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# THE STUDY OF ANTI MULLERIAN HORMONE IN WOMEN WITH INFERTILITY

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**Abstract**
**Background:** Anti Mullerian Hormone (AMH) is a new diagnostic biomarker of ovarian reserve and is indicative of infertility. Present study is an attempt to measure serum AMH and to compare it with different parameters like age, BMI, history of smoking in women with infertility
**Methodology:** This cross-sectional observational study was conducted in department of Biochemistry Darbhanga Medical College Laheriasaria. 60 women diagnosed with infertility in the age group of 21 to 55 years were consecutively selected from department of Obstetrics and Gynaecology. 60 age matched healthy controls were also taken from same department. Subjects were divided into two groups Study Group (Subjects with infertility) and Control Group (Healthy age matched controls). 2 ml venous blood was collected from antecubital vein of all subjects. Serum AMH was measured using Ultra-sensitive AMH/MIS ELISA AL-105i. BMI was calculated using Adolphe Quetlet method. History of smoking was taken while interviewing the patient. Sociodemographic data were recorded or sociodemographic performa-1. All observations were recorded and relation between different parameters was investigated using appropriate statistics.
**Results:** Serum AMH was lower in study group as compared to control group (p=0.001). Levels of AMH decreased with advancing age in both control and study group. AMH decreased as BMI increased in both the groups. Smokers in both groups had lower AMH than non-smokers.
**Conclusion:** AMH is a biomarker of infertility as it is low among women with infertility as compared to healthy women. Advancing age, weight gain and smoking decrease its levels.

**Keywords:** AMH, Infertility, BMI, Ultra-sensitive AMH/MIS ELISA AL-105i, smoking.

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## 1. Introduction

Infertility is defined as inability to conceive after more than one year of unprotected intercourse. When there is no history of a previous pregnancy, it is called primary infertility but when a woman has been previously pregnant even once, it is called secondary infertility.[1]
Globally infertility is estimated to affect 8% to 12% of reproductive age group couples.[2] Whereas, overall prevalence of infertility in India varies from 3.9% to 16.8%.[3]
There is a multifactorial causation associated with infertility. Delayed marriages, use of tobacco and alcohol, sedentary lifestyle, consumption of high fatty diet and weight gain have been attributed to cause infertility. To make things worse polycystic ovarian syndrome (PCOS), tuberculosis and pelvic infections have been on rise. Causes of infertility among men are abnormal sperm production and function, undescended testis, genetic defects, varicocele, diabetes, Chlamydia, gonorrhoea, mumps and HIV. Among females' common causes observed are decreased ovarian reserve, ovulatory disorder, tubal injury, blockade or Para tubal adhesion, Uterine infections, cervical and immunological factors.[4]
AMH is a recent biomarker for ovarian reserve.[5] It is a homodimeric disulphide linked glycoprotein with a molecular weight of 140kda. Human AMH has 560 amino acid precursors. It is a member of transforming growth factor beta super family. Its gene is located on short arm of chromosome 19 and band 19p.13.3 in humans.[6]
In mammalian ovary the role of AMH appears to be regulation of the primordial follicles pool by its inhibitory effect on formation and growth of primordial follicles.[7]
At menarche only 30,000 follicles are left. During reproductive life follicle depletion occurs exponentially with a sharp increase after the age of 35 year.[8]
At the mean age of 50 -51 years when the primordial follicle pool is exhausted.[9] AMH plays an important role in recruitment of primordial follicle and selection of a dominant follicle from a cohort of antral follicles. It is responsible for follicle growth also.[10]
Since AMH being a glycoprotein is catabolized in lysosomes with help of proteases and glycosidases with characteristic properties of soluble lysosomal hydrolases.[11]
With increasing age catastrophic oxidative events accumulate. This causes tissue damage and plays a significant role in the pathophysiology of many pathological situations including qualitative and quantitative decline in age related ovarian function.[12]

Concentration of AMH is lower in higher BMI.[13] AMH levels are significantly lower in obese compared with non-obese premenopausal women. But the antral follicles accounts did notdiffer by body size.[14] This suggests that lower AMH level in obese women did not result from decreased ovarian reserve but from other physiological process in hormone metabolism sequestration or clearance.
Smokers have a lower level of AMH than non-smokers, in smokers' level of AMH decreases faster and they reach the age of menopause earlier. Smoking is one of the strongest predictors of entry into early stages of the menopause transition and increases the likelihood of entering each transition stage by about 30%.[15]
A study found that age and smoking are significant and independent entities to the prediction of AMH. The mechanism behind this association is likely related to toxic effect of smoking on ovarian follicles which results in accelerated ovarian follicular depletion and that lead to diminished ovarian reserve at earlier reproductive ages. The mechanism behind this association is likely related to toxic effect of smoking on ovarian follicles which results in accelerated ovarian follicular depletion and lead to diminished ovarian reserve at earlier

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reproductive ages.[16]
Present study is aimed at measuring Serum AMH levels by Ultra-sensitive AMH/MIS ELISA AL-105i, comparing the levels to normal healthy controls and correlating them to other parameters like age, BMI and history of smoking.

## 2. Materials and methods

This cross-sectional observational study was conducted in department of Biochemistry Darbhanga Medical College Laheriasaria. 60 women diagnosed with infertility in the age group of 21 to 55 years were consecutively selected from department of Obstetrics and Gynaecology. 60 age matched healthy controls were also taken from same department. Subjects were divided into two groups Study Group (Subjects with infertility) and Control Group (Healthy age matched controls). 2 ml venous blood was collected from antecubital vein of all subjects. Serum AMH was measured using Ultra-sensitive AMH/MIS ELISA AL-105i. BMI was calculated using Adolphe Quetlet method. History of smoking was taken while interviewing the patient. Sociodemographic data were recorded or sociodemographic performa-1. All observations were recorded and relation between different parameters was investigated using appropriate statistics.

**Sample collection**

2 ml of venous blood collected from the anti-cubital vein by disposable syringe. All antiseptic precautions should be taken. Blood is placed in the clot activator vial and allowed to clot at room temperature for at least 30 minutes and then centrifuged at 2000 RPM for 20 minutes. The serum separated and stored at 2-8 degree centigrade and estimation was done on next day.

**Estimation of serum AMH levels**

**Method – Ultra sensititve AMH/MIS ELISA AL-105-i (Ansh Labs)Principle**

The antibody biotin conjugate binds to the solid phase antibody antigen complex which in turn binds to the streptovidin enzyme conjugate. The antibody antigen biotin conjugate SHRP complex bound to the wall is detected by enzyme substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelengths absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The observation measured is directly proportional to the concentration of AMH /MIS in the sample and calibrators.

**Components of the kit**

- 96 micro titration Wells with precoated a.m. antibody.
- serum a.m. standard 6 vials. (0ng/ml to. 16.9 ng /ml.
- Serum AMH controls 1 &2
- AMH streptovidin enzyme conjugate reagents- 12 ml.
- AMH biotin conjugate- 12 ml
- TBM (Tetra Methyl Benzidine chromogen - 12 ml)
- Stop solution (Sulfanic acid) 12ml
- Wash concentrate 25X - 60 ml

**Storage and stability**

Reagents are stable at 2-8 degree centigrade till expiry date.

**Preparation of working reagent**

**Preparation of wash buffer.**

Wash buffer was diluted 25-fold with deionized water and mixed well.
The wash solution is stable for one month at room temperature (23±2oC).

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**Calibration of standard**

AMH calibrator A to F were reconstituted with 1ml of deionized water and mixed well. Six wells were taken in the holder and 25µl of AMH standard were added into appropriate wells. Then 100 µl of AMH assay buffer was added to each well. These plates were incubated for 90 minutes in a orbital microplate shaker, shaking at a fast speed (600-800 rpm) at room temperature. Each well was then washed 5 times (350 µl/well) with wash solution. Then 100 µl of Antibody Biotin Conjugate was added to each well. The plate was incubated for 30 minutes on orbital microplate shaker at a fast speed (600-800 rpm) at room temperature. Each strip was washed 5 times with wash solution (350 µl/ wells). In next step 100 µl of Streptovidin enzyme conjugate was added to each well. The plate was anincubated for 30 minutes on an orbital microplate shaker at room temperature. Each well was washed 5 times with wash solution (350 µl/well). TMB Chromogen solution 100 µl was added to each well. The plate as incubated for 8 to 12 minutes on orbital microplate shaker at room temperature. At last, 100 µl of Stopping solution was added to each well. Absorbance of the solution in wells were taken within 20 minutes using ELISA reader at 450 nm.

| CONTROL VALUE (ng/ml) | ABSORBANCE (450 nm) |
|---|---|
| 0 | 0.1270 |
| 0.11 | 0.1317 |
| 0.49 | 0.1864 |
| 2.1 | 0.2720 |
| 6.7 | 0.7045 |
| 16.9 | 1.8164 |

**Reference range: -** <0.02 ng/mL to 10.40 ng/mL

**Estimation of serum AMH levels.**

Desired number of coated wells were taken in the holder, 25 µl of samples and controls were taken in appropriate wells. Other process was same as the calibration of standard curve.

**Precautions**

- The calibrator and controls contain human source components. Which have been tested and found non-reactive for hepatitis B surface antigenas well as HIV antibody with FDA licensed reagents.
- The plate should not be left to dry completely, as the dry condition can inactive the biological material on the plate.
- The TMB substrate B should be stored be stored in dark.
- The water in water bath should be heated if the crystalloid appears in the Wash buffer.
- The microplate should not be removed from storage bag until needed.[17]

**Statistics**

Data was collected from Study Group and Control Group and were expressed as Mean ± SD (standard deviation). Single and paired sample test was applied to determine the significance of biochemical parameters among groups. The entire data was analyzed by statistically programme SPSS 23.0.

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**Observations**

**Table 1: Showing comparison of Serum AMH level between controland study group.**

| | No. of subjects | Range (ng/ml) | Mean±S.D (ng/ml) | t value | P value |
|---|---|---|---|---|---|
| Control group | 60 | <0.02-10.40 | 3.68±3.0 | 1.047 | 0.001 |
| Study group | 60 | 0.00-1.24 | 0.48±0.62 | | |

Above table shows that serum AMH level in study group is low (0.48±0.62) as compared to control group (3.68±3.0). The difference was statistically significant (p=0.001).

**Table 2: Comparison of Serum AMH level (ng/mL) according todifferent age group in control and study group**

| Age (yrs) | Subjects | No. of cases | Mean±SD (ng/mL) | t value | p value |
|---|---|---|---|---|---|
| 21-30 | Control | 20 | 5.59±2.55 | 1.0158 | 0.001 |
| | Study Group | 20 | 0.808±0.257 | | |
| 31-40 | Control | 20 | 4.34±1.61 | 1.9312 | 0.002 |
| | Study Group | 20 | 0.521±0.78 | | |
| 41-50 | Control | 10 | 1.27±1.71 | 1.9167 | 0.003 |
| | Study Group | 10 | 0.239±0.46 | | |
| 51-55 | Control | 10 | 0.017±0.024 | 1.00061 | 0.001 |
| | Study Group | 10 | 0.0006±0.0008 | | |

Above table shows that in both study group and control group, as age increases AMH levels decrease.

**Table 3: Comparison of Serum AMH level (ng/mL) according todifferent BMI group in control and study group.**

| BMI ($kg/m^2$) | Subjects | No. of cases | Mean±SD (ng/mL) | t value | p value |
|---|---|---|---|---|---|
| <25 | Control | 20 | 5.59±2.55 | 1.0158 | 0.001 |
| | Study Group | 20 | 0.808±0.257 | | |
| 26-29 | Control | 20 | 4.34±1.61 | | |

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| | | | | | |
|---|---|---|---|---|---|
| | Study Group | 20 | 0.521±0.78 | 1.9312 | 0.002 |
| ≥ 30 | Control | 10 | 1.27±1.71 | | |
| | Study Group | 10 | 0.239±0.46 | 1.9167 | 0.001 |

Above table shows that as BMI increases, serum AMH levels decrease. In different BMI groups, serum AMH levels are significantly lower in study group as compared to control group.

**Table 4: Comparison of Serum AMH level (ng/mL) in smokers and non-smokers of control and study group.**

| | Subjects | No. of cases | Mean±SD (ng/mL) | t value | p value |
|---|---|---|---|---|---|
| Smokers | Control | 37 | 3.23±2.47 | | |
| | Study Group | 23 | 0.427±0.55 | 1.126 | 0.002 |
| Non-smokers | Control | 25 | 3.73±3.08 | | |
| | Study Group | 35 | 0.454±0.46 | 1.349 | 0.002 |

Above table shows that in both study group and control group, serum AMH levels were lower among smokers as compared to non-smokers.

## 3. Discussion

AMH is a new diagnostic marker of ovarian reserve. In present study, serum AMH was low in study group (0.48±0.62) as compared to control group (3.68±3.0). This finding of present study is concordant with Broekman's et al 2009 they also found that serum AMH level is less infertile women than the normal women, that is due to major individual variability that exists in ovarian ageing is predominantly due to different in pace of follicle pool depletion, coinciding with large range age of normal menopause between 40 – 60 years.[18]

Present study revealed that as age increased, Serum AMH levels decreased. The mean serum AMH in control group in the age group 21 – 30 years was 5.59±2.55 ng/ml, 4.34±1.61 ng/ml for 31 – 40 years age group, 1.27±1.71 ng/ml for 41 – 50 years age group, 0.0006±0.008 ng/ml for the age group 51 – 55 years age group. In study group mean serum AMH level was 0.808±0.257 ng/ml for 21-30 years age group, 0.521±0.78 ng/ml for 31-40 years age group, 0.239±0.46 ng/ml for 41-50 years age group and 0.0006±0.0008 ng/ml for 51-55 years age group. The data for decreasing serum AMH level with increasing age was statistically significant (p value <0.005). Bentzen et al 2013, also reported that serum AMH levels decrease as age advances due to increase prevalence of large follicle, which have a negative association with serum AMH level. They also reported that in women from 21 years of age the average decline of serum AMH levels calculated were 5.6%.[19] Hagen et al 2010 also studied that serum AMH levels were low during pre-pubertal development, rise during early puberty and reach at plateau around 20 – 25 years of age, followed by a gradual decline thereafter until becoming undetectable around menopause. [20]

In present study, it was observed that serum AMH in control group for BMI< 25 $kg/m^2$ was 5.59±2.55 ng/ml, for BMI between 26-29 $Kg/m^2$ was 4.34±1.61 ng/ml and for BMI > 30 $Kg/m^2$ was 1.27±1.71 ng/ml. Similarly, serum AMH in study for BMI< 25 $Kg/m^2$ was 0.808±0.257 ng/ml, for BMI between 26-29 $Kg/m^2$ was 0.521±0.78 and for BMI above 30 $Kg/m^2$ was 0.239±0.46. These findings were similar to Steiner et al 2010, where a negative correlation was established between BMI and serum AMH levels.[21] Lieman et al 2011 also

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studied that among women with diminished ovarian reserved mean serum AMH level was 33% lower in overweight and obese women compared with normal weight women. This is due to diminished ovarian reserve which causes low serum AMH level as BMI increases.[22] Present study also revealed that serum AMH levels were low among smokers as compared to non-smokers in both study group and control group. C. De Chanut et al 2011 also established negative relationship between smoking and serum AMH level.[23] Fuentes A et al 2012 and Plante B J 2010 reported that smoking was associated with lower AMH levels.[24] LaMarca et al 2013 reported the reason that smoking directly accelerate ovarian follicular depletion, thereby reducing serum AMH level and reducing age of menopause.[25]

## 4. Conclusion

This study concludes that serum AMH levels are significantly low among women with infertility as compared to normal women, which means that serum AMH is reliable diagnostic marker of ovarian reserve and thus of infertility. Furthermore, it becomes evident that as age increases, serum AMH levels decrease and likewise levels are also decrease as weight increases. The study clearly states that smokers have low serum AMH levels as compared to nonsmokers.

**Limitations of study**

Small sample size due to time limited nature of the study

**Conflict of Interest:** None

**Acknowledgement:** None

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