Anti-Müllerian hormone predictive levels to determine the likelihood of ovarian hyper-response in infertile women with polycystic ovarian morphology

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Abstract

Background: The objective of this study was to investigate how the serum levels of Anti-Müllerian hormone (AMH) in normal-ovulatory infertile women with polycystic ovarian morphology (PCOM) is associated with ovarian hyper-response?

Methods: This prospective cohort study was carried out on 100 infertile women with PCOM who were treated by antagonist/agonist-triggered stimulation protocol in Shahid Akbar-Abadi Hospital IVF center, Tehran, Iran. Serum AMH levels were measured before starting the ART cycle and the ovarian hyper-response was evaluated by retrieved oocyte numbers, estradiol levels on triggering day and the incidence of OHSS clinical signs and symptoms. Logistic regression and the area under the curve (AUC) were used to estimate the effects of AMH and accuracy of test.

Results: ROC curve analysis showed that AMH had a significant performance to predict ovarian hyper-response in PCOM patients (AUC = 0.73). The estimated threshold value was 4.95 ng/ml, specificity was 74.58% (95%CI: [50.85, 93.22]), and sensitivity was 73.17% (95%CI: [48.78, 92.68]). The results of logistic regression showed that there was a significant interaction between AMH and BMI ($P = 0.008$) so BMI had a moderation effect. In other words, the AMH cut-off values to predict the ovarian hyper-response were different for different BMI.

Conclusions: Considering the AMH cut-offs for different BMI categories would be valuable to adapt a tailored, effective and safe stimulation program for infertile women with PCOM.

Introduction

Anti-Müllerian hormone (AMH) is a member of the large family of Transforming Growth Factor-beta (TGF- β). It is secreted from the granulosa cells of the small antral and pre-antral follicles to set the initial stages of follicular evolution [1]. It is also a hormone marker suitable for evaluating the follicular number of the ovary and its serum levels indirectly show ovarian reserve[2]. The AMH level is independent of the hypothalamus-pituitary axis[3]. Therefore, there is little variation during a menstrual cycle and at intervals between cycles [4]. The serum levels of AMH are closely related to the number of primary antral follicles in normal women and women with Polycystic ovary syndrome[5]. As the low level of AMH represents low ovarian reserve, its high serum level also indicates increased ovarian reserve and can be a valuable tool for PCOS detection[6]. According to Rotterdam's criteria, PCOS is defined as the most common endocrinopathy in women of reproductive age, with the presence of two of the following conditions: Oligoavulation or non-ovulation, clinical or laboratory hyperandrogenism, and polycystic ovarian morphology (PCOM) in ultrasound. Failure of follicular maturation in patients with PCOS leads to non-ovulation, and accumulation of pre-antral and antral follicles; this is clearly associated with increased AMH secretion[7]. In Assisted reproductive technique (ART) cycles, infertile women with PCOS have a higher incidence of ovarian hyper-stimulation syndrome (OHSS) as a potential iatrogenic and potentially life-threatening problem [8, 9]. This risk is higher in women with higher serum AMH levels. On the other hand, a group of healthy women with regular menstrual cycles and normal ovulation, without clinical or lab evidence of hyperandrogenism, are other candidates for ART. They have PCOM only in ultrasonography. PCOM is the presence of at least one ovary with 12 or more follicles with a size between 2 to 10 mm in a single plane or a volume of ovaries greater than 10 ml in the absence of a dominant follicle greater than 10 mm, luteal corpus luteum or cyst. This condition is seen in the absence of PCOS in 25% of normal women [10]. The primary outcome of this study was to evaluate the AMH predictive level to determine the likelihood of ovarian hyper-response among normal ovulatory infertile women with polycystic ovarian morphology. According to recent data about the effects of body mass index (BMI) on AMH level [11–13], we also purposed a secondary objective to investigate the AMH cut off levels in different BMI categories among women with PCOM.

Materials And Methods

Study population

This prospective cohort study was carried out on 100 infertile women with PCOM referred to an IVF center of Shahidakbar-Abadi Hospital in Tehran. Women were between the ages of 20 and 40 years old, and candidates for ART with tubal or male factor. All participants with regular menstruation had no symptoms of clinical or laboratory evidence of hyperandrogenism and hyperandrogenemia. Based on International evidence-based guideline for the assessment and management of polycystic ovary

syndrome [14], women with an ovarian volume ≥ 10 ml on either ovary in endovaginal ultrasound assessment were considered as patients with polycystic ovarian morphology (PCOM).

Women with the following characteristics were excluded: age below 20 and over 40 years of age, with thyroid disorder or hyperprolactinemia, premature ovarian failure, abnormal karyotype, and clinical or laboratory hyperandrogenism. The method of study was approved by the Ethics Committee of Iran University of Medical Sciences and was registered with the code CIR.IUMS.RE 1394.92190025711. All participants signed a written informed consent. At the beginning of each cycle, demographic characteristics including age and BMI were recorded. Measurement of serum AMH levels was performed using Anti Mullerian Hormone (AMH) Gen II enzyme linked Immunosorbent Assay (ELISA) kit (Beckman Coulter Immunotech, USA). The lowest detection rate limit with a 95% probability is 0.08 ng/ml.

In the antagonist cycle, patients were monitored on sonography of the second day of the menstrual cycle and received recombinant FSH at a dose of 150 units per day (Gonal-F®; Merck, Geneva, Switzerland) and follicular growth was monitored by vaginal ultrasonography. The onset of 0.25 mg GnRH-antagonist daily was associated with follicular diameters at 13–14 mm (Cetrotide 0.25 mg, Merk Serono, Germany). With at least three follicles of 17 mm size, triggering was performed with 0.2 mg GnRH-Agonist injection (Decapeptyl 0.1 mg by Ferring Pharmaceuticals). Serum estradiol levels were measured on the triggering day and ovarian puncture was performed 36 hours later. The number of oocytes and the presence and severity of clinical symptoms of OHSS were documented. All embryos were frozen to transfer in subsequent FET cycles. A patient was considered as a hyper-responder when a triggering day estradiol level was more than 3500 pg/dl, and/or retrieved oocytes was more than 15 [15], and/or existing clinical manifestations of OHSS.

Statistical analysis

Data were analyzed using R software version 3.4.1, “pROC”, “plotROC”, “verification”, “ResourceSelection”, “multcomp”, and “ggplot2” packages [16–18]. Primary descriptive results were reported using Median and interquartile range (IQR) for quantitative non-parametric variables and mean $\pm$ standard deviation (SD) for normal variables, and percent (number) for qualitative variables. Normality of quantitative variables were assessed by Lilliefors test. Mann-Whitney U or independent sample t-test were used to compare the distribution of quantitative variables or mean as appropriate. Association of qualitative variables was evaluated using Chi-square test and p values estimated base on 10000 sampled tables by Mont Carlo method.

Binary logistic regression was used to estimate the effects of AMH and other factors on hyper-responding. Outputs of this method were reported using odds ratio (OR) and 95% confidence interval (95% CI). Receiver operating characteristics (ROC) analysis was used to evaluate the prediction performance of AMH. Accuracy of test was estimate by the area under the curve (AUC) and confidence interval of that was calculated using DeLong method. To get the best cut-off points and the clinical diagnostic ability of AMH, Youden's index (J) was used [19]. This index is defined as $J = \max [\text{sensitivity}(j) + \text{specificity}(j) - 1]$ where j is the cut-off point and it is a popular measurement for the ROC curve analysis and an optimal trade-off between sensitivity and specificity [20]. Confidence intervals of sensitivity and specificity were computed with 2000 stratified bootstrap replicates. A total sample size of 100 achieved 86% power to detect a change in sensitivity from 50–74.58% using a two-sided binomial test and 95% power to detect a change in specificity from 50–73.17%. The level of significant was set at $P < 0.05$.

Results

In this prospective cohort, the information from 100 infertile patients with PCOM was analyzed to determine the performance of AMH as a biomarker for hyper-responding during IVF cycles. Table 1 presents the demographic and biochemical baseline characteristics and the controlled ovarian stimulation (COS) outcome of PCOM patients with and without ovarian hyper-response. The hyper-response after COS was defined as retrieved oocyte number more than 15 and/or estradiol level on triggering day more than 3500 pg/ml. In total, 41% (n = 41) of the PCOM patients met the criteria for ovarian hyper-response. According to utilization of GnRH antagonist/agonist triggered/freeze all protocol and based on Navot's criteria (Navot et al 1992), we did not have any case of moderate, severe or critical OHSS. In the hyper-responder group, 20 patients (48.8%) represented some mild clinical manifestations of hyper-response including nausea and/or bloating which were symptomatically managed as outpatient.

The medians of number of oocytes in the suboptimal/normal responder and hyper-responder groups were 8 and 20, respectively. The difference in the two medians was statistically significant ($P < 0.001$). The serum estradiol level in the hyper-responder group

increased dramatically on the triggering day ($P < 0.001$). In addition, the average of BMI of patients in the hyper-responder group was significantly lower than that of the suboptimal/normal responder ($P = 0.027$). There was a difference in the medians of AMH between the two groups, suggesting that AMH positively affected the level of ovarian response ($P = 0.002$).

The main aim of the present study was to evaluate the performance and accuracy of AMH as a clinical predictor for the likelihood of ovarian hyper-response during ovarian stimulation in ART cycles in patients with PCOM. According to a crude analysis by logistic regression, the odds of hyper-responsiveness increased 1.28-fold with each one ng/ml increase in the level of AMH (OR = 1.28, 95% CI: [1.11, 1.5], $P = 0.001$) (Table 2). Interestingly, the Hosmer and Lemeshow test as a statistical method to evaluate the goodness of fit of a model, was not significant in this model, indicating that AMH is an appropriate biomarker to predict the ovarian response in patients with PCOM during IVF cycles (chi-squared = 9.76, degree of freedom = 8, $P = 0.28$).

Receiver operating characteristics (ROC) curve analysis of AMH showed that AMH had a significant performance to assign the PCOM patients to their true status of hyper and normal responder groups. Furthermore, the area under the curve (AUC) was equal to 0.73 which indicates reasonable accuracy of the test, and it was statistically different from a test that assigned patients to the groups randomly (AUC = 0.73, 95% CI: [0.63, 0.83], $P < 0.001$). In other words, 73% of patients were correctly assigned to the suboptimal/normal responder or hyper-responder groups by AMH. Figure 1 shows the ROC curve of the AMH marker. The point in the figure shown by the multiplication sign refers to the best cut-off point, which was estimated by Youden's index (J) (threshold value = 4.95, 95% CI: [3.85, 6.60]). According to the estimated threshold value by Youden's index (J), the specificity of AMH was 74.58% (95%CI: [50.85%, 93.22%]) and the sensitivity was 73.17% (95%CI: [48.78%, 92.68%]) (first row of Table 3).

Correlation analysis of BMI and AMH showed that there was an inverse correlation between these variables in the hyper-responder and the suboptimal/normal responder patients ($r = -0.311$, $P = 0.048$ and $r = -0.349$, $P = 0.007$ for the hyper-responder and suboptimal/normal responder groups, respectively). Generally, the correlation between AMH and BMI in the patients with PCOM was significantly negative ($r = -0.311$, $P = 0.002$) (Fig. 2). This negative correlation showed that different values of BMI could moderate the behavior of AMH as a biomarker in predicting ovarian hyper-response.

Based on the WHO classification, BMI is classified into three groups, included BMI less than 25 Kg/m², between 25 and 30 Kg/m², and greater than 30 kg/m². As described above, the crude analysis by logistic regression showed that there was a positive association between increasing crude AMH and a higher risk of hyper-responding (Table 2). Conversely, the association of BMI and a higher risk of hyper-responding were significantly negative. In other words, the odds of a hyper-response for a patient with a BMI from 25 up to 30 kg/m² was 0.14-fold less than a patient with a BMI < 25 kg/m² (OR = 0.14, 95% CI: [0.05,0.39], $P < 0.001$). Additionally, the odds of a hyper-response in a patient with a BMI greater than 30 kg/m² was 0.3-fold less than a patient with a BMI < 25 kg/m² (OR = 0.3, 95% CI: [0.09,0.9], $P = 0.035$).

Table 2 shows the results of the multivariate logistic regression, which estimated the effects of AMH, BMI, and their interactions. The effect of AMH on ovarian hyper-response at the different levels of BMI did not have the same slope because of the existing significant interaction between AMH and BMI (Deviance of likelihood ratio test = 7.51, degree of freedom = 2, P value = 0.023), so it was necessary to consider the relationship of AMH and hyper-responding in each subgroup of BMI separately. Consequently, there was an about a 2.38-fold increase in the odds of developing a hyper-response with each one ng/ml increase of AMH in BMI $\geq$ 30 kg/m² compared to BMI < 25 kg/m² (OR = 2.38, 95% CI: [1.19, 6.62], $P = 0.035$) (Table 2).

To better understand the behavior of the interaction effect, Fig. 3-A is presented. In this chart, the probability of developing a hyper-response is shown against the increase in AMH based on BMI groups. For AMH values less than about five ng/ml, patients with a BMI < 25 kg/m² had the highest probability of developing a hyper-response, but above five, the probability of a hyper-response was highest in the BMI > 30 kg/m² group. Overall, this chart shows that an increase of AMH increases the probability of developing a hyper-response in all three groups of BMI, but this increase was much steeper in PCOM patients with a BMI $\geq$ 30 kg/m².

According to the BMI classification, ROC curves of AMH showed that the accuracy of AMH for predicting hyper-responsiveness in all three classes of BMI constantly increased (Fig. 3-B). Advanced analysis revealed that there were different cut-off points for AMH by BMI classification (Table 3). These results were estimated using Youden's index (J) and they were consistent with the previous results of logistic regression.

Discussion

Serum AMH level is an indirect reflection of the ovarian follicular reserve and therefore, many researchers consider it to be a sensitive biomarker of ovarian aging and ovarian reserve [2, 8]. AMH serum levels are closely correlated with the number of early antral follicles in both healthy women and women with PCOS [5, 21] and it is mostly produced by granulosa cells of follicles from 2 to 9 mm in diameter. Impaired folliculogenesis in PCOS patients may cause excess accumulation of pre-antral and small antral follicles, which may ultimately lead to an increase in AMH level. Many studies have demonstrated high AMH levels in PCOS patients and although there is an absence of a worldwide standard for serum AMH assays and thus an inability to define thresholds, it has been previously suggested that a hyper-response or OHSS might be anticipated at around 3.5 ng/ml or higher during ART cycles [6, 8, 22].

In our study, we investigated the role of AMH as a predictor of ovarian hyper-response in a specific group of infertile women with PCOM, a group of women with regular menstrual cycles and normal ovulation, without hyper-androgenism, but with polycystic ovarian morphology on ultrasound examination. We observed that AMH levels in our PCOM hyper-responders (based on a triggering day estradiol level > 3500 pg/dl, and/or > 15 retrieved oocytes and/or, clinical manifestations of OHSS), were significantly higher than in the PCOM suboptimal/normal-responder group (6.8 versus 3.8 ng/ml, p value = 0.002). In addition, with each one ng/ml increase in the AMH level, the risk of hyper-responsiveness increased by 1.28 fold.

Different studies have calculated various AMH cut-off values for hyper-response in non-PCOS infertile women and in patients with PCOS. Vembu and Reddy (2017) in their study of 246 women (31% PCOS and 78% non-PCOS) suggested a cut-off value of 6.85 ng/ml with a sensitivity of 66.7% and a specificity of 68.7% in PCOS and 4.85 ng/ml with a sensitivity of 85.7% and a specificity of 89.7% in non-PCOS patients, to predict a hyper-response [9]. On the other hand, Zhang et al. (2017) proposed a lower cut-off value to predict ovarian hyper-response among their 120 PCOS patients, namely 2.84 ng/ml with a sensitivity and specificity of 72.7% and 65.9%, respectively [23]. These differences in AMH threshold could be related to various factors such as a lack of a well-defined population, stability and heterogeneity of circulating AMH, a wide range of reference values, inter-laboratory variability, and different immunoassays used worldwide [6].

We have calculated an AMH threshold specifically for a normal-ovulatory subgroup of infertile women with polycystic ovarian morphology, which, based on our literature reviews, has not been investigated previously. At a cut-off value of 4.95 ng/ml, the risk of hyper-response was increased in our studied PCOM patients with a specificity of 74.58% and a sensitivity of 73.17%. Because of the relatively limited numbers in our studied population, further studies with a larger number of PCOM patients are required to develop a more precise cut-off value. However, based on our findings, we suggest it is possible to tailor a safe stimulation protocols for normal-ovulatory infertile patients who have a polycystic ovarian appearance and an AMH level over 4.95 ng/ml.

It is also noteworthy that we had a group of poor/suboptimal-responders among our PCOM patients. Despite the increased antral folliculate count, the low follicle output rate (FORT) in this group of patients might be related to hypo-sensitivity/hypo-response to FSH due to genetic characteristics like FSH receptor polymorphism or LH-beta variants [24].

The average BMI in our PCOM hyper-responder group was significantly lower than among the suboptimal/normal responder ($P = 0.027$), and in the univariate analysis, the association between BMI and a high risk of hyper-response was significantly negative. On the other hand, the correlation analysis of BMI and AMH showed an inverse correlation between these two variables among both hyper-responder and suboptimal/normal responder PCOM patients ($r = -0.311$, $P = 0.002$). The correlation between AMH and BMI has also been investigated by some other recent studies and the results are controversial.

In their retrospective study of 951 non-PCOS women, Simões-Pereira et al. (2018), did not observe any significant effect of BMI on AMH levels [25]. In another retrospective cohort study, Kriseman et al. (2015) did not find any association between BMI and AMH levels in a general population of infertile women or in patients without PCOS [26]. However, BMI was significantly and inversely correlated with AMH among their 104 PCOS patients. In addition, Lefebvre et al. studied 691 women and found no effect of metabolic status on serum AMH levels in the non-PCOS group, but a significant, albeit weak, negative independent correlation was found between AMH and BMI for women with PCOS [27].

A recent meta-analysis showed that the AMH level is significantly lower in obese than in non-obese reproductive-aged women, and BMI is negatively correlated with AMH in PCOS and non-PCOS subjects. The authors concluded that PCOS and fertility status do not

appear to affect this association [12]. Interestingly, weight loss in adolescent girls with PCOS has been found to be associated with a significant drop in AMH concentrations and the hormone level becomes normalized [11]. Nilsson-Condori et al. (2018) also observed that AMH levels increased after a period of following a very low-calorie diet before bariatric surgery among 48 young obese women but then it decreased at 6 and 12 months after Roux-en-Y gastric bypass beyond the expected normal age-related decline[13]. However, they did not evaluate their subjects for ovarian morphology and PCOS. A negative impact of BMI on AMH levels has also been reported among women with a diminished ovarian reserve[28].

We hypothesized that the negative correlation between AMH and BMI could change the behavior of AMH as a biomarker in predicting an ovarian hyper-response in the presence of different values of BMI. BMI is also a possible predictive factor for ART outcomes, so it could be confounded with the relationship between AMH and an ovarian hyper-response. Therefore, it is statistically important to evaluate the performance of AMH as a predictor after adjustment for other important confounders and to consider the interaction effects between them. Our multivariate logistic regression analysis revealed that there was a significant interaction between AMH and BMI. In general, we observed there was an increase in the AMH level, increasing the probability of developing a hyper-response in all BMI groups, but this increase was more prominent in PCOM patients with a BMI over 30 kg/m². Consequently, the accuracy of AMH for predicting ovarian hyper-response in the three classes of BMI constantly increased and there were different cut-off values for AMH due to BMI classification in PCOM patients.

This finding suggests that the behavior of serum AMH level as a predictive biomarker for ovarian response might be more complicated in PCOM patients with a higher BMI, and it may not accurately present the true ovarian capacity to develop an exaggerated response in obese patients. Although there is no clear explanation for this issue, one possible explanation could be the positive correlation between AMH and LH levels[29]. LH levels are suppressed in obese women due to increased peripheral aromatization and estrogen production in fat tissue, which may result in lower serum AMH levels in these patients [30]. Recently, it has been demonstrated that serum AMH levels are positively correlated with antral follicular count but are also positively correlated with serum LH and free testosterone levels and negatively correlated with total body fat and percent body fat in PCOS patients [31]. In addition, it has been suggested that obesity may affect the catabolism of AMH [32]. These correlations have not been investigated in the present study but should be investigated in future RCTs. It would also be interesting to study the AMH predictive values for ovarian responses in relation with other predictive factors such as BMI in other groups of infertile women and especially among different subtypes of PCOS patients. These findings would be beneficial to develop an individualized controlled ovarian stimulation program for each infertile woman.

In conclusion, the findings of the present study suggest that infertile normal-ovulatory women with polycystic ovarian morphology are at risk of an ovarian hyper-response at AMH levels greater than 4.95 ng/ml. For this reason, individualized stimulation protocols for this group of patients with PCOM and AMH greater than 4.95 ng/ml may significantly reduce the chance of developing established moderate or severe forms of OHSS. Using lower starting doses of gonadotropins, antagonist/agonist triggered stimulation protocols and freezing all embryos are suggested effective strategies to achieve this goal. On the other hand based on our findings, women with polycystic ovarian morphology but with AMH level lower than 4.95 ng/ml are not considered high risk for hyper-response and using other stimulation protocols and fresh embryo transfer would be considered appropriate for them.

However, the AMH cut-off values to predict the ovarian hyper-response are different for different BMI categories among PCOM patients, and thus it becomes a more precise predictive marker as BMI rises. Considering the AMH cut-offs for different BMI categories would be valuable to develop an individually tailored, effective, and safe stimulation program for infertile women with PCOM.

Declarations

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Authors' contributions

AAS: Experimental design, Data Collection, Writing Manuscript. MA: Experimental design, Data Collection, editing Manuscript; NM: Data analysis, Writing Manuscript; MMA: Data Collection, editing Manuscript; AA: Project development, Experimental design, Data

Collection, Writing Manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The method of study was approved by the Ethics Committee of Iran University of Medical Sciences and was registered with the code CIR.IUMS.RE 1394.92190025711. The informed consent was obtained from all the participants.

Consent for publication

All authors provided final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions.

Competing interests

The author declares that there is no conflict of interest.

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Tables

Table 1. Demographical and biochemical baseline characteristics and COS outcomes of PCOM patients with and without ovarian hyper-response			
	PCOM women without ovarian hyper-response after COS (n=59)	PCOM women with ovarian hyper-response after COS (n=41)	P value
Characteristic data			
Age (year), Mean ±SD	31.02±4.29	30.59±5.89	0.690
FSH (IU/ml), Mean ±SD	5.54±2.61	5.93±3.10	0.474
Total gonadotropin dose (IU) , Mean ±SD	2800±101.54	1825±48.35	<0.001
Duration of stimulation (days) , Mean ±SD	12.5±1.3	12.15±1.1	0.134
AMH (ng/ml), Median (IQR)	3.8 (3.15,5.45)	6.8 (4.8,8.8)	0.002
BMI (kg/m ²), Mean ±SD	27.82±3.80	26.06±3.92	0.027
BMI categories (kg/m ²), % (n)			
<25	15.3(9)	48.8(20)	0.001
25-30	59.3(35)	26.8(11)	
≥30	25.4(15)	24.4(10)	
COS outcomes			
Number of follicles on triggering day, Median (IQR)	8 (5,10)	20 (16,27)	<0.001
Number of follicles on triggering day based on ovarian response, % (n)			
Poor-response (0-3)	8.47 (5)	0 (0)	<0.001
Suboptimal-Response (4-9)	61.02 (36)	0 (0)	
Normal- Response (10-15)	30.51 (18)	0 (0)	
Hyper-Response (>15)	0 (0)	100 (41)	
Estradiol level on triggering day (pg/ml), Median (IQR)	1590 (904.5,2252)	6768 (2710,9000)	<0.001
PCOM: polycystic ovarian morphology, COS: controlled ovarian stimulation, AMH: anti-müllerian hormone, FSH: follicle stimulating hormone, BMI: body mass index, ovarian hyper response: retrieved oocytes>15 and/or estradiol level on triggering day>3500 pg/ml.			

Table 2. Evaluating and estimating the prediction performance and the effects of AMH and BMI using univariate and multivariate logistic regressions and analysis of the area under the curve.

	Univariate Analysis			Multivariate Analysis		
	OR (95% CI)	P value	AUC of model (95% CI)**	AOR (95% CI)	P value	AUC of model (95% CI)**
AMH (ng/ml)	1.28 (1.11,1.5)	0.001	0.73 (0.63, 0.84)	1.17 (0.91,1.59)	0.264	0.82 (0.74, 0.91)
BMI (<25 kg/m ²)*	1	-	0.71 (0.61, 0.81)	1	-	
BMI (25-30 kg/m ²)	0.14 (0.05,0.39)	<0.001		0.14 (0.01,1.6)	0.113	
BMI (≥30 kg/m ²)	0.3 (0.09,0.9)	0.035		0.01 (0,0.37)	0.026	
Increase 1 ng/ml of AMH in BMI (25-30 kg/m ²) to BMI (<25 kg/m ²)	-	-	-	1.02 (0.71,1.43)	0.911	
Increase 1 ng/ml of AMH in BMI (≥30 kg/m ²) to BMI (<25 kg/m ²)	-	-	-	2.38 (1.19,6.62)	0.035	
Abbreviations: OR: odds ratio, AOR: adjusted odds ratio, CI: confidence interval, AUC: the area under the curve (calculated by predicted values of logistic regression).						
* Reference level.						
** Confidence interval was calculated using DeLong method.						

Table 3. Estimating best cut of points of AMH in total samples, and according to BMI classification using Youden's index (J).

		Threshold (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)
Overall		4.95 (3.85,6.6)	74.58 (50.85,93.22)	73.17 (48.78,92.68)
BMI (kg/m ²)	<25	9.8 (4.65,10.3)	100 (55.56,100)	50 (20,95)
	25-30	5.45 (5,8.05)	77.14 (60,94.29)	81.82 (54.55,100)
	≥30	3.85 (2.65,5.9)	86.67 (53.33,100)	90 (50,100)
CI: confidence interval.				

Figures

Figure 1

Receiver operating characteristics (ROC) curve of AMH. The point, shown by the “x” refers to the best cut-off point, which estimated by Youden’s index (J). The gray rectangle refers to a 95% bivariate confidence interval of sensitivity and 1-specificity.

Figure 2

Correlations and linear trend lines of BMI and AMH base on ovarian response groups and total patients with PCOM (overall).

Figure 3

The likelihood of hyper-response against AMH based on BMI groups (A) and Receiver operating characteristics (ROC) curve of AMH in three groups of BMI (B).