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Serum anti-Mullerian hormon seviyesi ve antral follikül sayısının stimüle edilmemiş menstruel siklustaki değişiminin saptanması

Variability of serum anti-Mullerian hormone level and antral follicle count in an unstimulated menstrual cycle.

MERVE DİZDAR [1] Orcid ID:0000-0002-6288-5815
İBRAHİM KALE [1] Orcid ID:0000-0001-7802-7199
İLGİ ESEN [1] Orcid ID:0000-0003-2770-2611
RAHİME NİDA BAYIK [1] Orcid ID:0000-0003-1805-2178

[1] Department of Obstetrics and Gynecology, Umraniye Training and Research Hospital.

ÖZ

Amaç: Çalışmada düzenli menstruel siklusu olan sağlıklı hastalarda, doğal siklusta serum anti-Mullerian hormon değerlerinin ve antral follikül sayımının, intra-menstruel değişiminin saptanması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya menstruel siklusu düzenli olan, sistemik hastalık ve geçirilmiş over cerrahi öyküsü olmayan, son iki ay içinde hormonal kontraseptif preparat kullanmamış olan 50 sağlıklı kadın dahil edilmiştir. Serum anti-Mullerian hormon seviyeleri ve antral follikül sayısı stimüle edilmemiş aynı siklus içerisinde erken folliküler faz, geç folliküler faz ve luteal fazda olmak üzere üç kez değerlendirilmiştir. Yaş gruplarına göre serum anti-Mullerian hormon seviyelerinin karşılaştırılması amacıyla da hastalar genç (<35 yaş) ve ileri yaş (≥35 yaş) olmak üzere iki gruba ayrılmıştır.

Bulgular: Hastaların yaş ortalaması 30.8, vücut kitle indeksi ortalaması 23.4 kg/m2 ve ortalama siklus uzunluğu 28.5 gün olarak bulunmuştur. Aynı menstruel siklusta erken folliküler faz, geç folliküler faz ve luteal faz olmak üzere üç kez değerlendirilen serum anti-Mullerian hormon seviyesi ortalaması 2.66 ng/dl ve ortalama antral follikül sayısı 17.4 olarak saptanmıştır. En yüksek serum anti-Mullerian hormon seviyesi ve antral follikül sayısı geç folliküler fazda bulunmuştur ve bu fark antral follikül sayısında olmasa da serum anti-Mullerian hormon seviyesi karşılaştırmasında istatistiksel olarak anlamlı bulunmuştur. (p = 0.307, p = 0.044). Serum anti-Mullerian hormon variabilitesi genç grupta, ileri yaş gruba kıyasla daha fazla olarak saptanmış olup istatistiksel olarak anlamlı bulunmuştur. (0.241 vs 0.132, p = 0.011)

Sonuç: Serum anti-Mullerian hormon seviyeleri menstruel siklus içinde dalgalanmalar göstermekte olup, en yüksek serum anti-Mullerian hormon seviyesi geç folliküler fazda saptanmıştır. Antral follikül sayımı menstruel siklus boyunca sabit kalmıştır.

Anahtar Kelimeler: Anti-Mullerian hormon, menstruel siklus, over rezervi, antral follikül sayısı, intra-siklik variabilite

ABSTRACT

Aim: To evaluate the variability of anti-Mullerian hormone (AMH) and antral follicle count (AFC) in an unstimulated menstrual cycle.

Materials and Method: The study was designed on 50 women who had regular menstrual cycles and did not have any systemic disease or previous ovarian surgery. Serum AMH levels and antral follicle counts of all participants were evaluated three times in the same menstrual cycle in the early and late follicular phase and luteal phase. To evaluate the intracyclic AMH fluctuation according to age, participants were divided into two groups; younger (<35 years) and older (≥35 years old).

Results: The mean age of the participants was 30.8, the mean BMI was 23.4 kg/m2, and the mean menstrual cycle duration was 28.5 days. Mean AMH values evaluated at three different times in the menstrual cycle, early follicular, late follicular, and luteal phase, were 2.66 ng/dl and mean AFC 17.4. The highest mean AMH level and AFC were detected in the late follicular phase and this was statistically significant for AMH level but not for AFC (p = 0.044, p = 0.307, respectively). The intracyclic fluctuation of AMH was greater in the younger patient group. The coefficient of variation was 0.241 in the younger patient group, and 0.132 in the older patient group. (p = 0.011)

Conclusion: Serum AMH levels fluctuate throughout the menstrual cycle and the highest serum AMH level was detected in the late follicular phase. AFC remained stable throughout the cycle.

Keywords: Anti-Mullerian hormone, menstrual cycle, ovarian reserve, antral follicle count, intra-cycle variability

Sorumlu Yazar/ Corresponding Author: Merve Dizdar
Adres: Department of Obstetrics and Gynecology, Umraniye Education and Research Hospital. Elmalıkent, Adem Yavuz Cd. 34764 Umraniye/Istanbul.
E-mail: mervedizdar@gmail.com

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INTRODUCTION

Due to the increasing tendency of women to postpone their pregnancy plans for various reasons in the modern world, the evaluation of ovarian reserve has gained great importance in recent years (1). Also, diseases that reduce ovarian reserve such as premature ovarian failure or endometriosis, in individuals without children, lead them to assisted reproductive techniques such as oocyte or embryo freezing (2). In addition, especially in young women diagnosed with malignancy, the possibility of ovarian failure after radiotherapy or chemotherapy brings future fertility concerns and creates extra stress on patients (3). Due to the reasons mentioned above, the interest and need for assisted reproductive treatments (ART) have increased in recent years.

For many years, anti-Mullerian hormone (AMH) and antral follicle count (AFC) have been routinely used to evaluate ovarian reserve. It is known that AMH secretion is independent of gonadotropins and therefore can be evaluated on any day of the menstrual cycle (4). AFC, on the other hand, is usually evaluated in the first days of the cycle before ART (5).

Individualization of the therapies in IVF depends on the prediction of ovarian response to controlled ovarian stimulation. Two sensitive ovarian reserve markers AMH and AFC are being used to foresee the poor and high responders to optimize stimulation protocol and gonadotropin dosage (6). Among ART, protocols in which ovarian stimulation is started randomly at any time of the cycle have been frequently used before chemotherapy or radiotherapy in patients with malignancy who desire fertility (7). In a study that evaluated the ovarian response to hyperstimulation in random start stimulation protocols, no detrimental effect of the presence of corpus luteum or dominant follicle was shown, supporting the applicability of random start protocols (8). In addition, dual stimulation in the same cycle has become a frequently used method as an assisted reproductive treatment method in patients with poor ovarian response in recent years (9). Considering these new ovarian stimulation protocols, we aimed to determine the variability of AMH and AFC in an unstimulated menstrual cycle in this study.I

MATERIAL AND METHODS

The participants included in this prospective study were randomly selected among the patients who applied to the gynecology outpatient clinic of the University of Health Sciences, Umraniye Training and Research Hospital, Istanbul, between July and September 2019, and they all provided written informed consent for participation.

We estimated the required sample size to test a moderate intracycle variability (effect size d: 0.4, two-tailed) between matched pairs with 80% power and 5% type I error rate. A total sample size of 52 patients was needed to show effect sizes 0.4 with adequate power. The effect size was estimated by assuming a mean change equivalent to 40-50% of the standard deviation for each parameter would be clinically significant.

Regular menstrual cycle is defined as a 25 to 35 days cycle and maximum 5 days difference between consecutive cycles. In light of this information, this study was designed on 50 women with regular menstrual cycles without any known history of infertility. Detailed reproductive history was taken. Age, BMI, chronic illness, medications, previous surgeries, and smoking details were recorded. Moreover, dysmenorrhea, dyspareunia, and dyschezia were questioned.

Exclusion criteria were irregular menstrual cycles, BMI >30 kg/m2. and <18 kg/m2., polycystic ovary syndrome, hormonal medicine use in the last 2 months, suspected or known endometriosis patients, previous ovarian surgery, any known chronic diseases, breastfeeding, ovarian mass, and malignancies.

Three evaluations for AMH level and AFC were performed for each woman in the same cycle. The first evaluation was done between days 2-4 and defined as early follicular phase. The second evaluation was done between days 8-10 and recorded as late follicular phase. Finally, the third evaluation was done between days 16-18 as a luteal phase evaluation. The third evaluation was confirmed to be in the luteal phase, with corpus luteum and/or free fluid in the pelvis as an indication of ovulation in the ultrasound examination. AFC was performed by the same gynecologist using a Voluson E6 device with the Sonography-based Automatic Volume Calculation (Sono AVC) technique which provides a 3-dimensional ovarian image and an automated antral follicle count. It saves time for both the patient and the sonographer, standardizes the measurements and allows quality control (10) (Figure 1).

Figure 1. Antral follicle counting - SonoAVC

Following the analysis of 150 serum samples for AMH; 50 participants were divided into two groups based on their ages as follows; younger patient group (<35 years) and older patient group (35 years).

The same kit, using Snibe Co. LTD Maglumi 4000+ AMH immunoassay device evaluated all samples. The kit used was a sandwich chemiluminescence immunoassay wherein one

antibody binds to the AMH mature-region and the other antibody binds to the AMH pro-region. The measuring range was indicated as 0.02-25 ng/ml.

Statistical Analysis

Statistics were performed with the SPSS 25.0 package program. The distribution of the data was found to be normal with the Kolmogorov Smirnov test. Variations through phases were evaluated with Anova. P-value < 0.05 was accepted as statistically significant. Every individual's AMH level coefficient of variation and the mean values were calculated. Spearman correlation coefficient was used to evaluate which of the two age subgroups has the greatest variation. The coefficient of variation (Cv), calculated as standard deviation (SD)/mean, was used as a measure to describe and compare variations between groups.

The study was conducted in accordance with Declaration of Helsinki Ethical Principles and Good Clinical Practices and approved by the ethics committee of Umraniye Training and Research Hospital (B.10.1.TKH.4.34.H.GP.0.01/129) (Date of approval: 26.06.2019)

RESULTS

The age of the women included in the study ranged from 18 to 44 years, with an average of 30.8 years. The mean BMI was 23.4 kg/m2 and the mean menstrual cycle duration was 28.5 days.

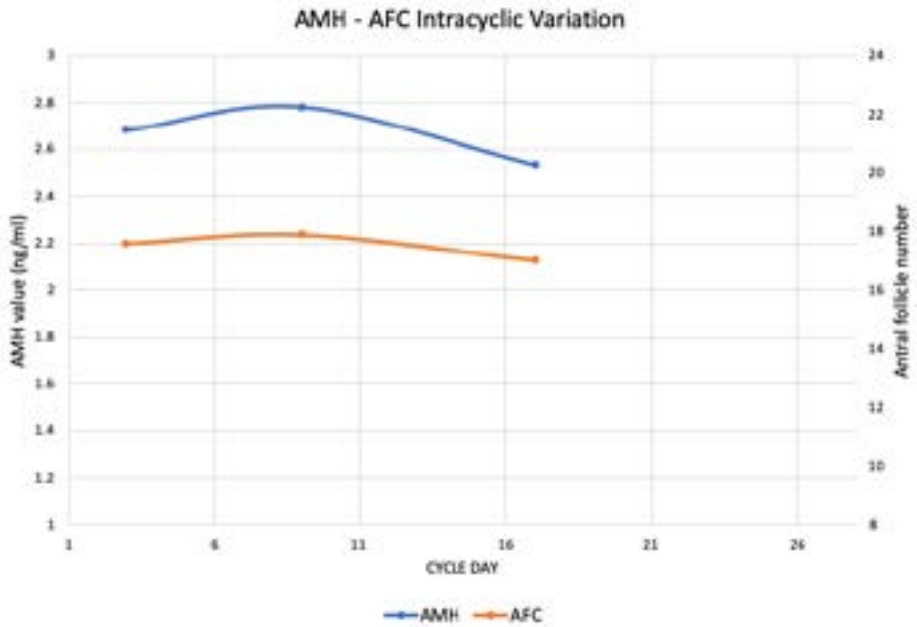
Mean AMH values evaluated at three different times in the menstrual cycle, early follicular, late follicular, and luteal phase, were 2.66 ng/dl and mean AFC 17.4. The mean AMH was 2.68 ng/dl in the early follicular phase, 2.78 ng/dl in the late follicular phase, and 2.53 ng/dl in the luteal phase. The highest mean AMH level was detected in the late follicular phase, which was statistically significant (p = 0.044) (Table 1).

Table 1 Mean AMH levels and AFC detected in early follicular phase, late follicular phase and luteal phase.

	Early Follicular Phase	Late Follicular Phase	Luteal Phase	p-value
AMH (ng/ml)	2.68 ± 1.97	2.78 ±2.02	2.53 ± 1.89	**0.044**
AFC	17.6 ±11.1	17.9 ±12.4	17 ± 11.2	0.307

*Values are shown as mean (± standard deviation)

The mean AFC was 17.6 in the early follicular phase, 17.9 in the late follicular phase, and 17.0 in the luteal phase (Figure 2).



The highest AFC was detected in the late follicular phase however the intra-cyclic difference was not statistically significant (p = 0.307) (Table 1). Variation of AMH levels between menstrual phases was greatest between late follicular phase and luteal phase (p=0.019, 95% CI (0.033, 0.464)) (Table 2).

Table 2 Comparison of mean AMH levels according to menstrual cycle phases

AMH (ng/ml)	Absolute difference	p-value	95% CI
Early Follicular vs Late Follicular Phase	-0.099	0.880	(-0.329, 0.132)
Early Follicular vs Luteal Phase	0.150	0.531	(-0.121, 0.421)
Late Follicular vs Luteal Phase	0.248	**0.019**	(0.033, 0.464)

Evaluation according to age subgroups revealed that the highest mean AMH level was detected in the late follicular phase in both the younger and older groups (Table 3).

Table 3 Mean AMH levels throughout the cycle according to age subgroups

	Age groups			
	18-34 years n=36	**35-45 years** n=14	**Total** n=50	**p-value**
AMH Cv	0.241	0.132	0.162	**0.011**
St. Dev	0.158	0.085	0.119	

*AMH Cv: AMH coefficient of variation. St. Dev: Standard Deviation

The intracyclic fluctuation of AMH was greater in the younger patient group. The coefficient of variation was 0.241 in the younger patient group, and 0.132 in the older patient group (p = 0.011) (Table 4).

Table 4 AMH- Coefficient of variation between age subgroups

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DİSCUSSION

In this study, we found that the AMH values in the late follicular phase of the menstrual cycle were significantly higher than in the early follicular and luteal phases, and the intracyclic variation was greater in the young age group whereas AFC was similar throughout the cycle.

In the literature, there are numerous studies about intracyclic variation of plasma AMH levels in different patient groups. In a study that focused on the AMH level variation in the follicular phase of 24 healthy regularly menstruating women; the blood samples were taken every other day until the LH peak in two consecutive cycles of the patients: one untreated and one FSH treated cycle. In the untreated cycle, AMH levels remained the same during the follicular phase (11). La Marca et al investigated day-to-day fluctuations in AMH levels in a study of 12 healthy, regularly menstruating women; they demonstrated no significant change in intracyclic AMH levels throughout the menstrual cycle (12). In contrast, various studies found a significant increase in AMH levels in the late follicular phase and pre-ovulatory phase compared to post-ovulatory phase and luteal phase. Hadlow et al conducted a study on 12 regularly menstruating, relatively old aged women (mean 36 years) and took 6 blood samples in the same menstrual cycle. They found AMH levels to be statistically significantly higher in the early follicular phase than the luteal phase. In addition, they evaluated AMH results against common cut-offs for definition of reduced ovarian reserve, and they determined that a minimum of 4 women out of 12 crossed the cutoff or not depending on which day the blood sample was taken (13). In a study including 36 regularly menstruating women, blood samples to evaluate serum AMH, FSH, E2, inhibin B, and free testosterone levels were taken every other day and every day periovulatory. Median AMH levels were significantly higher in the late follicular phase compared to the ovulatory and early luteal phase (14). In parallel to these studies we also found that AMH levels were significantly higher in late follicular phase compared to luteal phase.

In a study published in 2017, the intra-cyclic AMH variation was examined in 171 infertile women aged 18-42 years. In this study, in which the participants were divided into three groups as adequate, high and diminished according to their ovarian reserves, serum AMH levels were higher in all three groups during the follicular phase than the luteal phase (15). In 2019, Gorkem et al. searched the AMH fluctuations during the follicular and luteal phases of the menstrual cycle in 257 infertile women. In this study, in which the participants were divided into three groups as hypo-response, normo-response, and hyper-response, serum AMH levels in the follicular phase were found to be higher than those in the luteal phase in all three groups (16). Consistent with these studies, we also found that mean AMH level in the late follicular phase was significantly higher in the early follicular and luteal phases.

Sowers et al. evaluated the AMH fluctuations in the cycle in 20 women aged 30-40 years and stated that AMH has two cycle patterns. The first is “Aging ovary”, which is observed in women with AMH levels below 1 ng/mL, which includes shorter cycle lengths with little intra-cycle AMH changes, while the second, “Younger ovary” pattern included higher levels of AMH with significant changes during the cycle (17). In another study from Hehenkamp et al, periovulatory AMH increase in the young patient group is present but it is not statistically significant (18). Overbeek et al. reported in their study that the amplitude of fluctuations in serum AMH level was greater in young women than in older women (19). In a review published in 2013, La Marca et al evaluated the variability of AMH levels by age, BMI, ethnicity, and smoking. According to this review, ethnicity may affect AMH levels, smoking negatively affects AMH levels, there is a negative relationship between BMI and AMH levels, and younger women had significantly more intra-cycle fluctuations in AMH levels than older women (20). In line with these studies, we also found that the AMH fluctuation in the younger group was greater than the older patient group.

In addition to AMH, there are also studies in the literature on intra-cyclic variability of AFC, another indicator of ovarian reserve. In 2010, van Disseldorp et al. evaluated the inter and intra-cycle stability of AFC and AMH and demonstrated that serum AMH had less individual intra- and inter-cycle variation than AFC. They stated that serum AMH level is a more reliable and robust way of assessing ovarian reserve in subfertile women compared to AFC (21). Depmann et al. evaluated AMH and AFC fluctuations in the cycle of 44 women in a wide age range, including 25-46 years old. In this study, in which the median AMH level was 0.48 and the median AFC was five, they did not detect a statistical significance in the fluctuations of neither AMH nor AFC within the cycle (22). Deb et al. conducted a study on 36 healthy regularly menstruating women and they performed ultrasonographic examinations using SonoAVC on four different days in the same menstrual cycle. They found no significant intracyclic variation in small AFC(6mm) (23). In a study published by Mavrelos et al. in 2016, they investigated the variation of AFC in the early and late follicular phase and its clinical effect in infertile women. In this study, the authors stated that although the AFC in the early follicular phase was statistically significantly higher than the AFC in the late follicular phase, this did not significantly affect the ovarian stimulation protocol design and the prediction of excessive ovarian response in patients (24). In this study, in which AFC was evaluated 3 times during the cycle as early follicular, late follicular, and luteal phases, the highest number of antral follicles was detected in the late follicular phase, nevertheless it was not statistically significant. We think that these different results found in studies on intra-cycle fluctuations of AFC are related to the number of patients included in the study, the mean age of the patients, and ovarian reserves.

The strengths of this study are that the population studied were regularly menstruating women around 30 years old women and the variability of AMH in this population is of clinical interest in the era of women postponing the childbearing age. To these women, it is important to give comprehensive counseling on their fertility aspects (1). Also, it is important, especially in random start protocols and oncofertility patient populations to know whether an intracyclic fluctuation of AMH or AFC exists since the treatment protocol is determined on a cycle-independent measurement of AMH or AFC (25).

In this prospective study, all ultrasonographic evaluations were performed on the same ultrasound device by the same gynecologist to avoid interobserver variability. In addition, AMH values were determined accordingly on the same day of antral follicle count via the SonoAVC technique. However, the limited

number of participants for subgroup analyses and the fact that AFC and AMH evaluations were performed on only three different days representing the entire menstrual cycle are limiting factors.

CONCLUSION

We found that serum AMH levels fluctuate throughout the menstrual cycle and the highest mean AMH levels were detected in the late follicular phase. This fluctuation was much more significant in the younger patient group. AFC remains stable throughout the cycle. Despite statistically significant fluctuations in AMH levels, its clinical significance is still open to debate. We think that randomized controlled studies with larger series are needed to clarify the effect of serum AMH evaluation timing in the menstrual cycle on protocol selection and treatment success in ART.

Declarations

Ethics approval and consent to participate

All participants signed informed written consent before being enrolled in the study. The study was reviewed and approved by the ethics committee of the University of Health Sciences, Umraniye Training and Research Hospital. (Ethics approval reference number: B.10.1.TKH.4.34.H.GP.0.01/12 Date: 26.06.2019) All procedures were performed according to the Declaration of Helsinki Ethical Principles and Good Clinical Practices.

Availability of data and materialsThe data supporting this study is available through the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Concept & Design (MD, RNB) Data acquisition (MD), Analysis (MD, IE), Drafting (MD, IK), Critical revision (MD, RNB, IK, IE) Final approval ((MD, RNB, IK, IE,)

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TARTIŞMA

1. Sauer MV. Reproduction at an advanced maternal age and maternal health. Fertil Steril. 2015 May;103(5):1136-43.

2. Dolmans MM, Donnez J. Fertility preservation in women for medical and social reasons: Oocytes vs ovarian tissue. Best Pract Res Clin Obstet Gynaecol. 2021;70:63–80.

3. Korte E, Schilling R, Balcerek M, Byrne J, DIrksen U, Herrmann G, et al. Fertility-Related Wishes and Concerns of Adolescent Cancer Patients and Their Parents. https://home.liebertpub.com/jayao. 2020;9:55–62.

4. Loh JS, Maheshwari A. Anti-Müllerian hormone—is it a crystal ball for predicting ovarian ageing? Hum Reprod. 2011;26:2925–2932.

5. Fleming R, Seifer DB, Frattarelli JL, Ruman J. Assessing ovarian response: antral follicle count versus anti-Müllerian hormone. Reprod Biomed Online. 2015 Oct;31(4):486-96.

6. Sighinolfi G, Grisendi V, La Marca A. How to personalize ovarian stimulation in clinical practice. J Turk-Ger Gynecol Assoc 2017 Sep 1;18(3):148-153.

7. Cakmak H, Rosen MP. Random-start ovarian stimulation in patients with cancer. Curr Opin Obstet Gynecol. 2015;27:215–221.

8. Galati G, Serra N, Ciaffaglione M, Pinna M, Reschini M, Pisaturo V, et al. Folliculogenesis in random start protocols for oocytes cryopreservation: quantitative and qualitative aspects. Reprod Sci. 2022 Nov;29(11):3260-3265

9. Alsbjerg B, Haahr T, Elbaek HO, Laursen R, Povlsen BB, Humaidan P. Dual stimulation using corifollitropin alfa in 54 Bologna criteria poor ovarian responders – a case series. Reprod Biomed Online. 2019;38:677–682.

10. Ata B, Tulandi T. Ultrasound automated volume calculation in reproduction and in pregnancy. Fertil Steril. 2011;95:2163–2170.

11. La Marca A, Malmusi S, Giulini S, Tamaro LF, Orvieto R, Levratti P, Volpe A. Anti-Müllerian hormone plasma levels in spontaneous menstrual cycle and during treatment with FSH to induce ovulation. Hum Reprod. 2004;19:2738–2741.

12. La Marca A, Stabile G, Carducci Artenisio A, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod. 2006 Dec;21(12):3103-7.

13. Hadlow N, Longhurst K, McClements A, Natalwala J, Brown SJ, Matson PL. Variation in antimüllerian hormone concentration during the menstrual cycle may change the clinical classification of the ovarian response. Fertil Steril. 2013;99:1791–1797.

14. Wunder DM, Bersinger NA, Yared M, Kretschmer R, Birkhäuser MH. Statistically significant changes of antimüllerian hormone and inhibin levels during the physiologic menstrual cycle in reproductive age women. Fertil Steril. 2008 Apr;89(4):927-33

15. Gorkem U, Kucukler FK, Togrul C, Gungor T. Anti-Müllerian hormone exhibits a great variation in infertile women with different ovarian reserve patterns. Aust N Z J Obstet Gynaecol. 2017;57:464–468.

16. Gorkem U, Togrul C. Is There a Need to Alter the Timing of Anti-Müllerian Hormone Measurement during the Menstrual Cycle? Geburtshilfe Frauenheilkd. 2019;79:731–737.

17. Sowers M, McConnell D, Gast K, Zheng H, Nan B,

McCarthy JD, et al. Anti-Müllerian hormone and inhibin B variability during normal menstrual cycles. Fertil Steril. 2010; doi: 10.1016/j.fertnstert.2009.07.1674.

18. Hehenkamp WJK, Looman CWN, Themmen APN, De Jong FH, Te Velde ER, Broekmans FJM. Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab. 2006 Oct;91(10):4057-63

19. Overbeek A, Broekmans FJ, Hehenkamp WJ, Wijdeveld ME, Van Disseldorp J, Van Dulmen-Den Broeder E, et al. Intra-cycle fluctuations of anti-Müllerian hormone in normal women with a regular cycle: a re-analysis. Reprod Biomed Online. 2012;24:664–669.

20. La Marca A, Grisendi V, Griesinger G. How much does AMH really vary in normal women? Int J Endocrinol. 2013;2013:959487.

21. Van Disseldorp J, Lambalk CB, Kwee J, Looman CWN, Eijkemans MJC, Fauser BC, et al. Comparison of inter-and intra-cycle variability of anti-Müllerian hormone and antral follicle counts. Hum Reprod. 2010;25:221–227.

22. Depmann M, van Disseldorp J, Broer SL, Eijkemans MJC, Laven JSE, Visser JA, et al. Fluctuations in anti-Müllerian hormone levels throughout the menstrual cycle parallel fluctuations in the antral follicle count: a cohort study. Acta Obstet Gynecol Scand. 2016;95:820–828.

23. Deb S, Campbell BK, Clewes JS, Pincott Allen C, Raine Fenning NJ. Intracycle variation in number of antral follicles stratified by size and in endocrine markers of ovarian reserve in women with normal ovulatory menstrual cycles. Ultrasound Obstet Gynecol. 2013;41:216–222.

24. Mavrelos D, Al Chami A, Talaulikar V, Burt E, Webber L, Ploubidis G, et al. Variation in antral follicle counts at different times in the menstrual cycle: does it matter? Reprod Biomed Online. 2016;33:174–179.

25. Filippi F, Reschini M, Paffoni A, Martinelli F, Busnelli A, Somigliana E. Fertility

preservation in women with malignancies: The accuracy of AFC collected randomly during the menstrual cycle in predicting the number of mature oocytes retrieved. J Assist Reprod Genet. 2019 Mar;36(3):569-578