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Correlation of ovarian reserve in infertile women of different age groups

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Abstract

In recent years assessment of "ovarian reserve" became a strategy in treatment of female infertility. Accurate measurement of the ovarian reserve has long been a quest in reproductive medicine and recent years have seen a dramatic increase in research in this field. The measurement of anti-Mullerian hormone (AMH) in serum is a much more accurate measure of the ovarian reserve than the other hormones that have previously been available to us. The general purpose of ovarian reserve testing is to assess the quality and quantity of remaining oocytes in an attempt to predict the reproductive potential. The objectives of our study was i).To find out the ovarian reserve by measuring anti mullerian hormone (AMH), mid luteal progesterone levels, lutenizing hormone (LH), follicle stimulating hormone (FSH) and antral follicle count (AFC). ii).To correlate the ovarian reserve indicators with different age groups of infertile women. iii).To identify most reliable markers of ovarian reserve. The study comprises of 101 newly diagnosed PCOS women and they were all divided into three age groups. Group I consists of infertile women who were under the age of 26 years or less than 26 years. Group II consists of infertile women of age range between 27 to 35 years. Group III consists of infertile women who are 36 years and greater than 36 years of age. Patient history, health problem, other details were collected from each participant by using a pre-tested questionnaire and the data was noted. SPSS 20.0 version was used for statistical analysis. All the results were tabulated as mean and standard deviation. Comparisons between groups were performed by ANOVA, further post hoc comparisons were done by Tukey HSD test. Our results indicate serum AMH levels are having positive correlation with AFC, and also this relationship appears to be more significant. In our study serum AMH and the number of antral follicle measurements appears to be better predictors than conventional hormone assessments (like LH, FSH). The present study has revealed the importance of AMH, mid luteal progesterone and AFC as novel markers for ovarian reserve in the diagnosis and treatment of infertile women. In modern practice ovarian reserve assessment has a great value in the management of infertility.

Keywords: Anti Mullerian Hormone, gonadotropin hormones, ovarian reserve, antral follicle count

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Introduction

Our current understanding of female reproductive function presumes that the ovary contains a finite number of oocytes within primordial follicles and that their depletion proceeds and indicates the approach of menopause [1]. This pool of primordial follicles is formed during fetal life from approximately 18 weeks gestation, following oogonial proliferation, entry and arrest in meiosis, and interaction with somatic cells. A small number of this resting pool of primordial follicles is activated into growth every day throughout the reproductive life span of a woman with the vast majority destined to undergo atresia [1, 2]. Before puberty all growing follicles will become atretic, and after puberty only one a month may escape this fate and progress to ovulation. The pool of resting follicles is the true ovarian reserve.

This growing pool is perhaps better termed the functional ovarian reserve. One of the main causes of infertility is diminished ovarian reserve. In other words ovarian reserve is presence of number of good quality pre ovulatory oocytes in the ovaries. As a woman ages, her ovarian reserve decreases. In recent years assessment of "ovarian reserve" became a strategy in treatment of female infertility. Although the true and functional ovarian reserves reflect different stages of follicular development, they are inherently linked and both decline in parallel with increasing age. Accurate measurement of the ovarian reserve has long been a quest in reproductive medicine and recent years have seen a dramatic increase in research in this field, a large part of which has been fuelled by the recognition that measurement of anti-Mullerian hormone (AMH) in serum is a much more accurate measure of the ovarian reserve than the other hormones that have previously been available to us. Inhibin B also said to be a good predictor of oocyte yield after superovulation [3-5], requires measurement in the early follicular phase of the menstrual cycle and only decreases late in the reproductive years [6-8]. It has therefore not supplanted FSH as the most widely used marker of the ovarian reserve despite the latter hormone's well-recognized limitations. Factors such as genetic, lifestyle, medical issues-ovarian surgery, chemotherapy, radiation, environmental factors can influence quantity and quality of a woman's oocytes.

The age also appears to be a good marker for oocyte quality. Young women with diminished ovarian reserve may indicate reduced oocyte number but may have normal oocyte quality, while older women with normal ovarian reserve may have good number of oocytes but an age appropriate decrease in oocyte quality.

The general purpose of ovarian reserve testing is to assess the quality and quantity of remaining oocytes in an attempt to predict the reproductive potential. The availability of a test capable of providing reliable information regarding a woman's individual ovarian reserve within a certain age category would enable the clinician to provide an individually tailored treatment plan. The ideal screening test should be reproducible with low intercycle and intracycle variability. It should also demonstrate high specificity to minimize the risk of false positive determination of decreased ovarian reserve in a woman with normal ovarian reserve [9].

There are different tests to diagnose ovarian reserve. Traditionally, the age, follicle stimulating hormone (FSH), mid leuteal progesterone, estradiol (E2) levels andantral follicle count (AFC) by ultrasound at early follicular phase were used for evaluation of ovarian reserve.

At present the available tests for ovarian reserve include

1. Biochemical markers-serum FSH, serum Estradiol, AMH, Inhibin B and
2. Ovarian ultrasound imaging-AFC, Ovarian volume.

For example, the elevated serum FSH levels on day 3 of the menstrual cycle, low ovarian volume, an antral follicle count <5 per ovary and low inhibin B levels, <5 oocytes retrieved during an assisted reproductive technology (ART) cycle, are considered as a standard for decreased ovarian reserve. A new test for the assessment of decreased ovarian reserve is levels of anti-Mullerian hormone (AMH) [10 -12]. AMH, a member of the transforming growth factor, secreted in the human ovary by granulosa cells of primary growing follicles until the early antral stage. In females AMH regulates the growth of primary follicles by inhibiting further recruitment of other follicles during folliculogenesis. Serum AMH levels decline with increasing age and undetectable after menopause. AMH also decrease even before slight increase in baseline FSH [13-15].

FSH mostly indicates the past two week's follicular maturation. Whereas AMH indicates the pool of preantral follicles to the phase of folliculogenesis. Since age is only a rough estimate of ovarian reserve, many tests, such as FSH, LH, inhibin, AFC and total ovarian volumes, have been develop to predict ovarian reserve more precisely. A combination of tests can provide more accurate information of ovarian reserve. The AFC by ultrasound is promising and facilitates clinical use.

The objectives of our study was

1. To find out the ovarian reserve by measuring anti mullerian hormone (AMH), mid luteal progesterone levels, lutenizing hormone (LH), follicle stimulating hormone (FSH) and antral follicle count (AFC).
2. To correlate the ovarian reserve indicators with different age groups of infertile women.
3. To identify most reliable markers of ovarian reserve.

Materials and Methods

For this study purpose and recruitment of patients, visited places like public health centers (PHC), rural and urban health centers in different parts of the Andhra and Telangana districts. The study was conducted from the heterogeneous population. Women who are having the history of infertility, newly diagnosed infertility cases and those with primary infertility, were invited to Thumbay

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New Life Hospital, Hyderabad for the further evaluation and to confirm diagnosis of polycystic ovary syndrome. Diagnosis confirmation was done by a qualified gynecologist. The patients were addressed as one person at a time or in small groups of not more than 5 individuals at a time to fill the questionnaire. Each question was explained in detail well before hand to avoid ambiguity. The questionnaire was supplied to the rural women in regional language. After the clinical examination individuals were evaluated for infertility, the questionnaire was asked to fill by the individual. The case study form is filled with the information obtained. The questionnaire was given to each individual after taking an informed consent clearly explaining the purpose and the procedure involved in the study.

Study Design

This prospective study was conducted at Thumbay New Life Hospitals, Hyderabad, Telangana, India. The study comprises of 101newly diagnosed PCOS women and they were all divided into three age groups. Group I consists of infertile women who were under the age of 26 years or less than 26 years. Group II consists of infertile women of age range between 27 to 35 years. Group III consists of infertile women who are 36 years and greater than 36 years of age. Patient history, health problem, other details were collected from each participant by using a pre-tested questionnaire and the data was noted.

Ethical Considerations

The study was approved by Hospital-Institutional Ethics Committee. An informed consent was obtained from all the participants in the study.

Anthropometric data

Standard anthropometric data like age, height, weight were measured and noted from each subject. The BMI was calculated as the weight in kilograms divided by the square of height in meters.

Inclusion and Exclusion Criterion

Women with primary infertility with pure polycystic ovary syndrome were recruited for the study. Women with Secondary infertility, pelvic inflammatory diseases, endometriosis, other pelvic pathology and those women who are already on treatment are excluded from the study.

Patients with previous ovarian surgery, Polycystic Ovarian Syndrome (PCOS) and Premature Ovarian Failure (POF) were also excluded.

Diagnosis of PCOS was made on the basis of the Rotterdam criteria (The Rotterdam ESHRE/ASRM 2003 revised consensus, 2004). Two out of three of the following are required for diagnosis: oligo- and/or anovulation (defined by the presence of oligomenorrhea or amenorrhea); clinical and/or biochemical signs of hyperandrogenism [defined by presence of hirsutism (Ferriman–Gallwey score =6), acne or alopecia, and/or elevated androgen levels] and polycystic ovaries by gynecological ultrasound.

On 2-3 days of spontaneous menstrual cycle, all patients had a transvaginal scan by the same investigator using–Phillips HD 7 (Thiwan, 2012year) 2–5MHz multi-frequency ultrasound probe to assess the number of antral follicles, measuring 2-10 mm in size and counted in each ovary. The sum of both counts was the antral follicle count. Levels of LH, FSH and AMH were determined on the same days whereas progesterone levels were measured on the 21 day of the menstrual cycle.

Laboratory Investigation

On day 2 or 3of menstrual cycle, fasting blood samples were collected by vein puncture in plain tubes from the infertile women who were recruited for the study. Blood samples were centrifuged at 3500 rpm for 10 min to separate serum. Serum was isolated and frozen in aliquots at –20°Cuntil further analysis. Again blood sample was collected on day 21-23 of their menstrual cycle for the assessment of mid luteal progesterone. Hormones like anti Mullerian Hormone (AMH), luteinizing hormone (LH), follicle stimulating hormone (FSH) and mid luteal progesterone hormone was measured by chemiluminescence immunoassay (CLIA) method using Beckman Coulter Access-2 fully automated analyzer. The hormone kits used in the Beckman Coulter Access analyzer (USA) were from Beckman Coulter, Ireland. The manufacturer's instructions and procedure was followed for the measurement of all the hormones.

Statistical analysis

SPSS 20.0 version was used for statistical analysis. All the results were tabulated as mean and standard deviation. Comparisons between groups were performed by ANOVA, further post hoc comparisons were done by Tukey HSD test. All the variables followed normal distribution. The p-value of$<0.05$was considered to be statistically significant. Pearson correlation was done for the comparison of parameters among infertile women of three age groups. The p-value of<0.05 was considered to be statistically significant. ROC (Receiver Operating Characteristic) curve analysis was performed to know the good biomarkers for the assessment of ovarian reserve.

Results

A total of 101infertilewomen were enrolled for the study. All the study subjects were divided into three age groups. Group I includes women of age26 or less than 26 years, group II contains women between the age of 27 to 35 years and group III contains women of age 36 and above 36 years.

1. **To find out the ovarian reserve by measuring anti mullerian hormone (AMH), mid luteal progesterone levels, lutenizing hormone (LH), follicle stimulating hormone (FSH) and antral follicle count (AFC).**

Table 1 shows the mean and standard deviation of BMI, AMH, antral follicle count (AFC), LH, FSH and progesterone levels in infertile women of three different age groups. One way analysis of variance was used for the statistical analysis further post hoc comparisons were done by Tukey HSD test to find out the statistical significance between and within the three different age groups. The mean serum AMH levels were elevated in group I and group II while there is a slight decrease in group III.AFC, FSH levels showed consistent increase with the increase in age of infertile women, whereas mid-luteal progesterone levels showed consistent decrease with the increase in age of infertile women. Figure 1 shows the comparison of AMH, AFC and progesterone levels in infertile women of different age groups. Figure 2, 3, and 4 shows the mean plots of AMH, AFC and progesterone levels in three age groups of infertile women.

2. **To correlate the ovarian reserve indicators with different age groups of infertile women.**

Pearson correlation study revealed that there is a strong negative correlation of progesterone with the age of infertile women ($r = -0.606$ and $p < 0.01$) (Table 2). LH and FSH showed positive correlation with the age of infertile women ($r = 0.295$ and $p < 0.01$; $r = 0.358$ and $p < 0.01$ respectively). AMH levels and antral follicle count showed strong positive correlation ($r = 0.373$ and $p < 0.01$) indicating that they are good markers for ovarian reserve in infertile women (Table 2). A negative correlation was observed between progesterone and FSH levels ($r = -0.251$ and $p < 0.05$).

3. **To identify most reliable markers of ovarian reserve**

From the data present in table 2, it is evident that AMH, AFC and mid-luteal progesterone levels were having very good association. Therefore it can be suggested that AMH, AFC and were having very good association. Therefore it can be suggested that AMH, AFC and progesterone levels are the reliable markers in diagnosis and prognosis of infertility assessment progesterone in different age groups.

ROC (Receiver Operating Characteristic) curve (Figure–5) analysis was performed to identify good biomarker for the decreased ovarian reserve in infertile women of different age groups. Table 3 presents the characteristic features of AMH and AFC showed more area under curve indicates that they are good markers in assessing ovarian reserve.

Age groups	BMI	AMH	AFC	Progesterone	LH	FSH
Group I </=26 years	26.12±3.759^{a}	15.62±5.464^{a}	13.48±2.41^{a}	7.80±1.352^{a}	7.08±1.639^{a}	5.12±1.159^{a}
Group II 27-35 years	27.08±5.306^{a}	19.98±5.428^{b}	14.29±2.89^{b}	6.20±1.946^{b}	7.50±2.625^{a}	5.57±1.497^{b}
Group III >36 years	27.55±2.970^{a}	17.86±6.369^{a}	15.64±2.248^{c}	4.72±0.857^{c}	9.97±2.645^{b}	8.47±2.734^{c}

Table 1: Statistical analysis by ANOVA and Post HOC comparison by Tukey HSD test comparisons of parameters in three age groups. **Note:** Values are expressed as means±S.D. in each group. Values not sharing a common superscript differ significantly at $p<0.05$ (Post Hoc: Tukey HSD test).

	Age	BMI	AMH	AFC	Progesterone	LH	FSH
Age	1	0.184	0.076	0.196*	-0.606**	0.295**	0.358**
	--	0.066	0.447	0.050	0.00	0.003	0.000
BMI	0.184	1	0.156	0.098	0.015	0.030	-0.082
	0.066	--	0.120	0.329	0.884	0.769	0.413
AMH	0.076	0.156	1	0.373**	-0.027	-0.115	-0.104
	0.447	0.120	--	0.00	0.792	0.253	0.301
AFC	0.196*	0.098	0.373**	1	0.071	0.028	0.103
	0.050	0.329	0.000	--	0.480	0.783	0.307
Progesterone	0.606**	0.015	-0.027	0.071	1	-0.182	-0.251*
	0.00	0.884	0.792	0.480	--	0.068	0.011
LH	0.295**	0.030	-0.115	0.028	-0.182	1	0.162
	0.003	0.769	0.253	0.783	0.068	--	0.105
FSH	0.358**	-0.082	-0.104	0.103	-0.251*	0.162	1
	0.00	0.413	0.301	0.307	0.011	.105	--

Note: *. Correlation is significant at the 0.05 level (2-tailed).

Table 2: Pearson correlation test for all the parameters among infertile women

Test Result Variable(s)	Area Under Curve	Asymptotic 95% Confidence Interval	
		Lower Bound	Upper Bound
BMI	0.508	0.376	0.639
AMH	0.443	0.246	0.641
AFC	0.665	0.512	0.818
Progesterone	0.180	0.084	0.276
LH	0.787	0.667	0.908
FSH	0.830	0.705	0.955

Table 3: ROC curve analysis

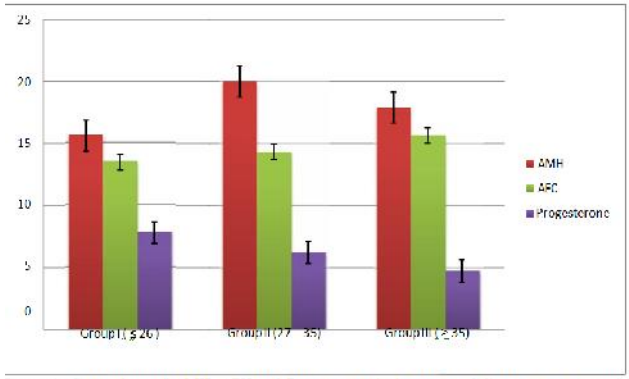


Figure 1: Comparison of AMH, AFH and Progesterone levles among different age groups of

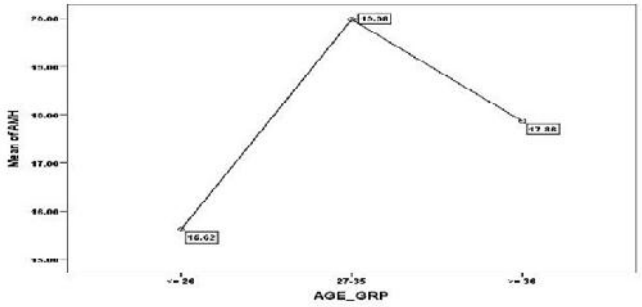


Figure 2: Mean values of AFC in three different age groups

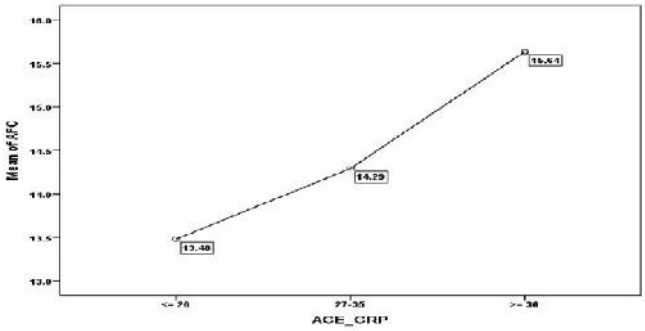
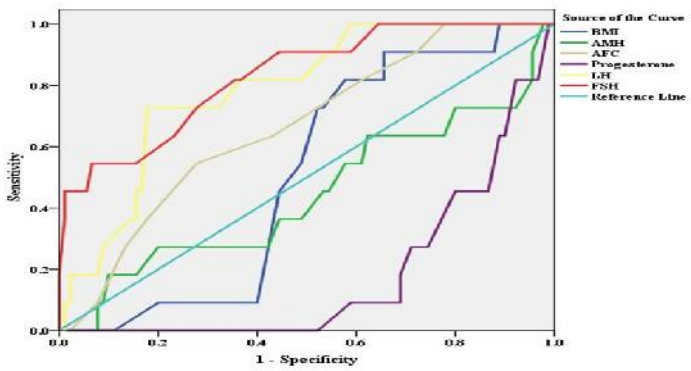
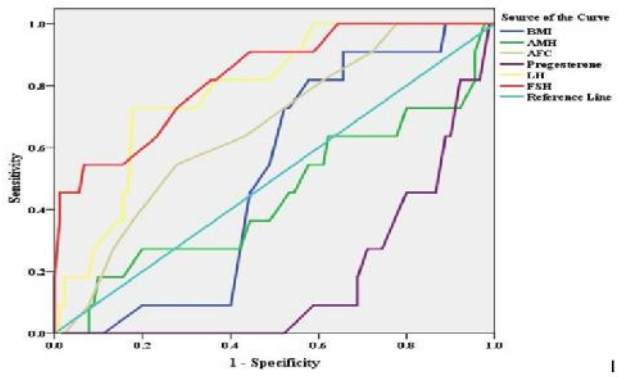


Figure 3: Mean values of progesterone in three different age groups

Figure 4: Mean values of progesterone in three different age groups

Figure 5: ROC analysis shows area under curve for BMI, AMH, AFC, Progesterone, LH and FSH in infertile women





Discussion

In this study, we evaluated how serum markers of ovarian reserve show variation with age in infertile women of Nellore, Andhra Pradesh. Of the markers considered, progesterone, AMH and AFC exhibited significant correlation with age. LH, FSH with AFC did not show any significant association with age compared to AMH, progesterone and AFC. Therefore, the data do not provide support for them as markers of ovarian reserve. The results obtained through our study show, that ovarian reserve assessment tests in each age group reflect age-specific changes. Above mentioned trends are also confirmed by other researchers [16-19]. It is interesting to note, that in our study AMH values were statistically significantly different from each other in all three age groups; whereas AFC values were statistically significantly higher in the group III compared with the group I, and in the group II compared with the group III; LH levels were statistically significantly higher only in group III compared with the group I. Thus, we can note that, AMH values better reflect age- specific changes, than other indicators. Our findings are relative with the study of de Vet et al [6]. where in early follicular phase hormone measurements at 3-year intervals revealed that serum AMH levels decline significantly whereas, we examined relationships between the age and ovarian reserve indicators in the whole study group and found that: age is in high significant negative correlation with AMH level and AFC, and in high significant positive correlation with FSH. However, the relation between the age and FSH was moderate ($p<0.01$). Thus, with the age AMH and AFC values strongly decline and the FSH levels moderately increase. The results of de Vet et al. also suggest that changes in serum AMH levels occur relatively early in the sequence of events associated with ovarian aging [6]. Elevated serum levels of FSH are not found until cycles become irregular [15,18-20]. Therefore, a marker that already shows a considerable change when cyclicity is still normal, would better identify women with declining fertility. Above mentioned results strongly suggest, that serum AMH level can be used as a marker of ovarian aging. In difference from the total study group comparison analysis within groups revealed quite interesting data in the group I and the most sensitive age group II (35-40 years), where the correlation between serum FSH levels and AFC was not statistically significant. Whereas AMH and AFC in all three study groups correlate positively and statistically significant. This positive correlation is confirmed by other researchers too [21-24].

Despite the fact, that at present there is no agreement on identification of the antral follicles by size, the majority of researchers imply the amount of 2-10 mm follicles counted in early follicular phase [25-27]. It has been reported that human antral follicles measuring <6mm express the great amount of AMH, and levels decline with antral follicles increase in size [17, 20]. In the study by Goksedef et al. the best correlation was found between AMH levels and5-6 mm antral follicles [15].

In our study the number of 2-10 mm antral follicles was counted in early follicular phase and positive correlation between AMH and AFC values with high significance was found in all age groups. According to data of one of the recent studies there Anti-Mullerian hormone (AMH) has recently been proposed as a parameter to replace ultrasonographic assessment of PCO morphology, with specificity and sensitivity of 97.1 and 94.6 % when using the Rotterdam criteria, or97.2 and 95.5 % using the NIH criteria [7, 23, 26]. Indeed, AMH levels correlate independently with both PCO morphology and androgenic profile [4, 22, 26]. Another parameter proposed as an adjunct to PCO morphology is an assessment of the ovarian stromal volume, measured as a ratio of the stromal area to total area of the ovary (S/A ratio). Although this S/A ratio performed well when discriminating between women with and without PCOS, and correlated with androgen levels, it has not been adopted as part of any of the existing diagnostic criteria [8, 13, 27-28].

Conclusion

In modern practice ovarian reserve assessment has a great value in the management of infertility. Our results indicate serum AMH levels are having positive correlation with AFC, and also this relationship appears to be more significant. In our study serum AMH and the number of antral follicle measurements appears to be better predictors than conventional hormone assessments (like LH, FSH). Using AMH measurement in combination with AFC may improve the assessment of ovarian reserve for evaluating the fertility potential and monitoring infertility treatment.

The present study has revealed the importance of AMH, mid luteal progesterone and AFC as novel markers for ovarian reserve in the diagnosis and treatment of infertile women. As limited data is available on the ovarian reserve indicators in infertile women of Nellore, Andhra

Pradesh population this data will be useful in suggesting assisted reproductive treatment for infertile women. This study gave exact means of management of infertile women with poor ovarian reserve. Our study further suggests that the assessment of ovarian reserve can be considered as a standard protocol in the evaluation of infertile women.

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References

1. Baarends WM, Uilenbrock JT, Kramer P, Hoogerbrugge JW, van Leeuwen EC. Themmem AP, et al. Anti-mullerian hormone and antimullerian hormone type II receptor messenger ribonucleic acid expression in rat ovaries during postnatal development, the estrous cycle, and gonadotrophin-induced follicle growth. J Endocrinol. 1995; 136(11):4951-62.
2. Howles CM. Role of LH and FSH in ovarian function. Mol Cell Endocrinol. 2000; 161:25-30.
3. Belosi C, Selvaggi L, Apa R, Guido M, Romualdi D, Fulghesu AM, et al. Is the PCOS diagnosis solved by ESHRE/ASRM 2003 consensus or could it include ultrasound examination of the ovarian stroma? Hum Reprod. 2006; 21(12):3108–15.
4. Rosenfield RL, Wroblewski K, Padmanabhan V, Littlejohn E, Mortensen M, Ehrmann DA. Antimullerian hormone levels are independently related to ovarian hyperandrogenism and polycystic ovaries. Fertil Steril. 2012; 98(1):242–9.
5. Jayaprakasan K, Deb S, Batcha M, Hopkisson J, Johnson I, Campbell B, et al. The cohort of antral follicles measuring 2-6 mm reflects the quantitative status of ovarian reserve as assessed by serum levels of anti-Mullerian hormone and response to controlled ovarian stimulation. Fertil Steril. 2010; 94 (5):1775-81.
6. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002; 77(2):357-62.
7. Eilertsen TB, Vanky E, Carlsen SM. Anti-Mullerian hormone in the diagnosis of polycystic ovary syndrome: can morphologic description be replaced? Hum Reprod. 2012; 27(8):2494–502.
8. Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti- Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprod. 2003; 18(2): 323–27.
9. Feyereisen E, Mendez Lozano DH, Taieb J, Hesters L, Frydman R, Fanchin R. Anti-Mullerian hormone: clinical insights into a promising biomarker of ovarian follicular status. Reprod Biomed Online. 2006; 12(6): 695–703.
10. Testing and interpreting measures of ovarian reserve: A committee opinion. Practice Committee of the American Society for Reproductive Medicine. Fertil Steril 2012; 98:1407-15.
11. Frattarelli JL, Levi AJ, Miller BT, Segars JH. A prospective assessment of the predictive value of basal antral follicles in in vitro fertilizationcycles. Fertil Steril. 2003; 80(2): 350-5.
12. ASRM pages, Committee Opinion No.589. Female age related fertility decline. American College of Obstetricians and Gynecologists. Obstet Gynecol 2014; 123:719-21.
13. Burger HG, Dudley EC, Hopper JL, Groome N, Guthrie JR, Green A, et al. Prospectively measured levels of serum follicle-stimulating hormone, estradiol, and the dimeric inhibins during the menopausal transition in a population-based cohort of women. J Clinical Endocrinol Metab. 1999; 84(11): 4025–30.
14. Fulghesu AM, Ciampelli M, Belosi C, Apa R, Pavone V, Lanzone A. A new ultrasound criterion for the diagnosis of polycystic ovary syndrome: the ovarian stroma/total area ratio. Fertil Steril. 2001; 76(2):326–31.
15. Goksedef BP, Idis N, Gorgen H, Asma Y R, Api M, Cetin A. The correlation of the antral follicle count and Serum anti-mullerian hormone. J Turkish-German Gynecol Assoc. 2010; 11: 212-5.
16. Hull MGR, Savage PE, Bromham DR et al. The value of single serum progesterone measurement in the mid-luteal phase as a criterion of a potentially fertile cycle ("ovulation") derived from treated and untreated conception cycles. Fertile Steril 1982; 37;355-60.
17. The Rotterdam ESHRE/ASRM-sponsored consensus workshop group. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS). Human Reproduction. 2004; 19:41–7.
18. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WHB. A validated model of serum anti-Mullerian hormone from conception to menopause. Reprod BioMed Online.2011; 23(2):204-6.

19. La Marca A, Volpe A. Anti-Mullerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool. Clin Endocrinol (Oxf). 2006; 64(6): 603–10.
20. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. Endocr Rev 2000; 21:200–14.
21. Mohan BS. Mid-luteal phase plasma progesterone levels in spontaneous and clomiphene citrate induced conception cycles. J Obstet Gynecol India 2005; 55(4): 350-352.
22. Nelson SM, Messow MC, Wallace AM, Fleming R, McConnachie A. "Nomogram for the decline in serum anti-mullerian hormone: a population study of 9,601 infertility patients." Fertil Steril. 2011; 95(2):736–41.
23. Noci I, Biagiotti R, Maggi M, Ricci F, Cinotti A, Scarselli G. Low day 3 luteinizing hormone values are predicitive of reduced response to ovarian stimulation. Hum Reprod 1998; 13:531-4.
24. Van Disseldorp J, Faddy MJ, Themmen APN, de Jong FH, Peeters PHM, van der Schouw YT, et al. Relationship of serum anti-mullerian hormone concentration to age at menopause. J Clinical Endocrinol Metab. 2008; 93(6): 2129–34.
25. Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. PLoS ONE 2010; 5:e8772.
26. Weenen C, Laven JS, von Bergh AR, Cranfield, M, Groome NP, Visser JA et al. Anti- Mullerian hormone expression pattern in thehuman ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004; 10(2): 77-83.
27. Welt CK, McNicholl DJ, Taylor AE, et al. Female reproductive aging is Marked by decreased secretion of dimeric inhibin. J Clin Endocrinol Metab 1999; 84:105–12.
28. Yong PTK, Baird DT, Thong KJ, et al. Prospective analysis of the relationships Between the ovarian follicle cohort and basal FSH concentration, the inhibin Response to exogenous FSH and ovarian follicle number at different stages of the normal menstrual cycle and after pituitary down-regulation. Hum Reprod 2003; 18:35–44.

