Basrah Journal of Surgery Original Article

ANTIMULLARIAN HORMONE VARIATION THROUGHOUT THE MENSTRUAL CYCLE

DOI: 10.33762/bsurg.2022.174670

Ali Falih Al-Assadi [@], Huda Abbas Jabbar AL.Sharae [*], Wafaa Sami [#], Safa Kifah [^]

[@] [*] Professor of Obstetrics and Gynecology - University of Basrah - College of Medicine - Department of Gynecology - Iraq, [*]F.I.C.M.S, Al-Basrah Hospital for Maternity and Childhood-Iraq, [#] F.I.C.M.S, Al-Basrah Hospital for Maternity and Childhood [^] M.B.Ch.B. Basrah Health Directorate-Iraq

Abstract

This prospective cross-sectional study is to assess the Anti-Mullerian hormone variation throughout the menstrual cycle in the number of women suffering from subfertility and attending the Basrah Fertility Center at Basrah Maternity and Children Hospital.

This study was conducted at Basrah Maternity and Children Hospital (Basrah city, south of Iraq) during the period from February 2019 to July 2020.The participants had not used combined oral contraceptive (COC) or being pregnant or breastfeeding for at least 2 months before the study cycle; midcycle was identified by subtracting 14 days from the mean cycle length. Fifty participants were included in this study; they were assessed using specially designed questionnaire. From each attendance 3 blood samples were taken for Anti-mullerian measurment, the first at the early follicular phase, the second one at the midcycle, and the third at the mid-luteal phase of the menstrual cycle.

Of our fifty participants, 64% were from the age group (20-29) year and 36% were from the age group (30-40) year.4% were underweight,40% were normal weight,32% were overweight and 24% were obese.62% were complaining of primary subfertility and 38% were with secondary subfertility.26% were employed, and 74% were housewives. There were statistically significant differences between AMH1 (early follicular phase) and AMH2 (midcycle) (p-value 0.0001) and between AMH2 and AMH3 (mid-luteal phase) (p-value 0.004), whereas there was no statistically significant difference between AMH1 and AMH3.

In conclusion, there is a significant intracyclic variation in the level of AMH and which tend to be lower at the midcycle phase.

Keywords: Antimullarin ,Infertility, Intracyclic variation.

Introduction

Infertility represents the inability to conceive naturally in one year of regular unprotected intercourse. Mostly, infertility is a sort of subfertility in which 1 in 7 couples need specialist help to get pregnant. Subfertility either primary or secondary. Primary subfertility is a delay for a couple who have had no previous pregnancies; and secondary subfertility is a delay for a couple who have conceived previously, although the pregnancy may not have been successful, for example, miscarriage and ectopic pregnancy [1].

OVARIAN RESERVE: According to the increasing demands of assisted reproduction technologies (ART), due to the modern trends of maternity adjournment, the evaluation of functional ovarian reserve has arisen in an attempt to advise interested couples [2]. Serum and ultrasonographic markers have been examined to reflect the gonadal reserve of infertile women, but none of them reflect the complex follicular dynamics or robustly correlated with the size and quality of primordial follicles remaining in the ovaries per wave of follicular growth. In other words, those tests do not ideally reflect the pool of un recruited follicles, which may be responsible for thecontinuity of ovulatory cycles and, therefore, for the long term reproductive potential [3].

The clinical markers include age [4]: Endocrine markers include Follicle-Stimulating Hormone, early follicular phase (basal) FSH is the most studied and used endocrine test in determining ovarian reserve [5]. Other endocrines markers including serum inhibin B; women with a low day three inhibin B concentration (<45 pg/ml) have an inadequate response to superovulation for IVF. They are less likely to conceive a clinical pregnancy [6].

Other marker included is Basal estradiol (E2) levels which may provide additional useful information for the evaluation of ovarian reserve [7].

Anti-Mullerian hormone (AMH) is a dimeric glycoprotein exclusively produced by granulosa cells of preantral (primary and secondary) and small antral follicles (AFs) in the ovary. The production of AMH starts following the follicular transition from the primordial to the primary stage, and it continues until the follicles reach the antral stages, with diameters of 2-6 mm [8-9]. The number of small AFs is related to the size of the primordial follicle pool. With the decrease in the number of AFs with age, AMH production appears to diminish and become undetectable at and after menopause. [10] AMH levels robustly correlate with basal antral follicle count (AFC) measured by transvaginal ultrasonography [11]. Various threshold values, 0.2–1.26 ng/ml, have been used to identify poor responders with 80–87% sensitivity and 64–93% specificity [12-13]. AMH is known to have an inhibitory effect on the pool of primordial follicles, acting on pregranulosa cells in order to limit the number of recruitable follicular units and, later, as a decisive factor in permitting the FSH-dependent growth of ovarian follicles [14-15]. At this moment, poor response can be associated with AMH serum levels<1 ng/mL, normal response with levels from 1to 4 ng/mL, and high response with levels>4ng/ml [16-20].

A high dispute about whether AMH significantly varies or not throughout the menstrual cycle. Old studies have suggested that serum AMH levels not fluctuate much throughout the menstrual cycle, as would be expected from the evidence that AMH is not secreted by the dominant follicular corpus luteum [21-22].

Two recognizable types of variability should be considered: the inter-individual and the intraindividual variability. The inter-individual variability of AMH refers to variations in AMH levels between different subjects and is first of all secondary to very high variability in the number of growing follicles within groups of women of similar age [23-24].

A very recent study found that serum AMH levels markedly lower in the luteal than follicular phase with a variation pattern similar to pituitary FSH, and the intraindividual variance of AMH was as high as 80% [25].

It may be accomplished that in patients with low ovarian reserve (usually aged women), AMH fluctuations haves little clinical relevance. In contrast, in young patients with usually high ovarian reserve, fluctuations of AMH might indeed impact on the preferential capability of diagnostic and predictive tests, respectively [26].

Patients & Methods

This prospective cross-sectional study was conducted at Basrah Fertility Center / Basrah hospital for Maternity and Children (Basrah city, south of Iraq) between February 2019 to July 2020. Women in a range of age (20–40) year old with subfertility background were all included.

The participants had not used COC or being pregnant or breastfeeding for at least 2 months before the study cycle; midcycle were identified by subtracting 14 days from the mean cycle length. The participants accomplished a specially designed written questionnaire, including:name ,age which divides the participants into two groups (20-29 year and 30-40 year)BMI which divided into four groups (underweight, normal weight, overweight and obese) ,Occupation , subfertility history (primary, secondary), Previous pelvic surgeries, History of PCOS, Smoking .

The exclusion criteria include (family history of premature menopause, childhood mumps infection, history of chronic disease, drug history, and previous pelvic radiation).They were informed about the method, blood sampling processing, and objectives of the study, and a verbal consents were obtained from them.

Statistical analysis: Analysis of the data was done using the statistical packages for social sciences (SPSS) version 23. Data were presented as numbers and percentages for nonparametric variables. Comparisons between variables and AMH hormone variability throughout the menstrual cycle were performed by cross tab using the chi-square test. The bivariate Odds Ratio (OR) and chi-square test were used to examine the association between variables and AMH variation; in all cases P. value <0.05 was considered to be significant.

Results

After analysis of the data available the following results were found:

Table (1) shows the demographic characters of patients under study.64% of them were from the age

group (20-29)year. 36% were from the age group (30-40)year.4% wereunderweight,40% were normal weight,32% were overweight and 24% were obese.62% were complaining of primary subfertility, and 38% were with secondary subfertility.26% were employed, and 74% were housewives.

Table 1: Demographic characters of patient understudy

Character		Number	Percentage
Age	20-29	32	64
	30-40	18	36
BMI	Under	2	4
	Normal	20	40
	Over	12	32
	Obese	16	24
Infertility	Primary	31	62
	Secondary	19	38
Occupation	Employed	13	26
	Housewife	37	74
PCOS	abcsent	32	64
	present	18	36

Table 2: **Level of AMH according to phases of the menstrual cycle**

Cycle phase	Mean	St.deveation	No.
AMH 1 Early follicilar	3.4668	1.46351	50
AMH 2 Midcycle	2.2894	1.06098	50
AMH3 midluteal	3.4938	1.23314	50

P value for high AMH level

P-VALUE	AMH1 VS AMH2	AMH1 VS AMH3	AMH2 VS AMH3
	0.0001	1.000	0.004

*The mean difference is significant at the 0.05 level.

This table shows that there are statistically significant differences between AMH1 and AMH2 (p-value 0.0001) and between AMH2 and AMH3 (p-value 0.004) whereas there is no statistically significant difference between AMH1 and AMH3.

Table 3: The effect of patients' AMH level on intracycle AMH variation

Patients' AMH level	AMH1 Mean /SD	AMH2 Mean/SD	AMH3 Mean/SD
Low AMH	0.634/0.3990989	0.354/0.403026	0.82/0.4658325
Normal AMH	3.44/1.0439317	2.86/1.068330	3.26/1.0560717
High AMH	5.66/0.861519	3.84/0.411401	5.86/0.9029915

P value for high AMH level

P-VALUE	AMH1 VS AMH2	AMH1 VS AMH3	AMH2 VS AMH3
	0.0001	0.258	0.001

p-value for normal AMH level

P-VALUE	AMH1 VS AMH2	AMH1 VS AMH3	AMH2 VS AMH3
	0.0069	0.3912	0.0597

p-value for Low AMH level

P-VALUE	AMH1 VS AMH2	AMH1 VS AMH3	AMH2 VS AMH3
	0.0006	0.0282	0.0001

This table shows the intracycle variation in the mean AMH level in a patient with low, normal, and high AMH. In a patient with low mean AMH value, the highest AMH value was at the mid-luteal phase, and the lowest was at the midcycle. The differences between these 3 values were statistically significant.

In a patient with a normal AMH level, the highest mean AMH value was at the early follicular phase, and the lowest was at the midcycle phase. The difference between AMH1 and AMH2 was statistically significant.

In a patient with a high AMH level, the highest mean AMH value was at the mid-luteal phase, and the lowest level was at the midcycle phase. The differences between AMH1 and AMH2 and between AMH2 and AMH3 was statistically significant.

Discussion

Compare to other ovarian reserve tests like FSH, AMH is thought, to have little variation throughout the menstrual cycle [13, 27, 28] ; that is why it can be used to assess ovarian reserve at any day of the cycle. However, in practice, we observed different AMH levels at different phases of the menstrual cycle during the management of patients presented for subfertility treatment.

Several factors were thought to be the cause of these variations, different machines, different laboratory techniques, sample preparation, and sample storage before analysis is among the factors that could alter the AMH level.In this study, 50 women were enrolled; all were attending the infertility center after spontaneous menstruation with no preceding hormonal therapy for the last 2 months. Samples were collected using a serum tube with gel separation to separate serum from cells, and samples were stored at 4^{o} c as this will provide reliable stability over more than 5 days [29].

These samples were analyzed using the same machine (minivans) and laboratory technique in patches every 5 days; all the above measures were taken to minimize technical effects on AMH measurement.

In this study, it was found that the higher level of AMH was at the early follicular phase(day 2-3 of the cycle). The lowest values were at the midcycle (day 12-16), which is the probable time of ovulation, and the AMH level increase again at the mid-luteal phase(day 19-25 of the cycle). The reduction in the midcycle AMH values was statistically significant compare to the early follicular phase and mid-luteal phase values. This finding is similar to that reported by Hadlow et al. [30-31] who investigated the variability of AMH concentrations in infertile women with an adequate ovarian reserve pattern. In that study, 82 blood samples were collected from a total 12 women to assess AMH variability. In line with our results, the mean AMH levels were significantly reduced in the luteal compared to the follicular phase.Wunder et al. and Cook et al [32-33] documented significant rises in the late follicular and preovulatory phases compared with lower levels after ovulation or in the early luteal phase. Hehenkamp et al. [34] reported a possible periovulatory rise in some younger patients with AMH levels.This pattern has been suggested to reflect the role of AMH in follicular growth i.e. AMH only reflects the growing follicle pole that is responsive to gonadotropins [34].

Regarding the effect of patient AMH level on intracycle variation in AMH level, the variations were noted in a patient with low, normal, and high-AMH with the lowest level at the midcycle phase. The differences were statistically significant in all patients.

In Sowers's study [35], who described 2 menstrual patterns, the aging ovary (low AMH level) pattern had little intracycle AMH variation, in contrast to the younger ovary pattern in which women had higher AMH levels with significant variation during the menstrual cycle.

Conclusion

We concluded that there is a significant intracyclic variation in the level of AMH with the lowest value at the midcycle phase.

Recommendation

As our study involving 50 infertile, we recommended that a larger study involving a larger sample size should be done in the future. The study should involve the comparison of different menstrual cycle phases AMH level to the patient response to stimulation to know which one is more representative to the true ovarian reserve. Also, we recommend including women with subfertility and normal fertility history to be included in a future study.

References

1. Taylor A. Extent of the problem .ABC of subfertility. 2003;327(7412):434-436.
2. Leridon H,"30 years of contraception in France ,"Contraception fertiliteSexualite ,vol.26,no.6,pp.435-438,1998.
3. Gougeon A, "Regulation of ovarian follicular development in primates: facts and hypotheses," Endocrine Reviews, vol.17, no. 2, pp. 121–155, 1996].
4. Faddy M J, Gosden R G, Gougeon A, Richardson S J, and Nelson J F, "Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause," Human Reproduction, vol. 7, no. 10, pp. 1342–1346, 1992.
5. Silberstein T, MacLaughlin D T, Shai I et al., "M¨ullerian inhibiting substance levels at the time of HCG administration in IVF cycles predict both ovarian reserve and embryo morphology," Human Reproduction, vol. 21, no. 1, pp. 159–163, 2006.
6. Seifer D B, Lambert-Messerlian G, Hogan J W, Gardiner A C, Blazar A S, Berk C A, et al. Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. FertilSteril. 1997;67:110–4. [PubMed] [Google Scholar]
7. Speroffand L. , Fritz M A, "Female infertility," in Clinical Gynecologic Endocrinology and Infertility, L.Speroff and M.A. Fritz, Eds., pp. 1015–1022, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 7th edition, 2005.
8. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: The role of anti Mullerian hormone. Reproduction. 2002;124:601–9. [PubMed] [Google Scholar]
9. Weenen C, Laven J S, Von Bergh A R, Cranfield M, Groome N P, Visser J A, et al. anti-Müllerian hormone expression pattern in the human ovary: Potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004;10:77–83. [PubMed] [Google Scholar]
10. de Vet A, Laven J S, de Jong F H, Themmen A P, Fauser B C. anti-Müllerian hormone serum levels: A putative marker for ovarian aging. FertilSteril. 2002;77:357–62. [PubMed] [Google Scholar]
11. Broer SL, Dólleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyper stimulation: A meta-analysis. Hum Reprod Update. 2011;17:46–54. [PubMed] [Google Scholar]
12. Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri DM, Serhal P. Antral follicle count, anti-Mullerian hormone and inhibin B: Predictors of ovarian response in assisted reproductive technology? BJOG. 2005;112:1384–90. [PubMed] [Google Scholar]
13. La Marca A, Giulini S, Tirelli A, Bertucci E, Marsella T, Xella S, et al. anti-Müllerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology. Hum Reprod. 2007;22:766–71. [PubMed] [Google Scholar]
14. Visser J A and Themmen A P N. "Anti-M¨ullerian hormone and folliculogenesis," Molecular and Cellular Endocrinology, vol. 234, no. 1-2, pp. 81–86, 2005.
15. Visser J A, Durlinger A L L , Peters I J J et al. "Increased oocyte degeneration and follicular atresia during the estrouscycle in anti-M¨ullerian hormone null mice," Endocrinology ,vol. 148, no. 5, pp. 2301–2308, 2007.
16. Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri D M, and Serhal P. "Antral follicle count, anti mullerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology ?" An International Journal of Obstetrics and Gynaecology , vol. 112, no. 10,pp. 1384–1390, 2005.
17. Marca La, Giulini S, Tirelli A et al. "Anti-M¨ullerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology," Human Reproduction, vol. 22, no. 3, pp. 766–771, 2007.
18. La Marca A, Sighinolfi G, Radi D et al. "Anti-M¨ullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART)," Human Reproduction Update, vol. 16,no. 2, Article ID dmp036, pp. 113–130, 2009
19. Nardo L G, Gelbaya T A, Wilkinson H et al. "Circulating basal anti-M¨ullerian hormone levels as predictor of ovarian response in women undergoing ovarian stimulation for invitro fertilization," Fertility and Sterility,vol.92,no.5,pp.1586–1593, 2009.
20. Nelson S M, Yates R W, Lyall H etal. "Anti-M¨ullerian hormone-based approach to controlled ovarian stimulation for assisted conception," Human Reproduction,vol.24, no. 4, pp. 867–875, 2009.
21. Hehenkamp W J K, Looman C W N, Themmen A P N, De Jong F H, TeVelde E R, and Broekmans F J M. "Anti-M¨ullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation," Journal of Clinical Endocrinology and Metabolism, vol. 91, no. 10, pp. 4057–4063, 2006.
22. Van Disseldorp J, Lambalk C B, Kwee J et al. "Comparison of inter-and intra-cycle variability of anti-M¨ullerian hormone and antral follicle counts," Human Reproduction,vol.25,no.1,pp.221–227, 2010.

23 La Marca A, Sighinolfi G, Giulini S et al. “Normal serum concentrations of anti-M¨ullerian hormone in women with regular menstrual cycles,” Reproductive BioMedicine Online,vol.21,no.4, pp. 463–469, 2010.

24. Almog B, Shehata F, Suissa S et al. “Age-related normograms of serum antim¨ullerian hormone levels in a population of infertile women: a multicenter study,” Fertility and Sterility,vol.95, no. 7, pp. 2359.e1–2363.e1, 2011.

25. Hadlow N, Longhurst K, McClements A, Natalwala J, Brown S J, and Matson P L, “Variation in antimullerian hormone concentration during the menstrual cycle may change the clinical classification of the ovarian response ,” Fertility and Sterility, vol. 99, no. 6, pp. 1791–1797, 2013

26. Overbeek A, F J Broekmans, HehenkampW J etal. “Intra-cycle fluctuations of anti-Mullerian hormone in normal women with a regular cycle: a re-analysis,” Reproductive BioMedicineOnline,vol.24,no.6,pp.664–669,2012.

27. Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, and Englert Y. “Stable serum levels of anti-M¨ullerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women,” Human Reproduction,vol.22,no.7,pp.1837–1840, 2007.

28. La Marca A, Broekmans F J, Volpe A, Fauser B C, Macklon N S. Anti-m€ullerianhormone (AMH): what do we still need to know? Hum Reprod 2009;24:2264–75.

29. Fleming R, Fairbairn C, Blaney C, Lucas D, Gaudoin M. Stability of AMH mea-surement in blood and avoidance of proteolytic changes. ReprodBiomedOnline. Published online November 21, 2012.

30. Hadlow N, Brown S J, Habib A, Wardrop R, Joseph J, Gillett M, et al. Quantifying the intraindividual variation of anti-müllerian hormone in the ovarian cycle. FertilSteril. (2016) 106:1230–7.doi: 10.1016/j.fertnstert.2016.06.009.

31. Randolph J F, Harlow S D, Helmuth M E, Zheng H, McConnell D S. Updated assays for inhibin B and AMH provide evidence for regular episodic secretion of inhibin B but not AMH in the follicular phase of the normal menstrual cycle. Hum Reprod. (2014) 29:592–600.doi: 10.1093/humrep/det447.

32. Cook CL, Siow Y, Taylor S, Fallat M. Serum Müllerian-inhibiting substance levels during normal menstrual cycles. Fertil-Steril. (2000) 73:859–61.doi: 10.1016/S0015-0282(99)00639-1.

33. Wunder D M, Bersinger N A, Yared M, Kretschmer R, Birkhäuser M H. Statistically significant changes of anti-Müllerian hormone and inhibin levels during the physiologic menstrual cycle in reproductive age women. FertilSteril. (2008) 89:927–33. doi: 10.1016/j.fertnstert.2007.04.054.

34. Gracia C, Shin S, Prewitt M, Chamberlin J, Lofaro L, Jones K, et al. Multicenter clinical evaluation of the Access AMH assay to determine AMH levels in reproductive age women during normal menstrual cycles. J Assist Reprod Genet.(2018) 35:777–83.doi: 10.1007/s10815-018-1141-5.

35. La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod. 2006;21:3103–7. [PubMed] [Google Scholar]