplained infertility, and a history of poor stimulation with injectable ovulation drugs are all known risk factors for DOR.[8] AMH is a dimeric glycoprotein composed of two monomers linked together by a disulfide bond. The 72-kD molecule is a member of the superfamily of transforming growth factors, which regulates tissue growth and differentiation.[9] AMH is produced by Sertoli cells during fetal sex differentiation in males, where it retards Mullerian duct development.[10] AMH (also known as Mullerian Inhibiting Substance) is produced postnatally in the granulosa cells of early-developing ovarian follicles in the female population and appears to be capable of inhibiting the initiation of primordial follicle growth and FSH-induced follicle growth.[11] When follicles progress from the primordial to the primary stage, AMH production begins and continues until the follicles reach the antral stages with a diameter of 2-6mm. The preantral and small antral stages of follicle development produce the most AMH.[12]-[14] As the follicle grows larger, production decreases and then stops. AMH is rarely produced in follicles that are larger than 8mm in diameter. As a result, the levels remain constant, and the AMH test can be performed at any point during a woman's cycle. Women with many small follicles, such as those with polycystic ovaries, have high AMH hormone levels, whereas women with few remaining follicles and those nearing menopause have low anti-Mullerian hormone levels.[15] Because human female serum contains measurable amounts of AMH throughout the reproductive life span,[9] and because AMH is only produced in growing ovarian follicles, serum level may be used as a marker for ovarian reserve, representing the quality and quantity of the ovarian follicle pool.[16] Furthermore, serum AMH correlates negatively with age and positively with antral follicle count at ultrasound.[17],[18] Furthermore, determining serum AMH levels in patients with premature ovarian failure (POF) can aid in the evaluation of follicle persistence and possibly fertility potential, and in some patients, it may aid in clarifying the mechanism of ovarian dysfunction.[19] In Bangladesh, subfertility is now emerging as a critical problem. A significant percentage of patients are attending infertility outdoors and seeking treatment. The present study was conducted to determine the possible correlation of serum AMH and patients with other fertility biomarkers like serum FSH, LH, and Estradiol.

II. Objective

General Objective

- To determine the possible association between patients' age and serum FSH, LH, Estradiol
- To determine the possible association between serum AMH levels and serum FSH, LH, and estradiol

III. Methods

This cross-sectional observational study was conducted at the Department of Obstetrics and Gynaecology, Infertility Unit, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. The study duration was 14 months, from October 2011 to December 2012. The current study included 86 women of reproductive age who were experiencing infertility. The samples were collected from the 86 study population using a purposive sampling technique. The study participants were chosen based on the inclusion and exclusion criteria. Each participant provided informed written consent, and the study hospital's ethical review committee granted ethical approval. A structured questionnaire was created that included all variables of interest such as age, educational status, occupational status, duration of subfertility, marital history, previous obstetric history such as parity, and the number of living children, a probable cause of subfertility. Anthropometric data, drug history, medical and surgical history, and relevant investigation were also included in the questionnaire. The questionnaire had both closed and open-ended questions. Following retesting and necessary modifications, the questionnaire was finalized. All previous investigation reports were thoroughly reviewed to determine the approximate cause of infertility. Following the aseptic procedure, blood samples for the assessment of FSH, LH, Estradiol, and AMH were obtained by venipuncture from patients who met the inclusion and exclusion criteria on days 2-3 of the spontaneous menstrual cycle.

Inclusion Criteria

- Women aged between 21-45 years
- Bangladeshi women diagnosed with subfertility
- Presence of both ovaries
- Patients who had given consent to participate in the study.

Exclusion Criteria

- Women older than 45 years of age

- Women who had surgery in one or both ovaries.
- Women receiving anti-cancer drugs
- Subfertility due to Turner's Syndrome, an Autoimmune disease.
- Exclude those affected with other chronic diseases etc.

IV. Results

Table 1: Distribution of biomarkers at basal level (D3) among subfertile patients (n=86)

Variables	Mean	±SD
AMH (Anti-Mullerian hormone**)**	2.72ng/ml	±3.61
FSH (Follicle-stimulating hormone**)**	6.20 miu/ml	±3.94
LH (Luteinizing Hormone**)**	6.9 miu/ml	±3.94
Estradiol	73.6 pg/ml	±28

Table 1 shows the distribution of different biomarkers at the basal level (D3) among the 86 subfertile patients. Results are expressed as mean ± SD.

Table 2: Distribution of Biomarkers at Basal Level (D_3) among different age groups of study subjects (n=86)

Name of Variables (Mean±SD)	**Age Groups**			
	21-30 years (n=26)	**31-35 years** (n=20)	**36-40 years** (n=21)	**41-45 years** (n=20)
AMH	3.91±3.4	3.07±4.5	1.87±2.8	1.25± 2.6
FSH	4.84±2	5.18± 3.5	5.32±2.9	9.85±5
LH	6.88±3.7	6.17±4.2	5.9±3	9.03±4.2
Estradiol	80.7±19.6	68. 3±23.4	77.5±36.2	66.82±30

Table 2 shows the mean ± SD value of different biomarkers among the four age groups of patients. There was a gradual linear decline of AMH observed with an increment of age. The mean value of FSH and LH gradually increased in the higher age groups of 40-45 years than in the lower age group of 21-30 years. Estradiol levels also decreased in a higher age group than in younger age group patients.

Table 3: Distribution of BMI among different age groups of study subjects (n=86)

Age Groups	BMI (Mean ± SD)
21-30 years (n=25)	25.2±3.8
31-35 years (n=20)	24±3.6
36-40 years (n=21)	25.6±3
41-45 years (n=20)	25±3.2

Table 3 revealed the mean BMI levels of different age groups. It was observed that the mean BMI levels were almost similar in all age groups, and no remarkable difference could be discerned.

Table 4: Correlation of age with Basal level of serum AMH, FSH, LH, and Estradiol (Pearson's Correlation)

Variables	Mean ± SD	r	P Value	Inference
AMH	2.72±3.61(ng/ml)	-.23	.03	Significant
FSH	6.20±3.95(miu/ml)	.43	.000	Significant
LH	6.97±3.94(miu/ml)	.16	.141	Not Significant
Estradiol	73.6±28.07(pg/ml)	-.12	.250	Not Significant

Correlation is significant at the 0.05 level (2-tailed).

Using Pearson's correlation test, the correlation between age and basal level of different serum parameters was observed. The table revealed a negative correlation between age and serum AMH (r=-0.23), and this negative correlation was statistically significant at a 0.03 level P-value. Serum FSH showed a positive correlation (r=0.43) with age, which was statistically significant ($p<0.01$). Serum LH had a positive correlation and Estradiol had a negative correlation with age, but they were not statistically significant.

Table 5: Correlation of basal level (D3) of serum AMH with basal level (D3) of serum FSH, LH, and Estradiol among subfertile patients (Pearson's Correlation).

		FSH	LH	Estradiol
AMH	**r**	**-.243**	**.149**	**.121**
	P value	**.02**	**.170**	**.275**
Inference		**Significant**	**Not Significant**	**Not Significant**

Correlation is significant at the 0.05 level (2-tailed).

Table 5shows a significant negative correlation between the basal level of serum AMH with serum FSH and a non-significant positive correlation of AMH with LH and Estradiol.

V. Discussion

The purpose of this cross-sectional observational study was to determine the mean serum AMH levels in infertile patients and to assess any observable relationship between age and serum AMH levels with other reproductive biomarkers such as FSH, LH, and Estradiol. AMH, or anti-Mullerian hormone, is a dimeric glycoprotein that is made up of two monomers connected by disulfide bonds and belongs to the transforming growth factor ß superfamily.[20] Early follicular Anti Mullerian hormone is a novel ovarian reserve indicator. A large number of studies have been conducted to investigate the relationship between age and serum AMH.[20]-[23]In the current study, the mean AMH at the basal level was 2.72 ng/ml. In our study, biomarkers of various variables (AMH, FSH, LH, Estradiol) were measured in participants of various ages. The participants were divided into four age groups: 21-30 years old (n=25), 31-35 years old (n=20), 36-40 years old (n=21), and 41-45 years old (n=20). Among these four subgroups, it was discovered that mean AMH levels decreased slightly in the first two age groups but significantly in the last two. Mean FSH levels increased slightly in the first three age groups but dramatically in the final age group of 41-45 years. Mean LH levels decreased between the ages of 31 and 40 but increased dramatically among participants aged 41 to 45. The mean Estradiol level did not consistently increase or decrease across all four age groups. The difference in AMH levels between age groups was statistically significant. Our research also discovered a significant drop in AMH levels after the age of 35. The mean value of AMH in the 35–40-year age group was 1.872.8 ng/ml. This finding was consistent with another study by Singer and Barad et al, which found a significant decrease in AMH above the age of 35, but no significant increase in FSH.[24] They discovered that the mean level of AMH in the age group 35 was 2.02.4 ng/mL, 1.40.7 ng/mL in the age group 35-37, and.8ng/mL in the age group 38-40. Other studies concluded that both AMH and FSH were probably at their best in reflecting ovarian function at the age of 35.[25],[26] Our analysis confirmed that basal AMH and basal FSH have a significant negative and positive correlation, i.e., AMH decreases with advanced female age while FSH increases. The day 3 FSH level was the old standard for ovarian reserve testing. Every lab uses a different scale to determine what a normal FSH cut-off value is for indicating a successful pregnancy. FSH levels below 10miu/ml (10) are considered normal, while levels above 10miu/ml (>10) are considered abnormal for a female during her reproductive years.[27] Our research found a significant positive correlation between age and FSH basal levels. The increase in FSH level (9.034.2) is more pronounced in women aged 41-45 years than in the other three age groups studied in this study. This was consistent with the findings of other studies.[28] Our study found that the age group 41-45 years had a significantly higher basal level of LH than the age groups 21-30 years and 31-35 years. Shin et al., on the other hand, discovered that serum levels of E2, LH, and inhibin B did not differ significantly by age group.[29] Their research also found that serum LH was higher and inhibin was lower in women in their 40s than in women in their 20s and 30s. The current study's basal LH also shows a weak positive correlation with age and AMH, but no significant association.

Limitations of The Study

The study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

VI. Conclusion

According to our findings, the AMH level decreases with age. Serum AMH was found to be negatively correlated with age, while serum FSH was found to be positively correlated with age. Age had a weak positive correlation with LH and a very weak negative correlation with estradiol, neither of which was statistically significant. Serum AMH and FSH had a significant inverse relationship. There was also a significant difference in mean serum AMH levels between age groups.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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