Original Article

Establishing an Anti-Müllerian Hormone Cutoff for Diagnosis of Polycystic Ovarian Syndrome in Women of Reproductive Age-Bearing Indian Ethnicity Using the Automated Anti-Müllerian Hormone Assay

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Abstract

Context: Polycystic ovary syndrome diagnosed by Rotterdam criteria, is the most common cause of anovulatory infertility. The criteria of polycystic ovarian morphology (PCOM) are subject to operator variability and technological advances. Serum anti-Müllerian hormone (AMH) level has been proposed as a more reliable alternative to antral follicle count. There is a paucity of data on use of AMH for diagnosis of PCOS in Indian women. **Aim and Objectives:** The aim of this study is to determine a cutoff level for AMH that could facilitate diagnosis of PCOS and its phenotypes in women of Indian origin using the automated (Roche) assay and to compare the competence of oocytes in PCOS and non-PCOS women undergoing *in vitro* fertilization-intracytoplasmic sperm injection (IVF-ICSI). **Materials and Methodology:** A total of 367 women undergoing treatment at our fertility center between February 2017 and August 2017 were prospectively enrolled in this study. Of these, 133 were diagnosed with PCOS, 69 had isolated PCOM, and 165 (controls) had normal ovaries on ultrasound examination. Serum AMH levels were assessed using the fully automated Roche Elecsys® immunoassay. Gonadotropin-releasing hormone antagonist protocol was used for IVF-ICSI in all patients. **Statistical Analysis Used:** Quantitative variables were compared using the Mann–Whitney test. Qualitative variables were correlated using the Chi-square test. $P < 0.05$ was considered to be statistically significant. **Results:** Mean AMH concentrations in women with PCOS was higher (7.56 ± 4.36 ng/mL) in comparison to PCOM and controls. Serum AMH concentration >5.03 ng/mL could facilitate diagnosis of PCOS (area under the curve = 0.826); sensitivity –70.68%, specificity of 79.91%. There was no difference in the ratio of mature to total oocytes retrieved in the three groups ($P > 0.05$). Mean number of mature oocytes was lower in controls than PCOS and PCOM ($P < 0.001$). **Conclusions:** Serum AMH concentration >5.03 ng/mL could be used as cutoff value for the diagnosis of PCOS in women of Indian origin.

Keywords: *Anti-Müllerian hormone, ovarian reserve, polycystic ovarian syndrome, Rotterdam criteria*

Introduction

Polycystic ovarian syndrome (PCOS), a polygenic, multifactorial syndrome, is one of the most frequently cited causes of an anovulatory infertility. It is the most common endocrine abnormality encountered

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in women of reproductive age.[1] Clinical manifestations of the syndrome are perceived from adolescence to menopause posing a major health risk throughout the lifetime of an individual. Obesity and insulin resistance are linked to severity of symptoms and the associated comorbidities of diabetes mellitus, hypertension, endometrial carcinoma, and psychological health issues.[2] Reproductive loss, infertility, implantation failure in assisted reproductive technique (ART), and the potentially life-threatening complication of ovarian hyperstimulation syndrome (OHSS) during infertility treatment, is a source of constant concern during the reproductive years.[3] Effects on the reproductive outcome in these women may be related to oocyte quality. Oocytes retrieved from PCOS patients in *in vitro* fertilization (IVF) exhibit developmental incompetence, probably as a result of epigenetic changes caused possibly by a hyperandrogenic follicular environment and oxidative stress.[4] An early and precise diagnosis is, therefore, essential to prevent long-term effects of the syndrome and improve fertility. Sadly, heterogeneity of the clinical and endocrine features creates a dilemma for precise diagnosis, leading to a wide range (5.6%–21.3%) in the reported prevalence of the syndrome.[5] There is ongoing research to identify the complex etiopathology of the syndrome and ascertain characteristics for a more accurate diagnosis.

Currently, diagnosis is based on the presence of two of the three following Rotterdam criteria-hyperandrogenism/hyperandrogenemia (HA), oligo/anovulation (OA) and polycystic ovarian morphology (PCOM), after excluding all other causes of HA and ovulatory dysfunction.[6] PCOM is diagnosed on transvaginal ultrasound by the presence of 12 or more follicles, 2–9 mm in diameter in the follicular phase.[7] The criteria of PCOM is subject not only to operator variability but also to technological advances, as smaller antral follicles are identified on 3D/4D ultrasound machines. There also appear to be ethnic and racial differences when defining cutoffs for diagnosis of PCOS based on the ovarian morphology and Asian women have been found to have a lower follicle number per ovary (FNPO) compared to the Caucasian women.[8] It has therefore been proposed that the criteria of antral follicle count (AFC) on ultrasound be replaced by the measurement of anti-Müllerian hormone (AMH) in serum, which has been established as a reliable marker of ovarian reserve.[9] AMH offers the convenience of blood sampling on any day of the cycle. In addition, the newly introduced automated AMH has overcome some of the drawbacks of the manual systems and become more precise, sensitive, and reproducible, adding to the value of this marker.

AMH, a dimeric glycoprotein, belongs to the transforming growth factor-β family and is secreted almost exclusively by the granulosa cells of the early antral follicles.[10] Serum AMH levels are high in women with PCOS due to an increase in the number of small antral follicles and an intrinsic defect of the granulosa cells. Ethnicity has been associated with altered age-specific levels of AMH, with Asian women having a lower AMH at a given age compared to their Caucasian counterparts.[11] A cutoff value of 4.7–5 ng/ml in Caucasian[12] and 10 ng/ml in Japanese and Korean women has been proposed for the diagnosis of PCOS.[13,14] There is, however, a paucity of data on the use of AMH for diagnosis of PCOS in women of Indian ethnicity.

Aim of the study

1. Primary aim: To determine if serum AMH levels estimated by the automated assay, could facilitate diagnosis of PCOS and its phenotypes in women of Indian origin
2. Secondary aim: To compare the age-related decline of AMH, ovarian response, and ratio of mature to immature oocytes in women with PCOS, PCOM, and controls.

Materials and Methodology

Source of data

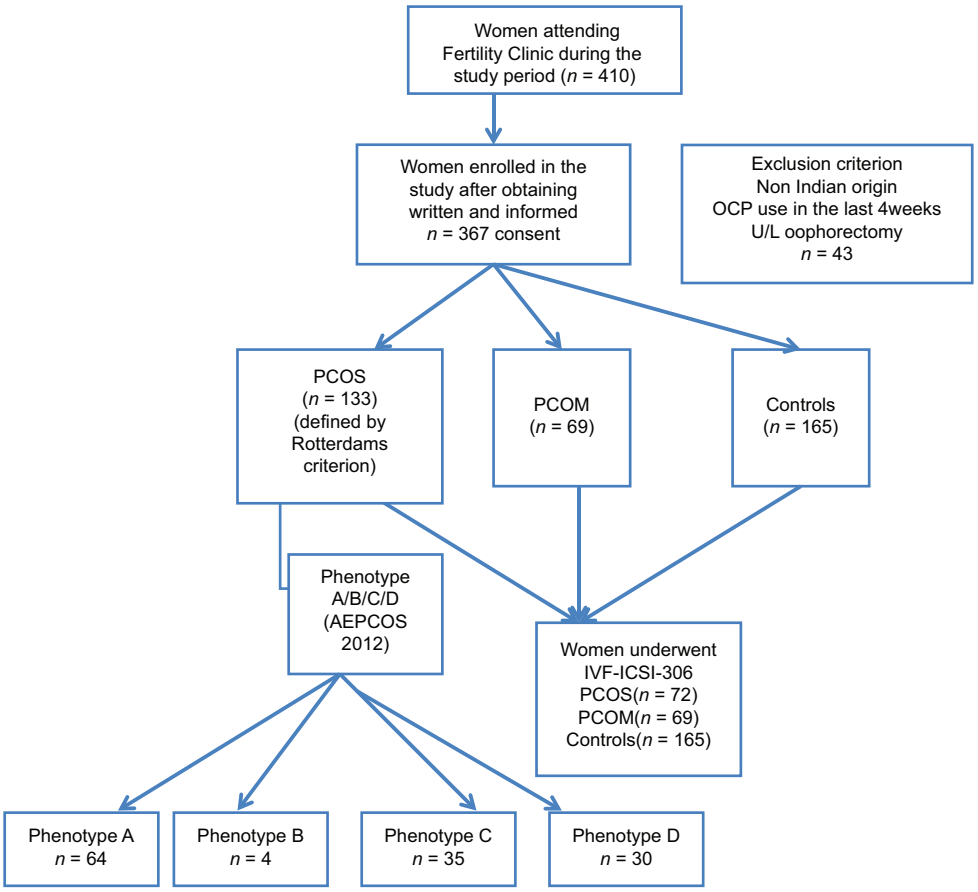
A total of 367 women who were undergoing treatment at our fertility center between February 2017 and August 2017 were prospectively enrolled in this study. Of these, 133 were diagnosed with PCOS according to the Rotterdam Criteria (2004), 69 had isolated polycystic ovaries on ultrasound examination (25 follicles of 2–9 mm on cycle day 2–5, in both ovaries using 8 MHz probe) and 165 had normal ovaries on ultrasound examination (controls). PCOS women were further divided into four phenotypes according to the Androgen Excess and PCOS Society (AEPCOS) Classification 2012 [Flowchart 1].[15] Phenotype A – clinical and/or biochemical HA + OA + PCOM; Phenotype B - HA + OA; Phenotype C - HA + PCOM; and Phenotype D – OA + PCOM. Of the 367 women recruited, 306 underwent IVF-intracytoplasmic sperm injection (ICSI): 72 with PCOS, 69 with PCOM, and 165 women in the control arm.

Ethical clearance

Approval for the study was obtained from the Institutional Review Board. AMH and AFC count are done routinely for ovarian reserve assessment in our clinic for all infertile patients.

Diagnosis of PCOS and PCOS phenotypes

A comprehensive clinical history was obtained from all the participants. Physical examination



Flowchart 1: Flowchart representing the study population

including assessment of body mass index (BMI) was done. Hirsutism was recorded according to the modified Ferriman–Gallwey score. Serum AMH, follicle-stimulating hormone (FSH) estradiol and luteinizing hormone (LH) levels were measured on Day 2 or 3 of the menstrual cycle for patients recruited in the study. AFC (the total number of follicles with a diameter between 2 and 9 mm in both ovaries) was assessed on day 2 or 3 of menstrual cycle using an 8 MHz transvaginal probe. Women recruited in the study were divided into three groups: Group A: Women with PCOS, Group B: Women with PCOM, and Group C: Controls. Women with PCOS were further assigned one of the four phenotypes according to the AEPCOS classification 2012.

Anti-Müllerian hormone estimation

About 2 ml of blood was collected in the early follicular phase (cycle day 2–5) for AMH estimation. AMH characteristics in terms of optimal cutoff and area under the receiver operating curve for diagnosing PCOS were estimated on a fully automated Elecsys® and cobas e immunoassay analyzers (Roche Diagnostics GmbH, Germany). Variations of AMH levels in different phenotypes of PCOS were also noted. The test design corresponds to a sandwich immunoassay, based on the streptavidin-biotin technology. The capture antibody is biotinylated; the detection antibody is covalently linked with a Ruthenium complex. Successfully formed antigen-antibody complexes can be detected via electrochemiluminescence within a total assay time of 18 min. The Elecsys® immunoassay detects AMH in the range of 0.01–23 ng/ml (0.07–164 pmol/L) and requires 50 µl of serum or lithium-heparin plasma. The automated Elecsys® AMH assay showed excellent precision, linearity, and functional sensitivity in comparison to the manual AMH assays and showed no interference in the results due to complement binding. Serum AMH levels measured by AMH Gen II are roughly 16% and 20% higher than those obtained with Access AMH and Elecsys AMH, respectively. In addition, serum AMH levels obtained with Elecsys AMH assay are approximately 5% lower than the access AMH assay.[16]

In vitro fertilization-intracytoplasmic sperm injection protocol

Of the 367 women recruited, 306 underwent controlled ovarian stimulation (OS) for ICSI: 72 with PCOS, 69 with PCOM, and 165 women in the control arm.

Gonadotropin-releasing hormone antagonist protocol was followed for all the patients enrolled in the study. Dose of gonadotropins was decided based on the patient's age, BMI, ovarian reserve, and previous response. OS was done as per our institutional protocol with recombinant follicle-stimulating hormone (follitropin-alfa Gonal-f®, EMD Serono, Inc.,) for the first 5 days followed by Menopur (highly purified HMG-Ferring Pharmaceutical Ltd.). Injection human chorionic gonadotropin 10,000 IU or triptorelin 0.2 mg s/c was given as the ovulation trigger and ovum pick up performed 36 h later. The number of oocytes retrieved and the number of MII were noted in each group.

Statistical analysis

Categorical variables were presented in number and percentage (%), and continuous variables were presented as mean ± standard deviation and median. Normality of data was tested by the Kolmogorov–Smirnov test. If the normality was rejected, then nonparametric test was used. Quantitative variables were compared using unpaired *t*-test/Mann–Whitney test (when the data sets were not normally distributed) between the two groups. Qualitative variables were correlated using Chi-square test/Fisher's exact test. Univariate and multivariate logistic regression was used to assess the association of CLABSI with various parameters. $P < 0.05$ was considered statistically significant. Data analysis was done using Statistical Package for the Social Sciences version 21.0 (IBM Corp., Armonk, NY, USA).

Results

The mean age of patients in the PCOS, PCOM, and control group was 33.66 ± 3.56, 24.07 ± 1.91, and 30.74 ± 3.72 years, respectively, which showed a statistically significant difference amongst the three groups ($P < 0.001$). The mean BMI (kg/m^2) was significantly higher in women with PCOS 27.72 ± 4.64 in comparison to women with PCOM (21.71 ± 2.79) and controls (26.01 ± 4.63) ($P < 0.001$) [Table 1]. BMI and age were lowest in the PCOM group which could be attributed to a vast majority of patients being young donors. After adjusting for age and BMI, the mean serum AMH concentrations showed a significant difference between women with PCOS, 7.56 ± 4.36 ng/mL; PCOM, 6.1 ± 3.78 ng/mL; and controls 2.25 ± 1.81 ng/mL ($P < 0.001$) [Figure 1].

Anti-Müllerian hormone differences in PCOS phenotypes

After adjusting for differences in age and BMI among the different PCOS phenotypes, it was observed that the mean AMH levels in women with PCOS (phenotype A) 9.05 ± 5 ng/mL were significantly higher than the other phenotypes ($P < 0.05$). In phenotype B, the mean AMH level of 3.32 ± 2.03 ng/mL was significantly lower than the other phenotypes. No significant difference was detected in the mean serum AMH levels between phenotype C – 6.31 ± 2.59 ng/mL and phenotype D – 6.39 ± 3.67 ng/mL ($P > 0.05$) [Figure 2].

Anti-Müllerian hormone cutoff

A receiver operator curve was generated to determine the ability of AMH to predict PCOS. Serum AMH concentrations >5.03 ng/mL discriminated women with PCOS from women with PCOM and normal ovaries with a sensitivity of 70.68% and specificity of 79.91% (area under the curve [AUC] = 0.826) [Figure 3]. Estimation of FSH and LH concentrations on cycle day 2 in the three groups revealed that FSH concentration was lower in women with PCOS in comparison to women with PCOM and controls ($P = 0.002$). The LH levels, in contrast, were higher in women with PCOS (5.7 IU/mL) compared to women with PCOM (4.1 IU/mL) and controls (3.75 IU/mL) ($P = 0.001$) [Table 2].

Age-related anti-Müllerian hormone decline in the three groups

We also found that the rate of decline of AMH with age was significantly slower in women with PCOS and PCOM than the control group; however, there was no statistically significant difference in the rate of fall of AMH between the PCOS and PCOM group [Table 3 and Figure 4].

Table 1: Age and BMI (Mean±SD) in women with PCOS, PCOM and controls

	Control	PCOS	PCOM	*P*	Control vs PCOS	Control vs PCOM	PCOS vs PCOM
AGE years (Mean±SD)	33.66±3.56	30.74±3.72	24.07±1.91	<.0001	<.0001	<.0001	<.0001
BMI (kg/m2) (Mean±SD)	26.01±4.63	27.72±4.64	21.71±2.79	<0.001			

Table 2: FSH, LH (Mean±SD) (Cycle Day 2/3) concentration in women with PCOS, PCOM and controls

CD2/3 FSH, LH (IU/L)	PCOS (Mean±SD)	PCOM (Mean±SD)	Control (Mean±SD)	PCOS vs PCOM *P* val	Controls vs PCOM *P* val	PCOS vs PCOM *P* val
FSH	4.23±1.29	5.34±1.37	6.68±2.22	<0.001	<0.001	<0.001
LH	6.86±4.72	4.13±1.9	4.22±2.26	<0.001	<0.001	<0.001

Oocyte maturity

Assessment of oocyte maturity and dose-related data from women who underwent IVF-ICSI in the three groups are as follows: The number of mature oocytes retrieved in the PCOM (17.68 ± 8.46) and PCOS (18.51 ± 8.99) groups were similar (P = 0.074), but significantly higher than the controls (8.3 ± 4.78) (P < 0.05). The mean number of total oocytes retrieved in the control group was (10.39 ± 6.03) significantly lower than in women with PCOS (23.03 ± 9.9) and PCOM (23.01 ± 10.93) (P < 0.001), while there were no differences between the PCOM and PCOS group. Interestingly, there was no difference in the ratio of the mature eggs to the total eggs retrieved in the three groups 80.5% in controls, 76.8% in PCOM, and 80.5% in PCOS (P > 0.05) [Figure 5]. The fertilization rates in the three groups were also not found to be statistically different 73% in controls and women with PCOM and 79% in women with PCOS. The total dose of gonadotropins required was similar in women with PCOM (2473.55 ± 538.02) and PCOS (2370.4 ± 724.38) which was significantly lower than the controls (2888.79 ± 787.21) (P < 0.001) [Table 4].

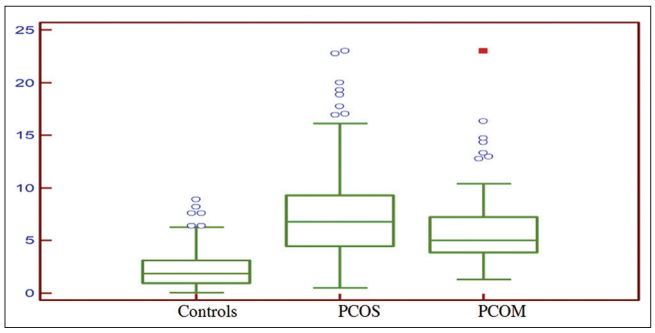


Figure 1: AMH concentration (ng/ml) Absolute values with median, interquartile range, maximum and minimum values excluding outliers

Discussion

Polycystic ovary syndrome is a complex, heterogeneous, endocrine disorder that affects women from adolescence to menopause. Disordered androgen production and metabolism define symptomatology of the syndrome. The associated metabolic syndrome (MS) of diabetes, hypertension, and endometrial cancer increases morbidity and has evolved as a major health concern. Obesity, insulin resistance, environmental, and lifestyle factors contribute to severity of the syndrome and the ever-increasing numbers. Timely diagnosis at a young age allows for acceptance of corrective lifestyle changes. Consequently, there has been a constant endeavor to establish accurate diagnostic criteria in all ethnic groups. Although many groups (National Institute of Health,[17] AEPCOS, ESHRE/ASRM) have attempted to define diagnostic criteria for PCOS, the widely-used criteria presently are the Rotterdam criteria. Of the three criteria – HA, OA, and PCOM, the long-standing debate revolves around the inclusion of the ultrasound criteria, which is subject to significant operator and instrument

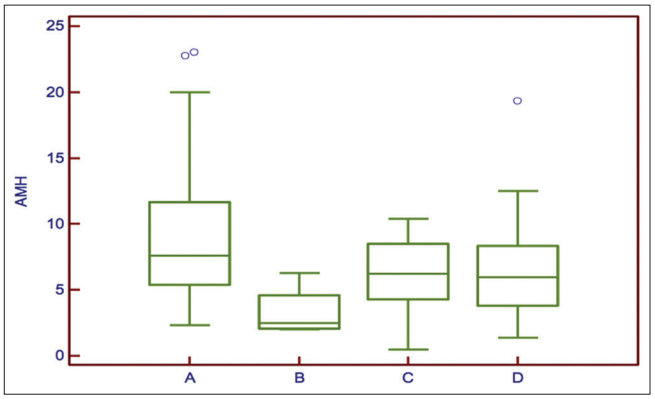


Figure 2: AMH concentration (ng/ml) in the various PCOS phenotypes. Absolute values with median, interquartile range, maximum and minimum values excluding outliers

Table 3: Correlation of Age and AMH in women with PCOS, PCOM and controls

	Control	PCOS	PCOM	Control vs. PCOS	Control vs. PCOM	PCOS vs. PCOM
AMH and Age						
Correlation Coefficient	-0.356	-0.102	-0.075	*P*	*P*	*P*
P	<.0001	0.244	0.538	val=0.0219	val=0.0419	val=0.857
Sample size	165	133	69			

Table 4: Total dose of GT required (IU) and number of oocytes and mature oocytes retrieved in women who underwent IVF-ICSI

	PCOS (Mean±SD)	PCOM (Mean±SD)	Controls (Mean±SD)	Controls vs. PCOM (*P* val)	Controls vs. PCOM (*P* val)	PCOS vs. PCOM (*P* val)
Dose of GT (IU)	2888.79±787.21	2370.4±724.38	2473.55±538.02	<0.001	0.825	0.0004
Number of oocytes retrieved	10.39±6.03	23.03±9.9	23.01±10.93	<0.001	0.002	0.113
Mature oocytes	8.3±4.78	18.51±8.99	17.68±8.46	<.0001	0.001	0.074

variability. The diagnosis of PCOM is based on the presence of 12 or more follicles, 2–9 mm in diameter in the follicular phase on transvaginal songraphy (TVS). This threshold was decided on the basis of a study by Jonard and Dewailly[7] and expert opinion by Balen *et al*. 2003.[18] An ovarian volume >10 cm^3, the necklace sign and an increase in stromal density were also considered, but the last two were omitted in the Rotterdam criterion.[6] A potential for overdiagnosis of PCOS with higher frequency probes, especially in younger women

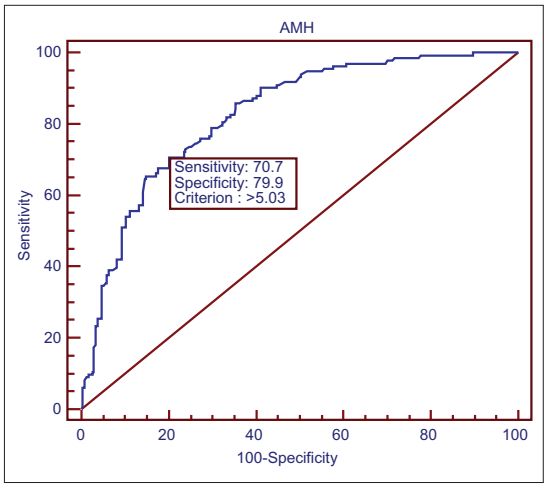


Figure 3: ROC curve of AMH concentration for the diagnosis of PCOS (AUC = 0.826)

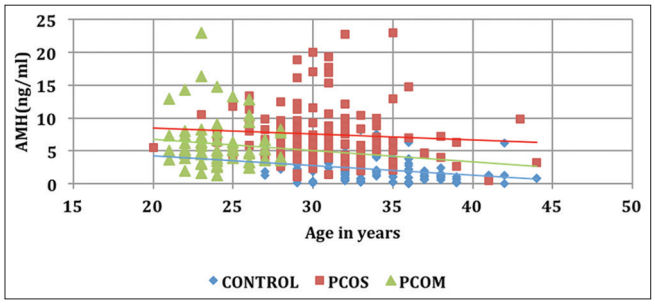


Figure 4: Correlation of Age and AMH in women with PCOS, PCOM and controls

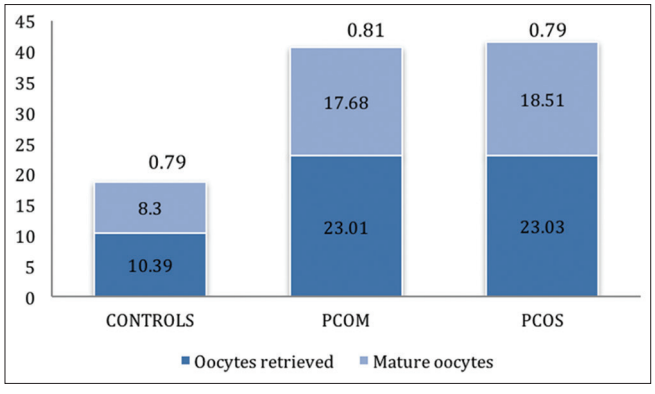


Figure 5: Ratio of Number of mature oocytes/total oocytes retrieved in women who underwent IVF-ICSI

was recognized,[19-21] and it was suggested that an FNPO of 18 should be applied with use of >8 MHz ultrasound probes,[22] since with this equipment a median FNPO in non-PCOS women was established at 11–13.[23-25] The International PCOS Guidelines 2018 suggest a threshold FNPO of ≥20 and/or an ovarian volume ≥10 ml for PCOM (when the image quality does not allow a reliable estimate of FNPO), using ultrasound transducers with a frequency bandwidth 8MHz.[26] Different FNPO thresholds have been recommended for PCOS diagnosis in different ethnic groups.[8,27,28] It has been suggested that Asian PCOS women have a lower FNPO compared to Caucasian women.[8] To overcome the variability of AFC estimation and improve the accuracy of PCOS diagnosis, use of serum AMH (using the automated AMH assay) has been proposed as a surrogate marker for PCOM instead of AFC despite it being slightly expensive test in comparison to AFC measurement. Lie Fong *et al*.[29] observed that the diagnostic performance of AMH (AUC = 0.903) to differentiate PCOS women from normal regularly cycling women was similar to that using the FNPO (AUC = 0.915) (P = 0.25). They further stated that in the older population (>30 years), the diagnostic performance of AMH was even greater than that of FNPO (AUCs = 0.948 vs. 0.874, respectively, P = 0.00035). AMH is released maximally from granulosa cells of antral follicles ≤6 mm and AMH levels in women with PCOS have been postulated to be 2–3-folds higher than in non-PCOS healthy women reflecting the increased number of small antral follicles.[29-33] AMH production on an average is 75 times higher per granulosa cell from women with anovulatory PCOS and 20 times higher from women with ovulatory PCOS compared to healthy controls.[34] Increased AMH concentrations are also found in follicular fluid,[35] with levels being 18 times higher in women with anovulatory PCOS in comparison with ovulatory PCOS. Various authors have validated these findings and demonstrated significantly higher serum AMH levels in PCOS women in comparison to normal controls, in Caucasian women,[36-40] and other ethnic groups.[41-43]

Our study on women of Indian ethnicity using the automated AMH assay concurs with these findings. We found significantly higher AMH levels in women with PCOS than PCOM and controls. Furthermore, the mean AMH levels in women with PCOS (phenotype A) was significantly higher than the other phenotypes (P < 0.05), while the mean AMH levels in phenotype B was significantly lower than the other phenotypes (P = 0.017). This study corresponds with other studies that suggest Phenotype A has the highest AMH levels and Phenotype B the lowest[44] providing confirmation of the view that increased number of antral follicles and not HA,

contributes to serum AMH levels. As in other studies, Phenotype A was the most prevalent PCOS phenotype and Phenotype B the least common in our PCOS population. With respect to IVF complications, it has been demonstrated that women with Phenotype A seem to be at the highest risk of OHSS and would benefit by taking steps for its prevention.[45] Bil *et al*. demonstrated that Phenotype A and B had the highest association with MS irrespective of BMI and that visceral adiposity index, the only independent predictor of MS in PCOS was also significantly higher in Phenotype B, when compared with the others ($P < 0.01$).[46] Phenotype identification would encourage timely monitoring for prevention of the MS. The need to establish a cutoff value of AMH for PCOS diagnosis has been recognized, and various studies have been conducted in the Caucasian population with suggested threshold values ranging from 3.5 ng/ml to 8.4 ng/ml.[9,37-39,47] [Table 5]. A systemic review and meta-analysis of ten studies by Iliodromiti *et al*. 2013[9] concluded that a value of 4.7 ng/ml could be used to diagnose PCOS with a sensitivity of 76.7% and specificity of 79.4% (AUC = 0.829). Lie Fong *et al*. 2017[29] using cluster analysis looked to define age-related AMH cutoff in PCOS women since AMH decreases with increasing age. They estimated that the cutoff value offering the best compromise between sensitivity and specificity for the diagnosis of PCOS to be 5.5 ug/l (AUC = 0.903) in young women and 5.0 ug/l (AUC = 0.948) in older women using the Gen II Beckman Coulter assay. A comparison to assess the efficacy of automated over manual AMH assay for PCOS detection was done by Pigny *et al*. 2016.[37] Although both systems were efficient, they suggested different threshold values for the diagnosis of PCOS on manual and automatic assays, the cutoff being 5.60 ng/mL (40 pmol/L) and 4.20 ng/mL (30 pmol/L), respectively. A study from Australia that used the automated Elecys assay suggested a cutoff for diagnosis of PCOS as 5.07 ng/ml (AUC-0.826 sensitivity 83.7% and specificity 82.3%).[39]

Keeping in mind, ethnic and racial differences in ovarian reserve attempts have been made to define an AMH threshold for PCOS diagnosis in different ethnic groups.[48] Our study in women of Indian ethnicity revealed that a serum AMH concentration >5.03 ng/mL could differentiate between women with PCOS, PCOM, and normal ovaries, with a sensitivity of 70.68% and specificity of 79.91% (AUC = 0.826). Our AMH threshold value in Indian women aligns with the suggested cutoff given by Pigny *et al*.[12] and Tremellen and Zander-Fox,[39] both done using the automated assay. Our findings are at variance with the study by Sujata

Table 5: Anti-Müllerian hormone cutoff (ng/ml) in the Caucasian population

Author	Ethnicity	Year	AMH assay	AMH cut off (ng/ml)	AUC	Sensitivity (%)	Specificity (%)
Pigny *et al*.	Caucasian	2006	IOT	8.4	0.851	67	92
Hart *et al*.	Caucasian/Australia	2010	IOT	4.2	0.641	53.1	69.8
Dewailly *et al*	Caucasian	2011	IOT	4.9	0.973	92	97
Homburg *et al*	Caucasian	2013	DSL	6.72	0.81	60.0	98.2
Casadei *et al*.	Caucasian/Italy	2013	IOT	4.62	0.97	95.0	95.0
Sahmay *et al*	Caucasian/Nordic	2013	DSL	3.94	0.92	80.0	89.8
Fong *et al*	Caucasian	2017	Gen II	5.5(young women <30 yrs)	0.903(young women)	82	84.1
				5(old women >30 yrs)	0.948(old women)		
Tremellen *et al*.	Austarlia/Caucasian	2015	Elecys automated	5.07	0.836	83.7	82.3
Pigny *et al*	Caucasian	2016	Automated	4.2	0.93	-	92
			Manual	5.6			
Iliodromiti *et al*	Metaanalysis	2013	IOT	4.7	0.87	82.8	79.4

Table 6: Anti-Müllerian hormone cutoff (ng/ml) in the Asian population

Author	Ethnicity	Year	AMH assay	AMH cut off (ng/ml)	AUC	Sensitivity (%)	Specificity (%)
Lin *et al*	Taiwan	2011	DSL	7.3	0.774	76	70
Chao *et al*.	Taiwan	2012	DSL	3.5	-	74.0	79.0
Woo *et al*	Korea	2012	IOT	7.82	0.86	75.9	86.8
Wiveko Budi *et al*	Iran	2014	Gen II	4.45	0.87	76	74.6
Song *et al*	Korea	2017	GEN II	10	0.876	71	93
Chao-Yan *et al*	Chinese	2018	Union	8.16 (20-29)	0.85	78.4	80.9
			Immunanalyser	5.89 (30-39)	0.86	82.6	79.8
Matsuzaki	Japan	2017	Elecsys	10	-	24.6	92.6
Mahajan & Jasneet	INDIA	2018	Elecsys	5.03	0.826	70.68	79.91

and Swoyam 2018[8] which suggested that FNPO in Indian women using high-frequency ultrasound should be set at 12 using a 2D and 10 using a 3D probe as opposed to 20 suggested by the International PCOS guidelines 2018. If FNPO is lower in Indian women, it should have reflected a lower AMH threshold value than the Caucasian population.

A much higher threshold value, however, has been given by other Asian groups [Table 6]. Woo *et al*. 2012,[42] using the Immunotech assay, found that a cutoff of 7.82 ng/ml in Korean women could help predict women with PCOS with a sensitivity of 75.9% and specificity of 86.8% (AUC 0.868), while Song *et al*. 2017[49] estimated an optimal cutoff value of 10 ng/ml (71% sensitivity and 93% specificity) (AUC = 0.876) in Korean women using the Beckman Coulter Gen II assay. Li *et al*.[43] obtained a cutoff value of 8 ng/mL, with a specificity of 70% and sensitivity of 61.7% (AUC 0.664) for diagnosis of PCOS in the Chinese population. An AMH threshold for PCOS diagnosis in Turkish and Japanese women has also been reported; 14.0 ng/ml (sensitivity of 48.8% and specificity of 77.1%, AUC 0.579) in the Turkish population[50] and 7.33 ng/ml (specificity of 76.8% and sensitivity of 44.7% AUC) in Japanese population;[14] the Japanese study was done on the Elecys automated AMH assay.

An age-related decline of AMH is recognized, and it has been suggested that a lower rate of AMH fall in PCOS women[30] increases their fertility window. We found that in our ethnic group too, the rate of decline of AMH with age was significantly lower in women with PCOS ($r = -0.102$) and PCOM (−0.075) than the controls (−0.356). An increase in number of recruitable follicles at an advanced age would allow for the better ART outcomes in these women. More embryos would allow the possibility of transferring euploid embryos after preimplantation genetic testing for aneuploidy (PGT-A). Aneuploidy is acknowledged to be the major reason for implantation failure at advanced maternal age.[51]

Polycystic ovary syndrome (PCOS) is associated with significant abnormalities of granulosa cell function, abnormal circulating hormones, and peri-follicular vascularity. Heterogeneity of steroidogenesis between individual follicles,[52] altered intrafollicular environment, increased granulosa cell apoptosis,[53] impaired mitochondrial function due to oxidative stress, and epigenetic modification[54] is implicated in poor oocyte quality and developmental competence. In our study, the mean number of oocytes retrieved in the control group was significantly lower than PCOS and PCOM group, while there were no differences between the PCOM and PCOS group ($P < 0.001$). The number of mature oocytes retrieved was also similar in PCOM and PCOS groups and were significantly higher than controls $P < 0.05$. However, the ratio of mature to total oocytes retrieved was similar in the three groups ($P > 0.05$). The fertilization rates were also similar. The higher numbers of oocytes obtained allow for the generation of proportionately higher number of good quality oocytes that have fertilization rates similar to oocytes obtained from non-PCOS patients. Ludwig *et al*.[55] also reported similar rates of oocyte maturation and embryo score in women with polycystic ovaries in comparison to those with normal ovaries, although they found a significantly higher miscarriage rate. Mikkelsen and Lindenberg[56] studied oocyte morphology, fertilization, and cleavage rates and found no difference in patients with and without polycystic ovaries. In contrast, lower fertilization and implantation rates (Chen *et al*. 2008)[57,58] and a lower proportion of meiotically competent oocytes (Chen *et al*.)[58,59] have been reported in PCOS patients.

Total dose of gonadotropins required for OS was similar in women with PCOM and PCOS which was significantly lower than the controls ($P < 0.001$), and these findings are in accordance with other studies.[40]

Conclusions

Our study suggests that a serum AMH concentration >5.03 ng/mL done on the automated assay may help facilitate diagnosis of PCOS in women of Indian origin. AMH also helps differentiate between PCOS phenotypes which are known to reflect severity of the syndrome. AMH showed a slower decline in PCOS patients. Even though women with PCOS and PCOM show a higher response to gonadotropin and a higher total number of mature oocytes than controls, there was no difference in rate of maturation between groups which substantiates the suggestion that there is a heterogeneity of steroidogenesis between individual follicles. Serum AMH is likely to emerge as an important marker of PCOS and may replace PCOM in the diagnostic criteria for women of reproductive age group.

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Conflicts of interest

There are no conflicts of interest.

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