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# STUDY OF ANTIMULLERIAN HORMONE (AMH) LEVEL VARIATION IN FEMALES IN ASSISTED REPRODUCTIVE TECHNOLOGY (ART) CENTRE

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**Abstract**

Study of AMH levels of patients of ART Centre and its variations has clinical relevance in the field of assisted reproductive technology. Diminished ovarian reserve has become a major cause of infertility. Anti-Mullerian hormone (AMH) seems to be a promising candidate to assess ovarian reserve and predict the response to controlled ovarian hyperstimulation (COH). The study is a prospective, observational study carried in Department of Anatomy in collaboration with Assisted Reproductive Technology centre at a tertiary care teaching hospital. The study is carried out on eighty females who had reported to ART centre for treatment for the first time, because of involuntary childlessness with at least 1 year of unprotected intercourse without pregnancy. In our study, eighty females were divided into four groups depending upon their AMH levels. The number of oocytes collected from each female on the day of oocyte pickup was documented. Comparison with previous studies was done. Knowledge of AMH levels will help gynaecologist to counsel the infertility patient prior to initiation of the IVF cycle. It not only allows the quantification of ovarian reserve but also predict the eventual ovarian response to ovarian stimulation and the clinical pregnancy.

**Keywords:** AntiMullerian Hormone, Assisted Reproductive Technology, Ovarian Reserve

## Introduction

Anti-Mullerian hormone (AMH), also known as Mullerian-inhibiting hormone (MIH), is a glycoprotein hormone structurally related to inhibin and activin from the transforming growth factor beta superfamily.AMH is a dimeric glycoprotein with a molar mass of 140 kDa. The molecule consists of two identical subunits linked by sulfide bridges and characterized by the N-terminal dimer (pro-region) and C-terminal dimer.[1] AMH binds to its Type 2 receptor AMHR2, which phosphorylizes a type I receptor under the TGF beta signaling pathway.[2]

In humans, the gene for AMH is located on chromosome 19p13.3.[3] The gene AMHR2, which codes for the receptor for AMH, is present on chromosome 12.[4] The key role of this hormone is growth differentiation and folliculogenesis.

AMH is activated by SOX9 in the Sertoli cells of the male fetus.[4] Its expression inhibits the development of the female reproductive tract which develops from Mullerian ducts also known as paramesonephric ducts. So in the male embryo, it arrests the development of fallopian tubes, uterus, and upper vagina.[2][3][4] The effect is ipsilateral, that is each testis suppresses Mullerian development only on its own side.[15] In humans, this action takes place during the first 8 weeks of gestation. If no hormone is produced from the gonads, the Mullerian ducts automatically develop, while the Wolffian( mesonephric) ducts, which are responsible for male reproductive parts, automatically die.[16]AMH expression is critical to sex differentiation at a specific time during fetal development and appears to be tightly regulated by nuclear receptor SF-1, transcription GATA factors, sex-reversal gene DAX1, and follicle-stimulating hormone (FSH).[5][6]

Amounts of AMH that are measurable in the blood vary by age and sex. AMH works by interacting with specific receptors on the surfaces of the cells of target tissues (anti-Mullerian hormone receptors). The best-known and most specific effect mediated through the AMH type II receptors, includes programmed cell death of the target tissue (the fetal Mullerian ducts).

After birth, AMH is also a product of granulosa cells of the preantral and small antral follicles in women. AMH is only present in the ovary until menopause.[9] AMH is expressed by granulosa cells of the ovary during the reproductive

years, and limits the formation of primary follicles by inhibiting excessive follicular recruitment by FSH.[11][17] AMH expression is greatest in the recruitment stage of folliculogenesis, in the preantral and small antral follicles. This expression diminishes as follicles develop and enter selection stage, upon which FSH expression increases.[18]Production of AMH regulates folliculogenesis by inhibiting recruitment of follicles from the resting pool in order to select for the dominant follicle, after which the production of AMH diminishes.[9][10]

Since granulosa cells envelop each oocyte and provide them energy. So AMH can also serve as a molecular biomarker for relative size of the ovarian reserve.[15] In addition, basal serum AMH concentrations predict ovarian response during IVF cycles. Concomitantly, oocyte quantity and embryo quality decrease with advancing age. Hence, AMH in serum constitutes a marker for embryo quality. Diminished ovarian reserve has become a major cause of infertility. AMH hormone seems to be a promising candidate to assess ovarian reserve.

Normal AMH levels in females are as per table I.

**Table I:** showing normal levels of AMH in females

| Age | Value(ng/ml) | Value(pmol/l) |
|---|---|---|
| Younger than 24 months | <5 | <35 |
| 24 months to 12 years | <10 | <70 |
| 13-45 years | 1-10 | 7-70 |
| More than 45 years | <1 | <7 |

**Aim:**

To study AntiMullerian Hormone (AMH) level variation in infertile females seeking IVF treatments.

**Objectives:**

1. To study the AMH levels in the infertile females seeking IVF treatment.
2. To study the comparison with previously done studies.
3. To study the number of oocytes collected in these females.

**Materials and Methods**:

The study is a prospective, observational study conducted in Department of Anatomy in collaboration with Assisted Reproductive Technology centre at a tertiary care teaching hospital in Western Maharashtra. A pre-structured study proforma was used to collect the data. The study was carried out on eighty females who had reported to ART centre for treatment for the first time, because of involuntary childlessness with at least 1 year of unprotected intercourse without pregnancy. On Day 3–5 of the women's normal cycle, serum AMH was measured. The females were divided into four groups depending upon their basal AMH levels. The distribution of frequency and percentage in each group as tabulated in table II:

**Table II:** Different groups with the number of females in each group.

| S/no | AMH level | No of females | Percentage of females |
|---|---|---|---|
| Group I | <1 | 20 | 25% |
| Group II | 1-2 | 17 | 21.25% |
| Group III | 2-4 | 20 | 25% |
| Group IV | >4 | 23 | 28.75% |
| Total | | 80 | 100% |

Regime for ovulation induction was given. Transvaginal ovum pickup was done after 36 hrs of Injection HCG. Oocyte pickup was done as aday care surgery .Oocyte retrieval was done transvaginally under short general anaesthesia in the ART centre. Ovaries were identified with transvaginal ultrasonography. Follicular fluid was collected with the precautions to prevent injury to the blood vessels. Follicular fluid was transferred to the sterile preplaced petridishes as seen in fig 1. Petridishes were examined under microscope for the number of oocytes. Oocytes were identified along with cumulus surrounding them. In the follicular fluid alongside the oocyte – cumulus complex, the haemorrhagic debris was also present giving the red colour to the fluid. Oocytes were separated from the debris with the help of sterile insulin syringe under microscope and separated oocytes- cumulus complexes were suctioned with small pipette and placed in another petridish. Separated oocytes were transferred twice so that no debris left. Number of oocytes collected was noted down for each female

**Figure 1:** showing the follicular fluid filled petridishes for microscopic examination

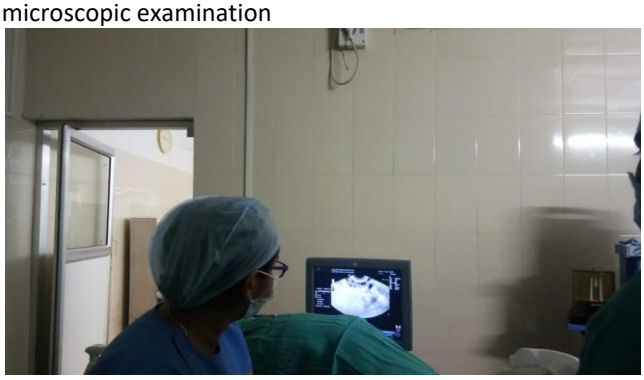


**Figure 2:** showing the oocyte retrieval under the ultrasonographic guidance

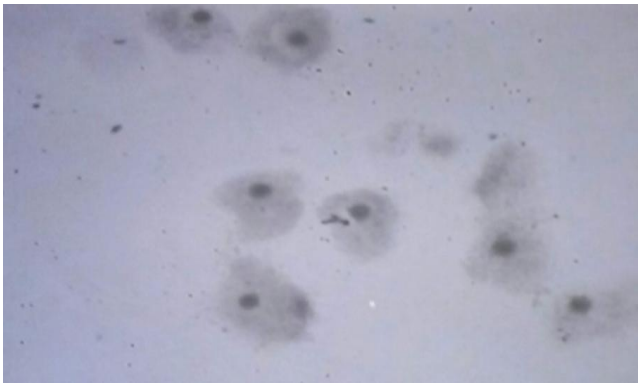


**Figure 3**: showing the oocytes with cumulus surrounding them

**Results:**

In our study, eighty females were divided into four groups (group I=20, group II = 17, group III=20, group IV=23) with different range of AMH as tabulated in table II. In each group, the maximum and minimum value is calculated. Mean for each group is calculated as tabulated in table III:

**Table III:** Different groups with mean, maximum and minimum AMH levels

| Group | AMH range( ng/ml) | Mean AMH | Max value | Min value |
|---|---|---|---|---|
| Group I | <1 | 0.57±0.31 | 0.99 | 0.02 |
| Group II | 1-2 | 1.33±0.25 | 1.84 | 1.03 |
| Group III | 2-4 | 3.11±0.58 | 3.84 | 2.01 |
| Group IV | >4 | 11.4 | 79 | 4.14 |

The number of oocytes collected on the day of oocyte pickup in each female of different groups is tabulated in table IV and maximum and minimum oocytes were noted down. In our study, maximum oocytes were 53, which were collected from female of group IV. Also no oocytes were retrieved in females of group I and group III( one female each).

**Table IV:** Different groups with maximum and minimum oocytes collected

| Group | AMH (ng/ml) | Maximum oocyte collected | Minimum oocyte collected |
|---|---|---|---|
| I | <1 | 09 | 00 |
| II | 1-2 | 20 | 01 |
| III | 2-4 | 24 | 00 |
| IV | >4 | 53 | 03 |

**Discussion:**

In the ART centre for in vitro fertilization treatment, measurement of AMH levels in females is important because it is not cycle dependent as seen in graph 1. Its independence of menstrual cycle stage and other influences like drugs and hormones makes it a good candidate for assessment of ovarian reserve status in the females. In our study with eighty females, AMH ranges from (0.02-79)ng/ml. AMH level is also related to number of oocytes collected after ovarian stimulation. Thus, oocyte grading and maturity is indirectly related to AMH levels which ultimately a factor for successful in vitro fertilization. Embryo quality and grading can also be predicted depending upon the maturity of oocytes retrieved.

The treating physician can use AMH as a guide to counsel the female about the chances of success of future in vitro fertilization. Also, the individualization of further treatment for in vitro fertilization may be selected for each female keeping in view the level of AMH.

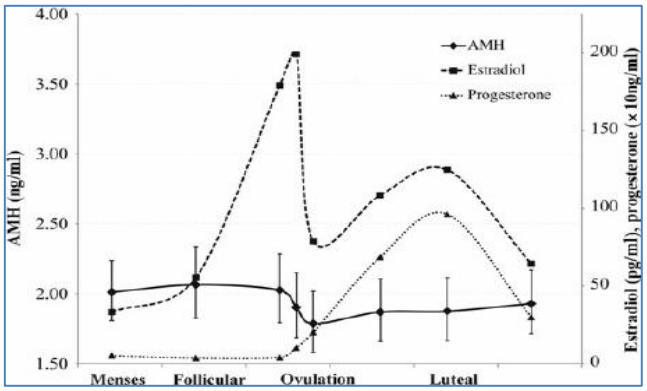
Comparison with the previous studies was done as tabulated in table V. Previous studies La marca et al and Jialyu et al with study population of 100 and 378 respectively also divided sample size into four groups. Range of AMH value for different groups in these studies is different as compared to our study. The percentage of study population under each group in previous studies as well as in present study is comparable as seen in table V.

Table V: showing comparison with previously done studies

| Groups | La Marca et al n=100 | | Jialyu et al n=378 | | Present study n=80 | |
|---|---|---|---|---|---|---|
| | n | AMH range | n | AMH range | N | AMH range |
| I | 25% | 00-0.4 (0.066±0.16) | 25.1% | < 1.43 | 25% | <1 (0.57±0.31) |
| II | 25% | 0.5-2.5 (0.9±0.79) | 25.1% | 1.44-2.55 (2.01) | 21.25% | 1-2 (1.33±0.25) |
| III | 25% | 2.6-6.9 (4.59±1.64) | 24.8% | 2.56-4.35 (3.28) | 25% | 2-4 (3.11±0.58) |
| IV | 25% | 7-11 (8.98±1.13) | 24.8% | >4.35 (6.31) | 28.75% | >4 (11.4) |

**Conclusion:**

The knowledge of AMH value is important for treating gynaecologist to predict the success rate of IVF and accordingly counsel the infertile patient prior to initiation of the IVF cycle. AMH level also allows the quantification of ovarian reserve and predict the eventual ovarian response to ovarian stimulation. Ultimately the clinical pregnancy can be predicted.




**Graph 1:** The AMH level during the menstrual cycle

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