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Research Article

The Clinical Role of Anti-Mullerian Hormone (AMH) in Assessing Ovarian Reserve among Polycystic Ovary Syndrome

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ABSTRACT
To assess the clinical implications of serum and follicular fluid AMH among polycystic ovary syndrome (PCOS) women receiving treatment with IVF/ICSI, a total of 168 women were enrolled in this study, 113 were PCOS while the remaining (n=55) were non-PCOS. Serum and follicular fluid AMH were analyzed, and compared to the endocrine profile and cycles outcomes. The results showed that both serum and follicular fluid AMH were increased significantly in PCOS women as compared to non-PCOS ones. A positive correlation was reported between them. The best cutoff points for them were detected by receiver operating characteristic (ROC) curve as 3.15 ng/ml and 3.65 ng/mg protein for serum and follicular fluid AMH, respectively. Using these cutoff points, the sensitivities were 94.2 % and 92, 9% and specificities were 100 % and 94.55 for serum and follicular fluid AMH, respectively. These data suggested that AMH level can offers a good diagnostic potency for PCOS, and it may be used as a marker of futility for counseling IVF/ICSI candidates.

Key words: Anti-Mullerian hormone, polycystic ovary syndrome, follicular fluid.

INTRODUCTION

Anti-Müllerian hormone (AMH), also known as Mullerian inhibiting substance (MIS), is a homodimeric glycoprotein member of the transforming growth factor-β (TGF- β) family. In females, AMH is mainly secreted by the granulosa cells of ovarian early developing follicles (1). The expression of AMH is localized in granulosa cells of primary, pre-antral and small antral follicles, suggesting an important role of AMH in human folliculogenesis (2). Since AMH is secreted exclusively in the gonads, its concentrations in females are thought to reflect the size of the ovarian follicle pool (3, 4). Moreover, AMH's role as a peripheral signal of the size of the growing follicle pool may have important clinical benefits. In women undergoing treatment for infertility, ovarian aging is characterized by decreased ovarian responsiveness to exogenous gonadotropin administration and poor pregnancy outcome. On one hand, correct identification of poor responders by assessment of their ovarian reserve before entering an in vitro fertilization (IVF) program is important. On the other hand, assessment of the ovarian reserve may also benefit patients that would generally be excluded from IVF programs because of advanced age (5). Polycystic ovary syndrome (PCOS), one of the most common endocrine disorders affecting 6.6 – 8% of women of childbearing age (6), is characterized by a marked increase in parental follicles number (7). PCOS encompasses a broad spectrum of clinical and biochemical characteristics. Although the mechanisms leading to PCOS are still poorly understood, the common denominator is a disturbance in the selection of the dominant follicle resulting in an ovulation (5). To date, controversial data are available regarding the relationship between the high AMH levels and the pre-antral follicles number in PCOS patients. Thus, is still unknown if the AMH excess in PCOS is secondary to the increase in pre-antral follicles number, or if an intrinsic increased AMH production by the granulosa cells is the cause of follicular arrest in PCOS (4).

In the current study, authors were concerned about the role of AMH as a marker for the quantitative aspect of ovarian reserve among PCOS patients. In addition, authors compared AMH levels in serum and follicular fluid from PCOS patients with age-matched non-PCOS individuals and compared them with the endocrine profile.

MATERIALS AND METHODS

Study population selection and stratification

Medical records were examined for patients who sought fertility consultation or treatment at the Infertility Division of the Department of Obstetrics-Gynecology, Ain Shams University Hospital. The study protocol was approved by the Medical Ethical Committee of Ain Shams University Hospital, Egypt, and was initiated after achieving written consent from the participants (n =168).

The inclusion criteria were patients who fitted the medical definition of infertility "One year of unprotected intercourse but not pregnant", with no previous in vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI) cycles, partner with normal semen parameters and

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Table 1: Clinical and endocrine parameters among investigated groups.

Characteristics	Non-PCOS (n= 55)	PCOS (n= 113)	*P*
Age (years)	28.2 ± 4	29.2 ± 3	0.099[a]
BMI (Kg/m^2)	26.6 ± 5	27.8 ± 4	0.084[a]
Duration (Days)	12.7 ± 1.8	11.8 ± 1	< 0.0001[a]
Total Oocyte retrieved	8.2 ± 5	15.6 ± 6	< 0.0001[a]
Mature oocyte	6.5 ± 4	13 ± 5	< 0.0001[a]
Fertilized oocyte	5 ± 3	10 ± 4	< 0.0001 [a]
Embryo number	6.7 ± 2.7	5 ± 1.9	0.587 [a]
FSH (IU/L)	6.7 ± 2.3	6.3 ± 1.7	0.208 [a]
LH (IU/L)	5.9 ± 2.5	6.3 ± 2	0.242 [a]
E2 (IU/L)	41 ± 0.13	40 ± 1.6	0.76 [a]
PRL (IU/L)	18 ± 8.6	13.5 ± 5.2	< 0.0001 [a]
TSH (IU/L)	2.1 ± 0.6	2.1 ± 0.8	0.909 [a]
Serum AMH (ng/ml)	2.2 ± 0.4	3.8 ± 0.5	< 0.0001 [a]
Follicular AMH (ng/mg protein)	2.7 ± 0.5	4.2 ± 0.5	< 0.0001 [a]
Chemical pregnancy			< 0.0001[b]
Non-pregnant (n= 67)	33 (60%)	34 (30.1%)	
Pregnant (n=101)	22 (40%)	79 (69.9%)	
Clinical pregnancy			< 0.0001[b]
Non-pregnant (n= 67)	33 (60%)	34 (30.1%)	
Pregnant (n=101)	22 (40%)	79 (69.9%)	

Statistical analysis using [a] analysis of variance (ANOVA), [b] Chi-square test.

matched, while the exclusion criteria was the presence of (1) history of ovarian or adnexal surgery, (2) suspicious findings of ovarian malignancy, and (3) presence of endocrine disorders such as diabetes mellitus, hyper-prolactinemia, thyroid dysfunction, congenital adrenal hyperplasia, cushing's syndrome, and adrenal insufficiency.

Accordingly, the enrolled patients were categorized into patients who were diagnosed for the first time with polycystic ovary syndrome (PCSO) (n=113) and patients without PCSO (n=55). The diagnosis of PCSO was done according to Rotterdam criteria (8). According to the Rotterdam criteria, we accepted the presence of two of the three following characteristics for inclusion in the study: (1) oligomenorrhea/amenorrhea, (2) clinical (hirsutism) or biochemical finding of hyper-androgenism, and (3) polycystic ovaries on transvaginal sonography. While the non-PCOS individuals were 23 normal females and their husbands were diagnosed with either oligosperms, or asthenosperms or azosperms,19 females diagnosed with tubal (mono1bi-late), and 13 females with endometriosis.

Ovarian stimulation protocols

All women underwent controlled ovarian hyper-stimulation (COH) with gonadotropin (GnRH) long

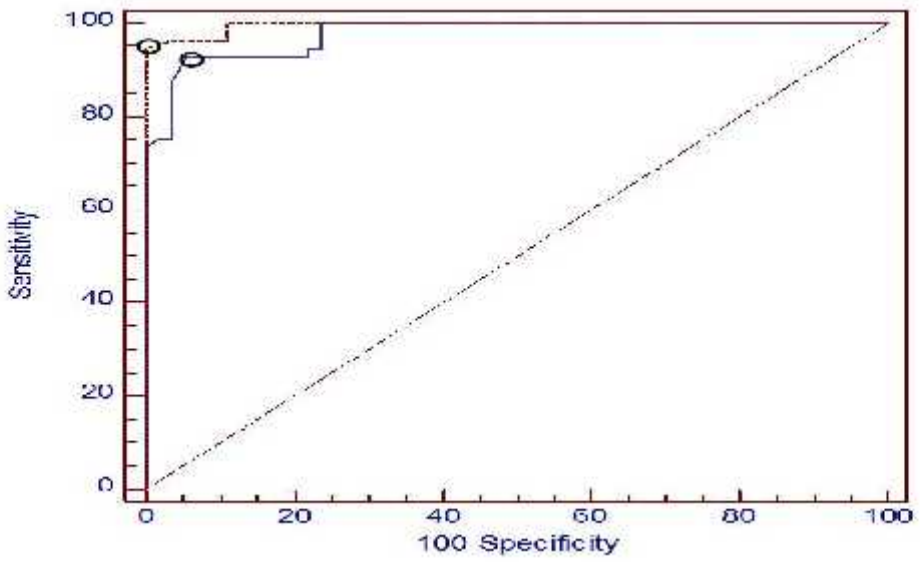

Figure 1: ROC curve analysis for AMH to calculate the best cutoff point that discriminates between PCOS and non-PCOS individuals. Open circle denotes best cut-off point of serum AMH (dashed line) as 3.15 ng/ml and follicular fluid AMH (solid line) as 3.65 ng/mg protein. Difference between areas (SE) 0.0193 (0.011) at 95% confidence interval -0.002 – 0.04 (P = 0.083).

protocol approved by Assisted Reproductive Treatment (ART) Unit, Ain Shams University Maternity Hospital. All participants received folic acid 400 mg/day before initiation the induction cycle, combined oral contraceptive pills on day 3 of the previous cycle. Then standard mid luteal protocol starts with daily subcutaneous injection of triptoreline acetate, on the day 21 of the previous cycle. Estradiol (E2)was measured on the 2nd day of menstrual cycle if less than 50 pg/ml, a daily human menopausal gonadotropin (HMG) (Ferring, Germany)or purified urinary FSH (Company, Country)injection was started. The starting dose of GnRH was prescribed according to age and body mass index (BMI) (9) of the patients. PCOS patients have received 150 IU of human menopausal gonadotropin (HMG; Menopur; Ferring GmbH, Kiel, Germany) regardless of the age. Then at the 6th day of stimulation the dose was adjusted according to ovarian response which was assessed by Transvaginal Ultrasound (TVU/S) (Siemens, Sonoline G20). After at least one follicle reached 14 mm in diameter, a daily injection of 0.25 mg of cetrorelix (Cetrotide; Serono, Baxter Oncology GmbH, Halle, Germany) was given until the day of HCG administration.

When at least two follicles reached 18 mm in diameter, 10,000 IU HCG (Pregnyl; Schering-Plough, Kenilworth, NJ, USA) was administered and oocyte retrieval was performed 34-36 hours later. Conventional IVF or ICSI was conducted 4-6 hours post oocyte retrieval. For IVF, each oocyte was inseminated with 20 X 103 motile spermatozoa in a single droplet containing 20 µl of fertilization medium (Quinn's Advantage Fertilization medium; SAGE IVF Inc. Trumbull, Connecticut, U.S.A.). For ICSI, 1-2 µl washed spermatozoa were placed in 7% polyvinylpyrrolidone (PVP; SAGE IVF Inc.) and a sperm was injected into each denuded oocyte using standardized techniques. Each embryo was cultured in a single droplet containing 20 µl of medium (Quinn's Advantage Cleavage medium; SAGE IVF Inc.) and incubated under the atmospheric composition of 5% CO2, 5% O2 and 90% N2 at 37°C. All embryo transfers were performed at 72 hours post oocyte retrieval.

Luteal support and confirmation of pregnancy

The luteal phase was supported by intramuscular injection of 50 mg of progesterone and vaginal supplementation of 300 mg micronized progesterone (Progeffik; Effik, Paris, France) or Crinone 8% progesterone gel (Columbia Laboratories, Inc., Livingston, NJ) once per day. Serum HCG was measured 14 days after oocyte retrieval and a value above 5 IU/ml was designated as positive pregnancy. Clinical pregnancy was defined as a pregnancy diagnosed by ultrasonographic visualization of the gestational sac. Viable pregnancy was defined asgestation age greater than 7th weeks with documented fetal cardiac activity by ultrasound.

Samples Collection

Serum collection: On the day of oocyte retrieval, the women underwent blood sampling by venipuncture at approximately 9:00 AM. Serum was separated and frozen in aliquots at –80 °C for subsequent centralized analysis.

Table 2: Predictability of AMH as s marker for ovarian reserve as compared to endocrine profile.

Mean ± SD	Total individuals (n=168)		PCOS (n=113)		Non-PCOS (n=55)	
	Non-Pregnant (n=67)	Pregnant (n=101)	Non-Pregnant (n=34)	Pregnant (n=79)	Non-Pregnant (n=33)	Pregnant (n=22)
Serum AMH (ng/ml)	3.3 ± 1	3.4 ± 0.7[a]	4.15 ± 0.4	3.7 ± 0.5[a]	2.1 ± 0.4	2.4 ± 0.4[a]
Follicular fluid AMH (ng/mg protein)	3.5 ± 0.9	3.9 ± 0.8[a]	4.3 ± 0.5	4.2 ± 0.5	2.7 ± 0.6	2.8 ± 0.4
FSH (IU/L)	6 ± 2.6	6.7 ± 1.7[a]	5.2 ± 1.9	6.7 ± 1.5[a]	6.8 ± 2.9	6.4 ± 2.3
LH (IU/L)	5.8 ± 2	6.4 ± 2.2[a]	5.4 1.9	6.7 ± 2[a]	6 ± 2.3	5.7 ± 2.8
PRL (IU/L)	16 ± 7	14.4 ± 6.5	14.4 ± 4.9	13 ± 5.4	17.6 ± 8.7	18.7 ± 8.6
E2 (IU/L)	4.3 ± 1.2	39 ± 1.6	42.6 ± 1.3	39.5 ± 1.7	43.8 ± 1	37.3 ± 1
TSH (IU/L)	2.2 ± 0.7	2 ± 0.8	2.3 ± 0.6	2 ± 0.8	2.1 ± 0.6	2.2 ± 0.5

[a] *Statistical analysis using analysis of variance (ANOVA) at P<0.04.*

Follicular fluid collection: Follicular fluid was collected from individual follicles at the time of laparoscopy, using a single lumen 17-gauge needle (Casmed, UK). Each sample was collected into a sterile tube without culture medium. No flushing medium was used. Follicular fluid from the first retrieved follicle of bilateral ovaries will be collected. Follicular fluid was collected from follicles > 17mm in diameter, free from blood contamination. The follicular fluid from all aspirated follicles from each patient was pooled and was immediately centrifuged at 3000g for 15 min at -4 C to eliminate cellular elements. Supernatants were removed and stored at -80 C prior to analysis of biochemical and hormonal analysis. Three to four follicles were aspirated from each patient in both groups.

All patients underwent the procedure in the early follicular phase of the menstrual cycle as assessed by the last day of the menstrual cycle, by their endocrine profile and by transvaginal ultrasound scan. In the PCOS group, the day of serum collection was either after a spontaneous period or randomly chosen because they were amenorrhoeic. All patients underwent a transvaginal ultrasound scan prior to the procedure in addition to an endocrine profile to confirm that they were in the follicular phase, as well as to confirm the absence of a dominant follicle.

Biochemical assessments

Assessments in serum: Serum AMH levels were determined using a commercially available "second generation" enzyme-linked immunosorbent assay kit (Glory Science Co., Ltd, USA). Intra-assay and inter-assay coefficients of variation were < 6% and < 10%, respectively, with the lower detection limit at 0.13 ng/mL and linearity up to 21 ng/ml for AMH. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), prolactin (PRL), and estradiol (E2) levels were determined with an automated multi-analysis system using a chemiluminescence technique (Advia-Centaur; Bayer Diagnostics, Puteaux, France).

Assessments in follicular fluid

Follicular fluid AMH measurement was done using the same methodology. To avoid possible bias due to follicular fluid volume variability, hormone concentrations in the follicular fluid were adjusted to its protein content, as previously reported elsewhere (10). Protein was measured according to the conventional Biuret reaction (11) using an automated multi-analysis system (AU640; Olympus, Rungis, France). Follicular fluid hormone level was expressed as ng/mg of protein for AMH.

STATISTICAL ANALYSIS

The threshold value for optimal sensitivity and specificity of AMH was determined by Receiver Operating Characteristics (ROC) curve, which was constructed by calculating the true-positive fraction (sensitivity %) and false-positive fraction (100- specificity %) of the above-mentioned markers at several cut-off points. The ROC curve can be used to select the best cutoff to investigate the predictability of AMH levels in both serum and follicular fluid among the investigated group that maximizes the

Table 3: Comparison between PCOS and non-PCOS individuals based on serum and follicular fluid AMH level.

	Cutoff point	AUC	Sen.%	Spec.%	PPV%	NPV%	Acc.%	*P*
Serum AMH (ng/ml)	3.15	0.996	94.2	100	100	90.2	96.4	0.0001
Follicular fluid AMH (ng/mg protein)	3.65	0.977	92.9	94.55	97.2	86.7	93.5	0.0001

sensitivity and minimizes the false-positive rate (12). Univariate analyses were performed using a Chi-square test, simple linear regression analysis was used to establish the relationships between the AMH in the follicular fluid and serum levels among other investigated hormones. The level of significance (P) was determined to be less than 0.05. All analyses were performed using Statistical Package for the Social Sciences software 16.0 (SPSS Inc., Chicago, IL), and MedCalc10.2 (MedCalc Software, Mariakerke, Belgium).

RESULTS

Overall data

A total of 168 participants were included in this study. The mean ± SD (range) women's age were 28.9 ± 3.5 (20 – 39 years), the mean BMI 27.5 ± 4.6 (20 – 42 Kg/m2) At baseline (on cycle day 3 before COH), transvaginal ultrasonography identified 17.2 ± 0.8 early antral follicles. Controlled ovarian hyper-stimulation (COH) lasted 12.08 ± 1.4 days and 101 out of 168 women developed pregnancy.

One hundred and thirteen were diagnosed with PCOS, 79 of them developed pregnancy and the remaining failed to be pregnant. Among the 55 enrolled non-PCOS individuals, 22 become pregnant and the rest (n=33) were non-pregnant. Clinical and endocrine parameters for both groups are shown in Table (1).

Correlation among AMH levels with clinical and endocrine parameters

Among the collective study population, a significant correlation was reported between serum and follicular AMH (R=0.711, P< 0.0001). Both of them showed significant negative correlation with PRL (R= -0.316, P< 0.0001 and R= -0.307, P< 0.0001, for serum and follicular AMH, respectively). As to oocyte and pregnancy; both of serum and follicular AMH showed significant correlation with oocyte (P<0.0001) and pregnancy (R= 0.220, P< 0.0001). Moreover, follicular fluid AMH showed a significant correlation with E2 (R=0.173, P=0.025).

Regarding the PCOS group significant correlation was reported between serum and follicular fluid AMH (R= 0.38, P< 0.0001). Serum AMH was significantly correlated with LH (R= -0.197, P= 0.037), also between follicular fluid AMH and E2 (R= 0.379, P< 0.0001), PRL (R= -0.226, P= 0.016), and both chemical and clinical pregnancy (R= -0.411, P< 0.0001). About the correlation between serum and follicular fluid AMH and clinical and endocrine parameters in non-PCOS group, significant correlation was reported between serum and follicular fluid AMH (R=0.401, P=0.002), also significant correlation was reported between both of them and pregnancy (R=0.328, P=0.015). In addition, significant correlation was reported between follicular fluid AMH with total oocyte retrieved (R= 0.279, P=0.039) and LH (R= 0.265, P=0.051).

Predictability of AMH as s marker for ovarian reserve as compared to endocrine profile

Authors tested the cycles outcome among all individuals regarding their endocrine profile and both serum and follicular fluid AMH. Significant results were reported with serum and follicular fluid AMH, FSH, and LH, as reported in Table (2).

Cutoff points of AMH in serum and follicular fluid among investigated groups

With respect to the prediction of PCOS patients from non-PCOS individuals, both serum and follicular fluid AMH demonstrated a high discriminative ability with respect to area under curve (AUC) of 0.996 ng/ml and 0.977 ng/mg protein, respectively as shown in Figure (1). The complete sensitivities, specificities, positive and negative predictive values and the accuracy are summarized in Table (3).

Using the above mentioned cutoff values, the distribution of AMH among the investigated groups showed increment of AMH in PCOS patients as compared to non-PCOS ones (P< 0.0001) as reported in Table (4).

Among all participants (n=168), by using the obtained cutoff points for serum and follicular fluid AMH, 73 out of 101 (72.3%) of the pregnant female showed positive (> cutoff point) serum and follicular fluid AMH (X2= 8.07, P =0.004, X2= 7.05, P =0.008, respectively).

Regarding the PCOS patients, only FSH, serum AMH and LH reported significant findings. When authors considered the AMH cutoff point, 73 out of 79 (92.4%) of the pregnant female showed positive (> cutoff point) serum and follicular fluid AMH (no significant results were recorded).

Moreover, the predictability of AMH in IVF/ICSI outcomes in PCOS women is presented in Table (5). Accordingly, women with positive AMH (> cutoff value) had higher fertilized oocyte, retrieved and mature oocyte

Table 4: Distribution of serum and follicular fluid AMH among investigated groups

	Non-PCOS (n= 55)	PCOS (n= 113)	*P*
Serum AMH (ng/ml)			< 0.0001
≤ 3.15 (n= 60)	55 (100%)	6 (5.3%)	
> 3.15 (n= 108)	0 (0%)	107 (94.7%)	
Follicular AMH (ng/mg protein)			< 0.0001
≤ 3.65 (n= 60)	52 (94.5%)	8 (7.1%)	
> 3.65 (n= 108)	3 (5.5%)	105 (92.9%)	

Statistical analysis using Chi-square test.

than those with negative AMH (≤cutoff value). Although no significant results were reported between AMH and either clinical or chemical pregnancy, women with positive pregnancy were having positive AMH.

DISCUSSION

Polycystic ovary syndrome (PCOS) is a common endocrine abnormality for women of reproductive age (13) and is the main cause of an ovulatory infertility (14). The pathogenesis of PCOS remains largely unknown though recent studies have suggested that anti-Mullerian hormone (AMH) may have a role to play in the disordered folliculogenesis in PCOS (15).

Our results revealed significant increment in the serum and follicular fluid of AMH for PCOS patients compared with age-matched non-PCOS individuals, these results are consistent with previous observations that levels of AMH in media conditioned in vitro by granulosa cells obtained from an ovulatory PCOS (16). This suggests that actual AMH production by granulosa cells in an ovulatory PCOS is significantly higher. Moreover, it has been suggested that increased number of follicles in PCOS are the source of elevated serum AMH (17) due primarily to the increased number of small antral follicles, assuming that each follicle produces a normal amount of AMH. In the current study that follicular fluid from size-matched follicles was pooled from each patient, thus the increased follicular fluid AMH produce greatly increased amounts of AMH in PCOS patients. No correlation between age and both serum and follicular fluid AMH was reported. Similarly, when authors consider the entire groups, no correlation was reported between AMH and BMI, these findings are in accordance with previous report (18). In the current study, Follicular fluid AMH concentrations in PCOS patients were 1.1 time higher than in serum, implicating the follicle is the major site of synthesis, further supported by the observed correlation between the follicular fluid and serum concentrations of AMH in PCOS patients (R= 0.38, P< 0.0001).

The endocrine profile was estimated among the study population. The authors reported increased levels of AMH,FSH, E2, and PRL while decreased levels of LH were reported in non-PCOS individuals as compared to PCOS ones. The increased level of LH in PCOS patients could be attributed to the finding previously reported that the appearance of LH receptors on follicles of smaller size in polycystic when compared with normal ovaries could be an important factor in the arrest of their future development (19). The high circulating levels of LH in our PCOS patients could have contributed to the increased levels of AMH we report. Also we report significantly increased AMH production in the PCOS follicles themselves, suggesting that the previously reported elevations in circulating levels of AMH are not solely due to increased numbers of AMH-producing follicles. Irrespective of the relationship between AMH and other circulating hormones, the observation that the PCOS follicles themselves are behaving in a fundamentally abnormal way regarding AMH productions, we believe, physiologically relevant.

Authors tested the diagnostic efficacy of AMH for PCOS by plotting the ROC curve. Interestingly, AMH showed significantly high sensitivity and specificity. On this basis it has been proposed that in situations where accurate ultrasound data are not available, AMH could be used instead of the follicle count as a diagnostic criterion for PCOS (20). We suggest that there is an intrinsic abnormality in the ovarian follicles themselves in PCOS, which cause them to produce more AMH thereby contributing to the follicular arrest of PCOS. This may also have clinical implications. It may be that high, localized concentrations of AMH could then act on the surrounding growing follicles to decrease their responsiveness to FSH, thereby contributing to follicular arrest. Indeed, it has been hypothesized that the excess of AMH at the level of selectable follicles could contribute to the follicular arrest of PCOS, mainly by an inhibitory action on FSH-induced aromatase expression (21). Further studies are required to settle these issues.

The most pertinent concern to the infertile couple has always been the success rate of specific ART treatments. Therefore, numerous studies have been dedicated to identify reliable markers that may help in predicting outcomes in IVF cycles, as well as the feasibility of

applying these markers in patient counseling prior to treatment initiation. A recent meta-analysis has concluded identifying patients of potential poor outcome, rather than a tool of exclusion. The patients should always have final

Table 5: Predictability of AMH in IVF/ICSI outcomes in PCOS women.

	Serum AMH > 3.65		Follicular fluid AMH	
	≤ 3.15 (n=6)	> 3.15 (n= 107)	≤ 3.65 (n= 8)	> 3.65 (n= 105)
Number of Fertilized oocyte	7 ± 3.2[a]	10.2 ± 3	6.7 ± 1.3	10.3 ± 4
	F= 4, *P* = 0.048		F= 6.19, *P* = 0.014	
Number of retrieved oocyte	13.3 ± 4	15.8 ± 6	10.25 ± 2	16 ± 6
			F= 6.2, *P* = 0.014 [a]	
Number of mature oocyte	7.3 ± 3	13.4 ± 4	7 ± 1.8	13 ± 4.8
	F= 9, *P* = 0.003		F= 14.3, *P* < 0.0001	
Number of embryo	5 ± 0.8	5 ± 1.9	4.7 ± 0.4	5 ± 1.9
Positive Clinical pregnancy (n=79)	6 (7.6%)[b]	73 (92.4%)	6 (7.6%)	73 (92.4%)
Positive Chemical pregnancy (n=79)	6 (7.6%)	73 (92.4%)	6 (7.6%)	73 (92.4%)

Statistical analysis using [a] ANOVA test, [b] Chi-square test.

that female age, duration of subfertility, basal FSH level and number of retrieved oocytes are all predictors of pregnancy in IVF cycles (22). However, serum AMH level, were not incorporated as the factors analyzed in that study. The latest study by Lee and his colleagues (23) concluded that serum AMH level may be used as a marker of fertility for counseling IVF/ICSI candidates. To our knowledge this is the first study concerning the assessment of both serum and follicular fluid AMH among patients with PCOS counseling IVF/ICSI. According to our results, pregnancy was reported in 79 of PCOs patients, 73 of them (92.4%) were positive for both serum and follicular fluid AMH (Table 5). Furthermore, the quality and quantity of oocytes are both important determinants for the success in ART and the most important predictor of pregnancy in IVF cycles among almost all of the analyzed studies (22).The observation drawn from the present study demonstrated the superiority of folicullar fluid AMH over serum AMH in its significance for predicting the quality and the quantity of oocytes and that pregnancy was still achievable in PCOS women after COH with GnRH long protocol and positive AMH.

One of the limitations of the present study was the relative small sample size (n = 168) enrolled. Being a retrospective study which targeted PCOS and other infertile population, a larger sample size is indeed needed in the future to confirm the results of this study. Furthermore, the results from the present study were to simply present an easier and more reliable counseling tool for evaluating and decision of whether or not to receive treatment.

In conclusion, to the best of our knowledge, this is the first study that investigated both serum and follicular fluid AMH among PCOS women under COH with GnRH long protocol and capable of achieving viable pregnancy by IVF/ICSI. In addition, from our data, we were able to derive the serum and follicular fluid AMH cut-off values that may reliably predict the PCOS patients. Moreover, the novelty of these data is that women with PCOS, in general, may have a less severe form of infertility compared to non-PCOS women with other forms of fertility. Thus, the higher AMH may be used to try 'easier,' less expensive and less risky fertility treatments first in PCOS women, such as metformin.

Conflict of Interest: The authors have nothing to disclose

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