

The Anti Mullerian Hormone- A Novel Marker for Assessing the Ovarian Reserve in Women with Regular Menstrual Cycles

V.S KALAISELVI, SAIKUMAR P, PRABHU K, PRASHANTH KRISHNA G

ABSTRACT

Background: Ovarian Reserve (OR) is a term which describes the functional potential of the ovary, which constitutes the size of the ovarian follicle pool and reflects the number and quality of the oocytes which are within it. Assessment of the OR helps in reflecting the reproductive potential of women. Various markers are available for assessing the OR and the best marker is the Anti Mullerian Hormone (AMH) which reflects the ovarian follicular pool in the ovary. In this study, the serum level of AMH/MIS(Mullerian Inhibiting Substance) was estimated to assess the ovarian reserve in both fertile and infertile women.

Objective: To assess the ovarian reserve in women of the fertile and subfertile groups with regular cycles, who were in the age range of 26 -33yrs, by estimating the level of AMH and those of other hormones like FSH and E2 and also to calculate the ovarian volume and the Antral follicular count by an ultrasonographic method.

Materials and Methods: Thirty fertile and thirty sub fertile women whose ages ranged from 26-33yrs were included as group 1 and group 2 respectively. The hormones like AMH ,FSH

and oestradiol were assayed. Measurement of the ovarian volume and the antral follicular count by doing a transvaginal ultrasonogram, was done in all the subjects who were involved in both the groups. The correlation test was studied between the variables and the test of significance of the variables between the 2 groups was also analyzed by the Statistical Package Of Social Sciences (SPSS).

Results: The Antral Follicular Count (AFC) and the ovarian volume were negatively correlated with the age. The ovarian volume was positively correlated with the AFC. The FSH negatively correlated with the AFC. The Anti Mullerian Hormone negatively correlated with the age, and it positively correlated with the AFC. The mean values of AFC, FSH, and AMH were also statistically significant between the two groups.

Conclusion: AMH can be considered as a marker for assessing the ovarian reserve, as it is cycle independent as compared to the other hormones. The women in the subfertile group with low levels of AMH should be insisted to proceed for ART as early as possible.

Key Words: Ovarian reserve,AMH,AFC

INTRODUCTION

Infertility (subfertility) is a social problem, even in a country like India, with a staggering population of about 1 billion, affecting 6.9-9.3% of the women. Ovarian Reserve (OR) is a term which is used to describe the functional potential of the ovary and it reflects the number and the quality of the oocytes which are within the ovary [1]. It constitutes the size of the ovarian follicular pool and the quality of the oocytes therein and it declines with an increase in age, resulting in a decrease in the female reproductive function. On an average, a female foetus contains about 7 million oocytes at birth, [1-2] million at puberty and about 40,000 oocytes at the onset of the menstrual cycle. A fixed proportion of the remaining oocytes become recruited i.e sensitized to the gonadotrophins, from which one or two will achieve dominance and will progress to ovulation.

The ovarian reserve declines with an increase in age of the women [2]. Various markers are available to assess the OR, which include age, the serum FSH, serum oestradiol and the serum anti mullerian hormone levels, the ovarian volume, the antral follicular

count, etc. The cycle fluctuation of the day 3 FSH level makes the OR estimation difficult and a single day 3 FSH measurement may not be very accurate [3]. However, E2 is never used alone as a marker for the OR. The ovarian volume and the antral follicular count are the predictors of the number of oocytes which is retrieved in the controlled ovarian hyperstimulation protocols and the cancellation rates in IVF [4,5] rather than assessing OR. So in the recent past, AMH has been considered as the best marker for assessing the ovarian reserve.

The anti mullerian hormone / mullerian inhibiting substance (AMH/MIS) is a glycoprotein dimer which consists of 72 KD monomers which are linked by disulphide bonds. It belongs to the member of the Transforming Growth Factor (TGF) superfamily.

During the foetal development, the embryo consists of mullerian and wolffian ducts. The mullerian ducts are the precursors of the uterus, the fallopian tubes and the upper part of the vagina and the wolffian ducts give rise to the epididymis and the seminal vesicles. In males, during the embryonic development, AMH is secreted by the Sertoli cells of the testis and it is responsible for

the regression of the mullerian ducts. In the female embryogenesis, the absence of AMH /MIS allows the development of the female sex organs. After birth, AMH is produced in small amounts by the ovarian granulosa cells and it becomes undetectable after menopause. Recently, it is been shown that AMH inhibits the recruitment of the primordial follicles and that it decreases the responsiveness of the growing follicles to the FSH [6].

In contrast to most of the hormonal biomarkers with a follicular status, AMH is exclusively produced by the granulosa cells, with the follicles ranging from the primary to the early antral stages. So, it has been shown that the AMH concentration in the serum is directly related to the antral follicle count and that it is a better indicator of the ovarian reserve than the 3rd day FSH, Inhibin B or the oestradiol levels.

The concentration of AMH is higher in the small antral follicles than in the preovulatory follicles, suggesting that the circulating AMH levels reflect both the quantity and the quality of the remaining follicles [7]. Since, there is no change in the AMH levels in response to the gonadotrophins, it can be measured throughout the cycle in contrast to the other hormones- the AMH intracycle and the cycle to cycle variation is also negligible. All these features support the feasibility of the AMH assessment throughout the cycle [8]. With this background, this work was focused on assessing the AMH levels in fertile and subfertile women with regular menstrual cycles.

MATERIALS AND METHODS

The subjects in this study included regular menstruating women with a cycle length of 28-35 days. Thirty fertile women (Group 1) with their ages ranging from 26-31 yrs and thirty subfertile women (Group 2) with their ages ranging from 29-33yrs were included in this study. After obtaining the institutional ethical committee clear-

ance and an informed consent from all the participants who were involved in this study, 3 ml of intravenous blood was taken from each participant. The serum samples were kept at -20°C and hormones like FSH, E2 on day 3 and AMH were assayed. The ovarian volume and the antral follicular count were measured by doing a trans vaginal ultrasonogram.

The FSH levels were analyzed by using MONOBIND Inc, E2 by Biosource, Belgium. AMH was estimated by an enzymatically amplified, two site immunoassay (Diagnostic system Lab Beckman coulter USA) with a sensitivity of 1pM or 0.14ng/ml. A correlation test and a test of significance were performed between the variables by using SPSS (Social Package for Statistical Sciences).

Exclusion criteria: Subjects with thyroid disorders and a history of an ovarian surgery, Diabetes mellitus, PCOS and male partner infertility were excluded from this study.

RESULTS

Elevated FSH levels, low AMH levels and low ovarian volumes and AFCs were seen in the subfertile women than in the fertile women. The antral follicular count negatively correlated with age ($r=-0.557$, $p=0.001$). The ovarian volume positively correlated with the AFC ($r=0.708$, $p=0.000$) and it negatively correlated with age ($r=-0.278$, $p=0.075$). The FSH negatively correlated with the AFC ($r=-0.182$, $p=0.061$).

The anti mullerian hormone negatively correlated with age ($r=-0.263$, $p=0.005$) and it positively correlated with the AFC ($r=0.303$, $p=0.002$), as has been shown in [Table/Fig-1].

The mean values of AFC and AMH were low in the subfertile group and a higher value of FSH was observed in group 2. Their mean values showed statistical significance between the two groups, as has been shown in [Table/Fig-2].

	AGE	BMI	OV/TVS	AFC	FSH	E2	AMH
AGE	1	-0.002 0.990	-.278 0.075	-0.557** 0.001	0.106 0.276	0.109 0.264	-0.267 0.005
BMI	0.002 0.990	1	0.194 0.218	0.034 0.858	0.154 0.113	0.072 0.464	0.136 0.163
OV/TVS	-.278 0.075	0.194 0.218	1	0.708** 0.000	0.182 0.075	0.067 0.405	0.278 0.075
AFC	-0.557** 0.001	0.038 0.858	0.708** 0.000	1	-0.182 0.061	-0.216 0.091	0.303 0.002
FSH	0.106 0.276	0.154 0.113	0.182 0.075	-0.182 0.061	1	0.078 0.467	0.220 0.023
E2	0.109 0.264	0.072 0.464	0.067 0.405	-0.216 0.091	0.078 0.467	1	0.036 0.716
AMH	-0.267 0.005*	0.136 0.163	0.278 0.075	0.303 0.002*	0.220 0.023	0.036 0.716	1

[Table/Fig-1]: Bivariate Correlation Test Between the Variables

	Fertile-1	Sub Fertile-2	Student t-Test
AGE (YRS)	29.1± 2.56	31.75± 2.16	0.001
BMI (kg/m2)	24.5± 2.07	23.05± 1.96	NS
OV (Cm3)	11.8± 3.5	9.40 ±2.96	NS
AFC	9.1± 4.56	7.1± 3.5	0.001
FSH mlu / mL	7.0 ±2.6	9.5± 3.1	0.025
E2 Pg/mL	139± 56	156 ± 67	NS
AMH ng/mL	3.697 ±1.6	2.911 ±0.98	0.001

[Table/Fig-2]: Test of Significance Between Group 1 and Group 2

DISCUSSION

Elevated FSH levels, low AMH levels, and low ovarian volumes and AFCs were seen in the subfertile women than in the fertile women. A significant number of women with normal FSH values had reduced egg supplies. The lower egg supply was not reflected in their FSH values [9]. The level of FSH has been proven to increase with the age of the follicles and its fluctuation in the day 3 FSH levels makes the ovarian reserve estimation difficult. Its higher levels represent a declining OR and an increased day 3 FSH level is considered as a late indicator of the marked de-

creased fertility potential [3]. The elevated basal oestradiol levels poorly predict the OR, even when the basal level is normal. So, the third day oestradiol level in the prediction of the ovarian reserve is still debatable [10]. As with FSH, the oestradiol testing is also available and however, it is never used alone as a biomarker for assessing the OR. Even in women with regular periods, mobilization of the patients for the 3rd day sampling is difficult and that is why the AFC and the AMH values are considered to be more significant [9].

The antral follicular count, even though it is an indirect measurement of the ovarian reserve, is a useful tool and it is rather superior to the basal oestradiol level in the prediction of a poor ovarian response. The AFC visualization with a transvaginal ultrasonogram is practically difficult, as it requires a 3D ultrasonogram which may not be available at many centres.

In this study, the number of antral follicles which were 2-10mm in diameter, as was measured by vaginal sonography during the early follicular phase, appeared to correlate well with age in a selected group of women who had regular cycles. The number of antral follicles also correlated with the presumed basal markers for the reproductive age, which included the FSH and the ovarian volume.

With regards to the ovarian volume, a low value correlates with the number of growing follicles but not with the number of oocytes which are retrieved [11]. The human ovary changes its size, shape and activity throughout its life. The largest published data on the ovarian volume, which was related to age [12], showed a statistically significant decrease in the ovarian volume with each decade of life, from 30 to 70 years. In a study on both fertile and infertile women who were aged 35-45 years, a negative correlation between the mean ovarian volume and age was seen in both the groups [13].

But interestingly, the basal FSH and the antral follicle count values did not differ between the infertile and the fertile women. But in this study, the mean basal FSH and the mean AFC values were different between the fertile and the infertile groups.

The most appropriate serum marker is the one that reflects the number of follicles that have made their transition from the primordial pool into the growing follicle pool, during the gonadotrophin – independent phase, prior to the follicular recruitment. At present, AMH seems to fulfill this requirement completely and it has the advantage of cycle independence.

Our study showed that as the age advanced, the AMH level decreased. This finding was found to correlate with the findings of many studies [14,15] and also with the basal FSH values. In subfertile women, the level of AMH is significantly lower as compared to that in fertile women. A slight positive correlation of AMH with the AFC and the ovarian volume was also observed and a significant reduction of AMH was observed in the subfertile group.

The average level of AMH for a woman who is aged 35 years is 2.0ng/ml. A low AMH level which is less than 1.0ng/ml indicates a decreased ovarian reserve, particularly when it is associated with a low AFC of less than [8-10,16]. AMH, which is produced by the granulosa cells of the recruited follicles, has been identified as a regulator of the recruitment, it prevents the depletion of all the primordial follicle pools at once and it has been suggested as a better predictor of the ovarian response [16].

AMH is the only marker of the ovarian reserve that can be tested in both the follicular as well as the luteal phases, As it is cycle independent [17]. Moreover, its sampling is easy as compared to the ovarian volume and the AFC, which require technical experts to assess them and also the availability of a 3D ultrasonogram. AMH may be used as a marker to assess the ovarian reserve in future, although much work is needed to be done on it before it can be routinely used.

CONCLUSION

Elevated FSH levels were seen in subfertile women than in the fertile women. The AMH level was significantly lower in the subfertile women than in the fertile women. AMH can be considered as a marker for assessing the ovarian reserve in women with normal menstrual cycles, as it is cycle independent as compared to the other hormones like FSH. The women with low levels of AMH in the subfertile group should be insisted to proceed for ART as early as possible. The relationship between the AMH levels and the ovarian response during the ovarian stimulation for IVF requires further extended studies.

REFERENCES

- [1] Te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum Reprod. Update.* 2002; 8: 141 – 54.
- [2] De Vet A, Laven JS, De Jong FH, Themmen AP, Fauser BC. Anti-Mullerian hormone serum levels: A putative marker for ovarian ageing. *Fertil Steril.* 2002; 77: 357 – 62.
- [3] Perloe M., Levy DP, Sills ES Strategies for ascertaining ovarian reserve among women suspected of subfertility. *Int J Fertil Womens Med.* 2000; 45, 215-24.
- [4] Tomas C, Nuojua-Huttunen S, Martikainen H. Pretreatment transvaginal ultrasound examination predict ovarian responsiveness to gonadotrophins in in-vitro fertilization. *Hum. Reprod.* 1997;12:220-23. [PubMed].
- [5] Frattarelli JL, Lauria-Costab DF, Miller BT, Bergh PA, Scott RT. Basal antral follicle number and mean ovarian diameter predict cycle cancellation and ovarian responsiveness in assisted reproductive technology cycles. *Fertile. Steril.* 2000;74:512–17. [PubMed]
- [6] Visser JA, De Jong FH, Laven JS, Themmen AP. Anti Mullerian Hormone: A new marker of ovarian function. *Reproduction.* 2006;131: 1-9.
- [7] Feyereisen E, Mendez Lozano DH, Taieb J, Hesters L, Frydman R, Fanchin R. Anti-Mullerian hormone: clinical insights into a promising marker of ovarian follicular status. *Reprod Biomed Online.* 2006; 12: 695 – 703.
- [8] Cook CL, Siow Y, Taylor S, Fallet ME. Serum Mullerian Inhibitory substance levels during normal menstrual cycles. *Fertil Steril.* 2002;73: 859-61 .
- [9] Abha Maheswari, Paul Fowler, Siladitya Battacharya. Assessment of ovarian reserve-should we perform routinely? *Human Reprod.* 2006; 21: P 2729-35.
- [10] Bukman A, Heineman MJ. Ovarian reserve testing and the use of prognostic models in patients with subfertility. *Hum Reprod Update.* 2001; 7: 581 – 90.
- [11] Tomas C, Nuojua-Huttunen S, Martikainen H. Pre treatment transvaginal ultrasound examination predicts ovarian responsiveness to gonadotrophins in vitro fertilization. *Hum Reprod.*1997;12:220-23 .
- [12] Pavlik F, De Priest PD, Gallion Hh, Ueland FR, Reedy MB, Kruscio RJ et al. Ovarian volume related to age. *Gynecol Oncol.*2000; 77: 410-12.
- [13] ErdemM, ErdemA, Biberoglu K, Arslan M. Age related changes in ovarian volume, antral follicle counts and basal follicle stimulating hormone levels comparison between fertile and infertile women. *Gynecol, Endocrinol.* 2003;17:199-205.
- [14] Mulders AG, Laven JS, Eijkemans MJ, de Jong FH, Themmen AP, Fauser BC. Changes in anti-Mullerian hormone serum concentrations over time suggest delayed ovarian ageing in normo gonadotrophic anovulatory infertility. *Hum Reprod.*2004; 19: 2036 – 42.
- [15] VanRooji IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong et al. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod.*2002; 17: 3065 – 71.

- [16] Nelson SM , Yates RW, Lyall H, Jamieson M, Traynor I, Gaudoin M, Mitchell P, Ambrose P, Fleming F. Anti-mullerian hormone based approach to controlled ovarian stimulation for assisted conception. *Human Reprod.* 2009; 24 : 867-75.
- [17] La Marca ,Volpe A. Anti Mullerian Hormone in female reproduction is measurement of circulating AMH a useful tool? *Clin Endocrinol.* 2006 ;64: 603-10.

AUTHOR(S):

1. Dr. V. S Kalaiselvi
2. Dr. Saikumar P
3. Dr. Prabhu K
4. Dr. Prashanth Krishna G

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor Department of Biochemistry, Sree Balaji Medical College and Hospital, Chennai, India.
2. Professor Department of Physiology, Sree Balaji Medical College and Hospital, Chennai, India.
3. Associate professor Department of Anatomy, Sree Balaji Medical College and Hospital, Chennai, India.
4. MBBS Student Sri Ramachandra Medical College, Chennai, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. V. S Kalaiselvi,
Associate Professor Department of Biochemistry,
Sree Balaji Medical College and Hospital,
No 7, Clc Works Road, Chrompet,
Chennai 600044, India.
Phone: 9884218580
E-mail: kalai.selvi51@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS:

None.

Date of Submission: **Sep 20, 2012**
Date of Peer Review: **Oct 15, 2012**
Date of Acceptance: **Oct 27, 2012**
Date of Online Ahead of Print: **Nov 12, 2012**
Date of Publishing: **Dec 15, 2012**