OPEN ACCESS
Scholars International Journal of Obstetrics and Gynecology
Abbreviated Key Title: Sch Int J Obstet Gynec
ISSN 2616-8235 (Print) |ISSN 2617-3492 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Association of Serum Anti-Mullerian Hormone Level with Age in Subfertile Patients

Dr. Rumana Sultana[1], Dr. Mohammad. Monir Hossian[2], Prof. Parveen Fatima[3]

[1]Assistant Professor, Department of Gynecology, Dhaka Medical College Hospital, Dhaka, Bangladesh
[2]Assistant Professor, Department of Pediatrics, Sir Salimullah Medical College & Mitford Hospital, Dhaka, Bangladesh
[3]Ex. Professor and Department Head, Department of Gynecology and Obstetrics, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

DOI: 10.36348/sijog.2022.v05i12.004 | **Received:** 09.10.2022 | **Accepted:** 15.11.2022 | **Published:** 08.12.2022

***Corresponding author:** Dr. Rumana Sultana
Assistant Professor, Department of Gynecology, Dhaka Medical College Hospital, Dhaka, Bangladesh

Abstract

Introduction: Infertility affects approximately 10%-15% of reproductive-aged couples. Ovarian reserve describes the number of good-quality oocytes remaining within the ovaries. As a women ages, her ovarian reserve declines, principally due to apoptotic loss of primordial follicles. Serum AMH level is being considered a possible testing method for determining ovarian reserve. The aim of the study was to observe any association between serum Anti- Mullerian Hormone (AMH) level with patient age among subfertile patients. ***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:*** 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. The mean BMI levels were almost similar in all age groups, and no remarkable difference could be discerned. The difference in AMH levels among the different age groups was statistically significant. a statistically significant negative correlation between age and serum AMH was observed, while a significant positive correlation was observed between FSH and age. ***Conclusion:*** As the age of a women advances, the AMH level decreases. The serum AMH is negatively correlated with age and serum FSH is positively correlated with age.

Keywords: Serum, fertility, Subfertile, Ovarian, Reproductive.

INTRODUCTION

Childlessness is a life crisis. Data from population-based studies suggest that 10-15% of couples in the western world are affected by impaired fertility [1, 2]. Infertility is emerging as a conjugal problem with a greater magnitude due to delayed marriage and childbearing of women for their carrier. Recent studies revealed that Infertility affects approximately 15-20% of reproductive-aged couples [3]. Childlessness may be a tragedy to the married couple, especially for the women, and can be a cause of marital upset as well as personal unhappiness and ill-health [4]. In a series of infertile marriages, the main etiological factor is found among the female population in about 40% of cases, and the absence of ovulation or infrequent ovulation is seen in a fifth of all women with infertility [4, 5]. Ovarian reserve refers to the number and quality of oocytes that at any given age are available to produce a dominant follicle late in the follicular phase of the menstrual cycle [6]. Diminished ovarian reserve refers to a group of patients at any age whose ovaries and the eggs contained within, have a markedly decreased ability to produce pregnancies. Diminished ovarian reserve (DOR) or function is an important cause of infertility. DOR has been associated with a poor response to ovarian stimulation, lower number of oocytes retrieval during the in-vitro fertilization (IVF) cycle, lower pregnancy rates after ART cycles, a greater likelihood of cycle cancellation as well as higher miscarriage and aneuploidy rates [7]. Some known risk factors of DOR are age>35 years,

previous ovarian surgery, single ovary, unexplained infertility, and history of poor stimulation with injectable ovulation drugs [8]. Anti-Mullerian hormone (AMH) is a dimeric glycoprotein made up of two monomers attached by a disulfide bond. The 72-kd molecule belongs to the transforming growth factor β superfamily which acts on tissue growth and differentiation [9]. In the male, AMH is produced by Sertoli cells during fetal sex differentiation, in which it regresses the Mullerian duct development [10]. In the female population, AMH (also known as Mullerian Inhibiting Substance) is produced in the granulosa cells of early developing ovarian follicles postnatally and seems to be able to inhibit the initiation of primordial follicle growth and FSH induced follicle growth [11]. After follicles differentiate from primordial to the primary stage, production of AMH starts and it continues until the follicles have reached the antral stages with a diameter of 2-6mm. AMH production is highest in preantral and small antral stages of follicle development [12-14]. Production decreases and then stops as the follicle grows further. AMH is seldom produced in follicles larger than 8mm in diameter. As a result, the levels remain constant, and the AMH test can be performed on any day of a woman's cycle. Women with many small follicles, such as those with polycystic ovaries have high AMH hormone values, and women that have few remaining follicles and those who are close to menopause have low anti-Mullerian hormone levels [15]. As human female serum contains measurable amounts of AMH during the reproductive life span, [9] and AMH is solely produced in the growing ovarian follicles, serum level may be used as a marker for ovarian reserve representing the quality and quantity of ovarian follicle pool [16]. In addition, serum AMH shows a negative correlation with age and a positive correlation with antral follicle count at ultrasound [17, 18]. Moreover, the determination of serum AMH levels in premature ovarian failure patients (POF) can help in the evaluation of the persistence of follicles and possibly of the fertility potential, and in some patients could also help 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 outdoor and seeking treatment. Few data is available regarding the research of AMH in our country. So we want to determine serum AMH levels to see the correlation between age with AMH. By estimating AMH, a prediction of the remaining reproductive lifetime could be assessed as well as the likely success of assisted reproductive techniques such as IVF. It will also help in counseling patients about their chances for pregnancy either spontaneous or during their fertility therapy. AMH also helps in predicting the onset of menopause or women who are at risk of developing premature ovarian failure.

OBJECTIVE

General Objective

- To find out the association of serum Anti-Mullerian Hormone level with age in subfertile patients.

Specific Objectives

- To find out the possible association of other reproductive biomarkers with age in subfertile patients.

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. A purposive sampling technique was applied to collect the samples from the selected 86 study population. The study subjects were selected following the inclusion and exclusion criteria. Informed written consent was obtained from each participant, and ethical approval was obtained from the ethical review committee of the study hospital. A structured questionnaire was prepared which include all the variables of interest like demographic and socio-economic data such as age, educational status, occupational status, duration of subfertility, marital history, previous obstetric history like parity, and the number of living children, a probable cause of subfertility. The questionnaire also included anthropometric data, drug history, medical and surgical history, and relevant investigation. The questionnaire was both closed and open-ended. The questionnaire was finalized following retesting and necessary modification. All the previous investigation reports were reviewed thoroughly to find out the approximate cause of subfertility. Blood sample collection following the aseptic procedure, on day 2-3 of the spontaneous menstrual cycle the blood samples for the assessment of FSH, LH, Estradiol, and AMH were obtained by venipuncture from the patients who fulfilled inclusion and exclusion criteria.

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, autoimmune diseases.
- Exclude those affected with other chronic diseases etc.

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: Difference of Mean Serum AMH with different age group

Age Group	Mean AMH (ng/ml)	F	P Value	Inference
21-30(n=25)	3.91±3.4	2.72	0.48	Significant
31-35(n=20)	3.07±4.5			
36-40(n=21)	1.87±2.8			
41-45(n=20)	1.25±2.5			

Table 4 showed the difference in mean serum AMH among the different age groups. The difference in AMH levels among the different age groups was statistically significant (P<0.05).

Table 5: 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.

DISCUSSION

This cross-sectional observational study was conducted to find out the mean serum AMH levels among subfertile patients, and to evaluate any observable relation between age and serum AMH levels, along with other reproductive hormones like FSH, LH, and Estradiol. AMH or Anti-Mullerian hormone is a member of the transforming growth factor ß superfamily and is a dimeric glycoprotein that is made up of two monomers attached to each other by disulfide bonds [20]. Early follicular Anti Mullerian hormone is a novel measure for ovarian reserve. A large number of studies have described the correlation between age and serum AMH [20-23]. In the present study, we observed that the mean AMH at the basal level was 2.72 ng/ml. Biomarkers of different variables (AMH, FSH, LH, Estradiol) were measured among the participants of different age groups in our study. The participants were divided in 4 subgroups based on their age, 21-30 years (n=25), 31-35 years (n=20), 36-40 years (n=21) and 41-45 years (n=20). Among these 4 subgroups, it was observed that mean AMH levels continued to decrease slightly in the first two groups but had a much larger decrease in the last two age groups. Mean FSH levels showed a slight increase between the first three age groups but had a drastic increase in the last age group of 41-45 years. Mean LH levels decreased between the age of 31-and 40 years but showed a drastic increase among the participants of the age group 41-45 years. The mean Estradiol level had no consistent increase or decrease among the participants of all 4 age groups. The difference in mean AMH levels among the different age groups was statistically significant. Our study also revealed a marked fall in AMH levels after 35 years of age. We found the mean value of AMH in the age group 35-40 years to be 1.87±2.8 ng/ml. This finding was consistent with another study by Singer and Barad *et al.,* who revealed marked a fall in AMH above the age of 35, but the increase in FSH was not marked [24]. They found the mean level of AMH in the age group <35 was 2.0±2.4 ng/mL; among the age group 35-37 it was 1.4±0.7 ng/mL and among the 38-40 age group was .8ng/ml. Other studies also concluded that at age 35, both AMH and FSH were probably at their best in reflecting ovarian function [25, 26]. Analysis of our study confirmed that basal AMH and basal FSH demonstrate a significant negative and positive correlation respectively, i.e., AMH decreases with advanced female age whereas FSH increases. The most appropriate serum marker is the one that reflects the number of follicles that have their transition from the primordial pool into the growing follicular pool, during the gonadotrophin–independent phase before the follicular recruitment. Over the past two decades, several ovarian reserve tests (ORTs) have been designed to answer that particular question. A new potential test in this field is measuring the anti-Mullerian hormone. It shows progressive decline throughout life and is a direct indicator of ovarian aging The advantage of AMH over other hormones is that it is the only marker of the ovarian reserve that can be tested in both the follicular as well as the luteal phases, it is cycling independent, remain stable throughout the menstrual cycle and hence serum can be drawn any day of the cycle. So doing a blood test AMH is easy and convenient to measure and is an attractive test for ovarian reserve.

Limitations of the Study

The weakness of this study is cross-sectional data, as a case-control study could not be done due to the high cost of kit. The study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

CONCLUSION

Our study shows that as age advances, the AMH level decreases. The serum AMH is negatively correlated with age and serum FSH is positively correlated with age. There is a weakly positive correlation between age with LH and a very weak negative correlation between age and estradiol and these correlations are not statistically significant. There is a significant inverse relationship between serum AMH and FSH. A significant difference between the mean serum levels of AMH in different age groups was also found.

FUNDING

No funding sources.

CONFLICT OF INTEREST

None declared.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

REFERENCES

1. Templeton, A., Fraser, C., & Thrompton, B. (1990). The epidemiology of infertility in Aberdeen. *Br Med J*, 301, 142-52.
2. Evers, J. L. H. (2002). Female Subfertility. *The Lancet*, 360, 151-159.
3. Roudebush, W. E., Kivens, W. J., & Mattke, J. M. (2008). Biomarkers of Ovarian Reserve. *Biomarker Insights*, 3, 259- 268 available from http://

creativecommons.org/licences/by/3.0, visited on 2011, October 26.

4. Kumar, P., & Malhotra, N. (2008). Jeffcoates Principles of Gynaecology.7th International edition. :pp 699,700.New Delhi: *Joypee Brothers Medical Publishers.*
5. Edmonds, D. K. (2006). Dewhurst's Textbook of Obstetric & Gynaecology, *7th edition Blackwell Publishing*: pp-440,441.
6. Baird, D. T., Collins, J., Egozcue, J., Evers, L. H., Gianaroli, L., Leridon, H., Sunde, A., Templeton, A., … & Steirteghem, A. V. (2005). Fertility and aging. *Hum Reproduction Update*, 11(1), 261-267.
7. Greenseid, K., Jindal, S., Zapantis, A., Nihsen, M., Hurwitz, J., & Paul, L. (2009). Declining ovarian reserve adversely influences granulose cell viability. *Fertil Steril*, 91(6), 2611-2615.
8. Lenton, E. A., Landgren, B. M., Sexton, L., & Herper, R. (1984). Normal variation in the length of the follicular phase of the menstrual cycle: effect of chronolocal age. *Br J Obstet Gynecol*, 91, 681-684.
9. Lee, M. M., Donahhoe, P. K., Hasegawa, T., Silverman, B., Crist, G. B., Best, S., Hasegawa, Y., Noto, R. A., Schoenfeld, D., & Maclaughlin, D. T. (1996). Mullerian inhibiting substance in human: normal levels from infancy to adulthood. *J Clin Endocrinol Metab*, 81(2), 571-6.
10. Cate, R. L., Mattaliano, R. J., Hession, C., Tizard, R., Farber, N. M., Cheung, A., ... & Donahoe, P. K. (1986). Isolation of the bovine and human genes for Müllerian inhibiting substance and expression of the human gene in animal cells. *Cell*, *45*(5), 685-698.
11. La Marca, A., & Volpe, A. (2006). Anti-mullerian hormone (AMH) in female reproduction: is measure measurement of circulating AMH a useful tool? *Clinical Endocrinology*, 64, 603-610.
12. Durlinger, A. L., Kramer, P., Karel, B., de Jong, F. H., Uilenbroek, J. T., Grootegoed, J. A., & Themmen, A. P. (1999). Control of primordial follicle recruitment by Anti-Mullerian hormone in the mouse Ovary. *Endocrinology*, 140, 5789-5796.
13. Durlinger, A. L., Visser, J. A., & Themmen, A. P. (2002). Regulation of ovarian function: the role of anti-mullerian hormone. *Reproduction*, 124, 601-609.
14. Weenen, C., Laven, J. S., Von Bergh, A. R., Cranfield, M., Groome, N. P., Visser, J. A., ... & Themmen, A. P. (2004). Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *MHR: Basic science of reproductive medicine*, *10*(2), 77-83.
15. Advanced Fertility Centre of Chicago. Anti-Mullerian Hormone Testing of Ovarian Reserve available on www.advanced fertility.com/amh-fertility test.html visited on 2011 Oct 26.
16. Te Velde, E. R., & Pearson, P. L. (2002). The variability of female reproductive age. *Hum Reproduction Update*, 8(2), 141-154.
17. De Vet, A., Laven, J. S., de Jong, F. H., Themmen, A. P., & Fauser, B. C. (2002). Anti-Mullerian hormone levels: a putative marker for ovarian aging. *Fertil Steril*, 77, 357-362.
18. van Rooij, I. A., Broekmans, F. J., Scheffer, G. J., Looman, C. W., Habbema, J. D. F., de Jong, F. H., ... & te Velde, E. R. (2005). Serum antimüllerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. *Fertility and sterility*, *83*(4), 979-987.
19. Meduri, G., Massin, N., Guibourdenche, J., Bachelot, A., Fiori, O., Kuttenn, F., ... & Touraine, P. (2007). Serum anti-Müllerian hormone expression in women with premature ovarian failure. *Human reproduction*, *22*(1), 117-123.
20. La Marca, A., Sighinolfi, G., Radi, D., Argento, C., Baraldi, E., Artenisio, A. C., ... & Volpe, A. (2010). Anti-Müllerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Human reproduction update*, *16*(2), 113-130.
21. Fiçicioğlu, C., Kutlu, T., Baglam, E., & Bakacak, Z. (2006). Early follicular antimüllerian hormone as an indicator of ovarian reserve. *Fertility and sterility*, *85*(3), 592-596.
22. Visser, J. A., JD, de Jong, F. H., Laven, J. S., & Themmen, A. P. (2006). Anti-Mullerian hormone a new marker for ovarian function. *Reproduction*, 131, 1-9.
23. Van Rooij, I. A. J., Broekmans, F. J. M., Te Velde, E. R., Fauser, B. C. J. M., Bancsi, L. F. J. M. M., De Jong, F. H., & Themmen, A. P. N. (2002). Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. *Human reproduction*, *17*(12), 3065-3071.
24. Singer, T., Barad, D. H., Weghofer, A., & Gleicher, N. (2009). Correlation of antimüllerian hormone and baseline follicle-stimulating hormone levels. *Fertility and sterility*, *91*(6), 2616-2619.
25. Gleicher, N., Weghofer, A., & Barad, D. H. (2011). Defining ovarian reserve to better understand ovarian aging. *Reproductive biology and endocrinology*, *9*(1), 1-11. http://www.rbej.com/content
26. Barad, D. H., Weghofer, A., & Gleicher, N. (2011). Utility of age-specific serum anti-Müllerian hormone concentrations. *Reproductive biomedicine online*, *22*(3), 284-291.