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ORIGINAL ARTICLE

Evaluation of Anti-Mullerian Hormone, Follicular-stimulating Hormone, Antral Follicle Count, and Ovarian Volume as a Different Predictor for Ovarian Response in Cases of Polycystic Ovary Syndrome

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Abstract

Background: **Anti-Mullerian hormone, also referred to as AMH, is a glycoprotein that belongs to the transforming growth factor-B family of proteins. Recent research has suggested that AMH may be a useful indicator of ovarian follicular development. Its reliability superpasses that of inhibin B, estradiol, and follicular-stimulating hormone (FSH). It demonstrates four biochemical parameters for follicular state that are not shared by the traditional hormone predictors.**

Aim: **The primary goal of this research is to examine the connection that exists among different parameters (AMH, FSH, antral follicle count, and size of the ovaries) in persons with polycystic ovary syndrome (PCOS) and the response of these patients to ovulation induction.**

Patients and methods: **This prospective trial was executed in Infertility Clinics at Al-Azhar University Hospitals and El Fayoum General Hospitals. This study includes 50 patients diagnosed to have PCOS in accordance to Rotterdam criteria 2003. These criteria contained the following: clinical hyperandrogenism (hirsutism using modified Ferriman−Gallwey score ≥8) or biochemical hyperandrogenism (either higher total testosterone >2.1 nmol/l or free testosterone above 0.03 pmol/l); oligomenorrhea (<6−9 menses per year) or oligo-ovulation; and polycystic ovaries on ultrasound (≥12 antral follicles in each ovary measuring 2−9 mm in diameter, and/or ovarian volume equal to 10 cm^3 or more).**

Results: **We found that cases with elevated AMH levels responded less favorably to ovulation induction than those with low AMH levels. Ovarian size, FSH, and age all had positive associations. Ovarian response and hormonal status in those with PCOS can be predicted by AMH.**

Conclusion: **Ovulation-induction outcomes in females with PCOS may be predicted by measuring serum AMH concentrations before treatment, which could make ovulation-induction procedures more individualized as well as economical.**

Keywords: Follicular hormone, Mullerian hormone, Ovary syndrome

1. Introduction

There are a lot of ladies of reproductive age who suffer from polycystic ovary syndrome (PCOS), a gynecologic−endocrine condition. Ovarian enlargement in addition to dysfunction, elevated testosterone levels, insulin resistance, and so on are common symptoms of this disease. Premenopausal onset of PCOS and its associated difficulties impact about one in 10 women.[1]

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Although there appears to be an advantageous relationship among anti-Mullerian hormone (AMH) and the presence of ultrasonographically visible antral follicles, AMH measures a distinct population of follicles and hence may supplement ultrasound data. The combined use of antral follicle count (AFC) as well as AMH did enhance the prediction of poor ovarian response, but this improvement was not statistically significant.[2]

Reduced negative feedback by inhibin B and estradiol as a result of a smaller follicular-stimulating hormone (FSH)-sensitive follicle pool is the biological reason behind the early follicular FSH surge. The hormonal marker of reproductive aging is undeniably elevated by FSH levels. Because of this, high levels of FSH cannot be utilized as an indicator of impending infertility. Basal FSH levels taken at regular intervals may serve as a short-term predictor.[3] In order to diagnose PCOD in PCOS, it is not sufficient to simply note the multiple follicles, rather in addition to the multiple small follicles, the ovaries of cases with PCOS also exhibit increased size and stromal volume, and along with the follicles, have a conventional topographic connection to the surrounding stroma. In a polycystic ovary, the many tiny follicles are dispersed toward the periphery as well as surround the dense core stroma. The Rotterdam consensus criteria for an ovary to be thought about polycystic weighs equally on the ovarian volume (above 10 ml) or the total number of small follicles calculating among 2 and 9 mm in size (>20); the existence of either of both of these morphological ultrasonographic results in a single ovary satisfies the Rotterdam criteria for a polycystic ovary.[4]

2. Patients and methods

This prospective trial was performed in Infertility Clinics at Al-Azhar University Hospitals and El Fayoum General Hospitals. This study includes 50 patients diagnosed to have PCOS regarding Rotterdam criteria 2003. These criteria included clinical hyperandrogenism (hirsutism using modified Ferriman–Gallwey score $\geq$8) or biochemical hyperandrogenism (either elevated total testosterone >2.1 nmol/l or free testosterone >0.03 pmol/l); oligomenorrhea (<6–9 menses per year) or oligo-ovulation; and polycystic ovaries on ultrasound ($\geq$12 antral follicles in each ovary ranging 2–9 mm in diameter, and/or ovaries' size $\geq$10 cm^3). For diagnosis of PCOS, the patient must have at least two of the previous criteria. The patients eligible for this study had the following:

Inclusion criteria include: represented by age from 20 to 35 years old, duration of infertility more than 1 year, PCOS-approved criteria (Rotterdam criteria), normal hysterosalpingogram, and normal seminal profile.

Exclusion criteria include: individuals with any of the following criteria eliminated from this trial: age <20 or >35 years old, endocrinological diseases (diabetes mellitus, thyroid abnormalities, and hyperprolactinemia), hyperandrogenemia due to other causes rather than PCOS, patients having unilateral or bilateral tubal block as seen in hysterosalpingogram, and history of laparoscopic ovarian drilling.

2.1. History taking

History taking included the following: personal history (oligomenorrhea or amenorrhea, detailed menstrual history, acne, hirsutism, infertility, and galactorrhea); diabetes mellitus, past history of autoimmune disease, hypertension, thyroid abnormalities; and family history of PCOS.

Detailed clinical examination: included checking of heart rate, blood pressure, complexion, temperature, and jaundice, along with examination of the chest and heart. In addition to this, we performed a P/V examination to rule out the presence of uterine in addition to adnexal masses, palpated for pelvi-abdominal masses, and also inspected the distribution of hair.

2.2. Transvaginal ultrasound

All patients underwent transvaginal ultrasound in lithotomy position using Mindray DC-70 machine and E6 machine GE with frequency of 8 MHz with vaginal probe to assess the pelvic organs, uterus, endometrium, and adnexa measuring ovarian volume and AFC.[5]

By inserting an ultrasound probe into the vagina, a doctor can look for abnormalities in the reproductive system. The endometrial layer can be evaluated for its thickness. 'The revised Rotterdam criteria now define polycystic ovary with findings of 12 or smaller (2–9 mm) follicles in each ovary.' After identifying the ovary, the probe was moved to record a single frame on two screens that represented the ovary's largest diameter. To determine the ovary's actual transverse axis, the probe was turned through a full 900°. We measured the biggest ovarian diameters in the sagittal, coronal, and anteroposterior planes to determine the ovarian volume. The AFC as well as location were determined in the ovary's longest segment, in addition to more accurate outcomes, a two-dimensional sweep was performed across the whole ovary. When there is a high volume of follicles, as in polycystic ovary,

this technique proves to be both feasible and efficient.

2.3. Laboratory investigations

Basal hormone analysis on day 2 of menstruation included AMH, FSH, AFC, and prolactin.

2.4. Determination of anti-Mullerian hormone

AMH was determined using ELISA kit procured from Kamiya Biomedical Company (Seattle, Washington, USA). After letting the samples wait for the clot to form for a 2-h period at room temperature or overnight at 4 °C in a serum separator tube, we centrifuged them for a 20-min period at around 1000*g*. Freshly prepared serum was assayed immediately or stored in an aliquot at −20 °C for later use. Repeated freeze/thaw cycles were avoided. Hemolytic samples were excluded. Hemolysis was avoided as sample hemolysis will influence the results. For an analysis, this assay has a sensitivity of 0.014 ng/ml. Intra-assay and also inter-assay coefficients of disparity were less than and equal to 12.3 % and less than and equal to 14.2 %, respectively.

2.5. Determination of serum follicular-stimulating hormone

It was done by ab108678 FSH human ELISA kit that is a method for the quantitative detection of FSH in serum that uses an immunoenzymatic colorimetric approach. The kits were manufactured by Abcam Inc (Cambridge, Massachusetts, USA). The next table represented the reference values reported by the ELISA kit manufacturer. However, every laboratory establishes its normal values based on populations.

2.6. Measurement of antral follicle count (cycle day 2)

A longitudinal image of one of the ovaries was obtained and adjusting the focus optimized the image with magnification. Serial scans were obtained by making a slow sweep with the transvaginal probe, as these sweeps were performed through the whole ovary, the antral follicle was assessed on a frozen image (that was defined as all echo-lucent rounded structures seen within the ovarian stroma).

2.7. Determination of prolactin

On day 2 of the cycles leading up to the stimulation of the ovaries, a specimen of blood of 10 ml was acquired using vein puncture in the early morning hours. After allowing the samples to clot at room temperature for at least 1 h, the blood sample was used. Within 2 h of the end of the withdrawal period, all of the samples were put through a centrifuge, and then they were frozen at −20 °C until the basal hormone levels were determined. Prolactin was calculated in a sample gathered with radioimmunoassay using Gamma counter immunoassay analyzer model (ISOGP-1 MGM), using a commercial kit according to the manufacturer's sensitized assay protocol (coat-A-count IRMA; Siemens Medical Solutions Diagnostics, Flanders, New Jersey, USA).[6]

2.8. Methodology

Fifty women underwent ovulation induction by using highly purified human menopausal gonadotropin (HMG) (Menopure-ampoule 75 Ferring, Germany) daily, by deep intramuscular injection. Each ampoule contains FSH 75 IU–luteinizing hormone 75 IU. Induction was starting out with day two after spontaneous or progestin-induced withdrawal bleeding. Transvaginal ultrasound was utilized for the procedure of folliculometry.

The monitoring of ovarian response was done by transvaginal ultrasound folliculometry every 2 days starting on the fifth day of the treatment. Human chorionic gonadotrophin 10.000 IU (Pregnyl, NV Organon International, Jersey City, the Netherlands) was put into the person with a single injection into the muscle on the day that the diameter of the dominant follicle is at least 18 mm. If we find more than three follicles larger than 16 mm in diameter and/or serum estrogen more than 1000 pg/ml, stimulation was cancelled, and HCG was not be prescribed to avoid ovarian hyperstimulation syndrome and the patients were instructed to use barrier contraceptive method.

Patients were followed up 7–10 days after HCG administration using transvaginal ultrasound to confirm ovulation as denoted by the disappearance of the previously growing follicle. Treatment with HMG was stopped in the absence of follicular development after 15 days of stimulation.

2.9. Statistical analysis

In our trial, there is evidence that there are statistically significant positive correlations among AFC and both ovarian volume and age. But, there are nonsignificant correlations between each of AMH and AFC and either BMI or FSH. Epi-Info, version 6 and SPP for Windows version 8 were

utilized throughout the information entry, checking, and analysis processes.[7]

3. Result

In total, there are 50 people participating in this study with polycystic overy. Age ranges from 18 to 29 years with mean 22.66 years. BMI among 17 and 32 kg/m^2 with mean 20.94 kg/m^2. Larger percentage of patients had normal BMI, while 14 % were overweight and obese (Table 1, Fig. 1).

Serum FSH ranged from 4 to 8 with mean 5.8. Serum AMH ranged from 3 to 9 with mean 5.74 as in Table 2.

With early ultrasonography, AFC ranged from 15 to 30 with mean 21.3. With follow-up, 29 patients showed follicles, while 21 patients showed no follicles. Fifteen patients had one follicle and the remaining 14 had two follicles. Ovarian volume ranged from 7 to 16 with mean 11.38 (Table 3).

Concerning ovarian response, 58 % showed successful response, while 42 % had failed to respond (Table 4, Fig. 2).

There is an association that is statistically significant among ovulation and age. Age was significantly increased in those with failed ovulation. There is no

Table 1. Distribution of the examined individuals by BMI and age.

	$N = 50$
Age (year)	
Mean ± SD	22.66 ± 3.21
Range	19–29
BMI (kg/m^2)	
Mean ± SD	20.94 ± 3.83
Range	17–32
Underweight [*n* (%)]	6 (12)
Normal [*n* (%)]	37 (74)
Overweight and obese [*n* (%)]	7 (14)

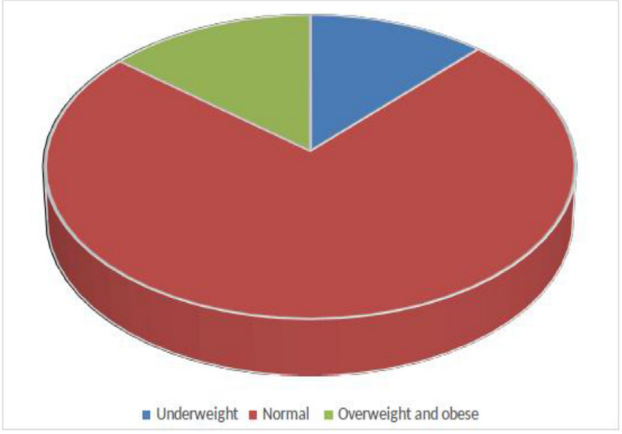

Fig. 1. Pie chart showing the distribution of cases in accordance with BMI.

Table 2. Distribution of participants examined corresponding to their hormonal profile.

	Mean ± SD	Range
FSH	5.8 ± 1.43	4–8
AMH	5.74 ± 2.05	3–9

AMH, anti-Mullerian hormone; FSH, follicular-stimulating hormone.

Table 3. Distribution of the examined individuals due to the ultrasonographic findings.

	Mean ± SD	Range
AFC	21.3 ± 3.91	15–30
Follicles	0.86 ± 0.83	0–2
0	21	42 %
1	15	30 %
2	14	28 %
Ovarian volume	11.38 ± 2.4	7–16

AFC, antral follicle count.

Table 4. Distribution of the studied participants according to ovulation.

	$N = 50$ [*n* (%)]
Ovulation	
No	21 (42)
Yes	29 (58)

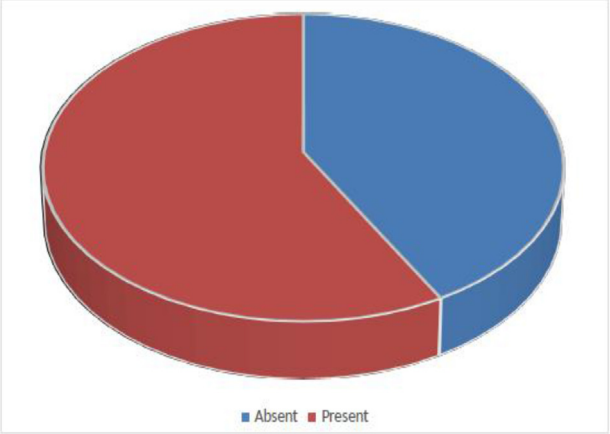

Fig. 2. Pie chart showing the distribution of participants was examined based on ovarian response.

Table 5. Relation between ovulation and baseline data among the studied patients.[a]

Parameters	Ovulation			Test
	Present ($N = 29$)	Absent ($N = 21$)	*t*	*P*
	Mean ± SD	Mean ± SD		
Age (year)	21.31 ± 2.61	24.52 ± 3.06	3.999	<0.001[b]
BMI (kg/m^2)	21.62 ± 3.35	20.0 ± 4.31	1.495	0.141

t, independent sample *t*-test.

[a] *P* value less than 0.05 is statistically significant.

[b] *P* value less than or equal to 0.001 is statistically highly significant.

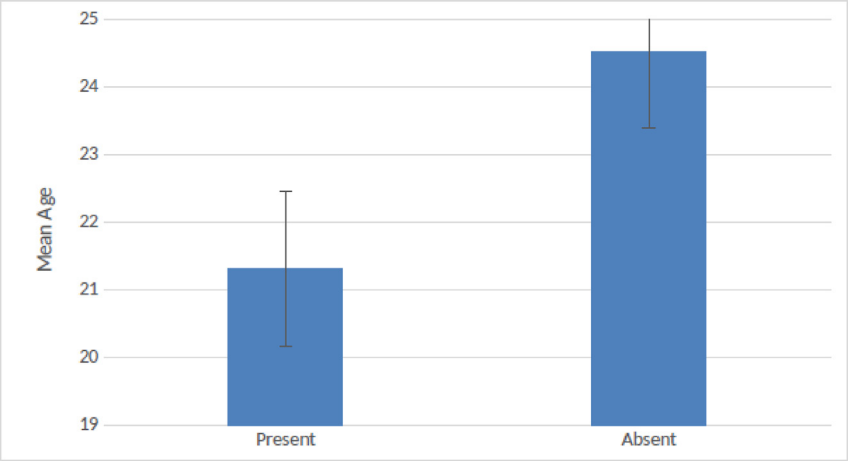

Fig. 3. Simple bar chart displays the relation among ovulation and age.

statistical significant relation among ovulation and BMI as in Table 5, Fig. 3.

There is a correlation that can be supported by statistical analysis that exists among ovulation and AMH. The levels of AMH in the serum are dramatically elevated in women who are unable to ovulate. There is a statistically nonsignificant connection among ovulation and also serum FSH (Table 6, Fig. 4).

There is an association that is statistically significant among ovulation and both ovarian volume and AFC. Those with ovulation had significantly lower ovarian volume and AFC as in Table 7, Figs. 5 and 6.

Table 6. Relation between ovulation and hormonal profile among the studied patients.

Parameters	Ovulation			Test
	Present ($N = 29$)	Absent ($N = 21$)	t	P
	Mean ± SD	Mean ± SD		
FSH (mIU/ml)	5.83 ± 1.58	5.76 ± 1.22	0.159	0.874
AMH (ng/ml)	4.52 ± 1.3	7.57 ± 1.47	7.768	<0.001[a]

AMH, anti-Mullerian hormone; FSH, follicular-stimulating hormone.
[a] P value less than or equal to 0.001 is statistically highly significant.

Table 7. Relation between ovulation and ultrasonographic data among the studied patients.

Parameters	Ovulation			Test
	Present ($N = 29$)	Absent ($N = 21$)	t	P
	Mean ± SD	Mean ± SD		
Ovarian volume (ml)	10.0 ± 1.41	13.29 ± 2.17	6.065	<0.001[a]
AFC (ovary)	18.9 ± 2.35	24.62 ± 3.12	7.4	<0.001[a]

AFC, antral follicle count.
[a] P value less than or equal to 0.001 is statistically highly significant.

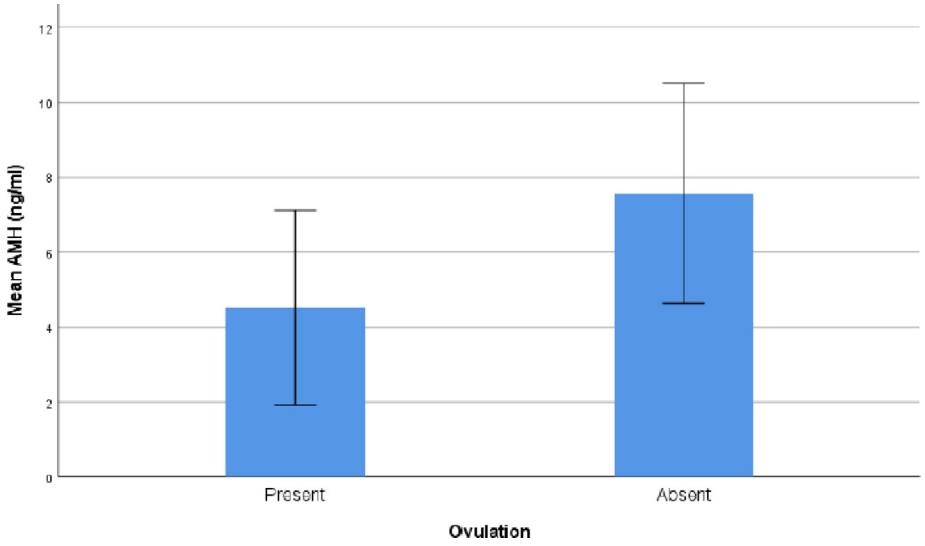

Fig. 4. Simple bar chart demonstrating the relation among ovulation and AMH. AMH, anti-Mullerian hormone.

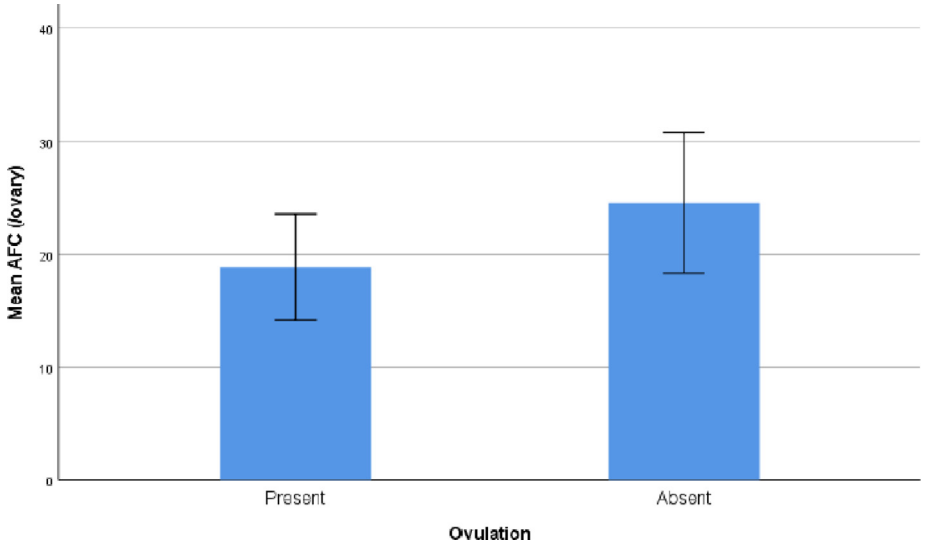

Fig. 5. Simple bar chart showing the relation between ovulation and AFC. AFC, antral follicle count.

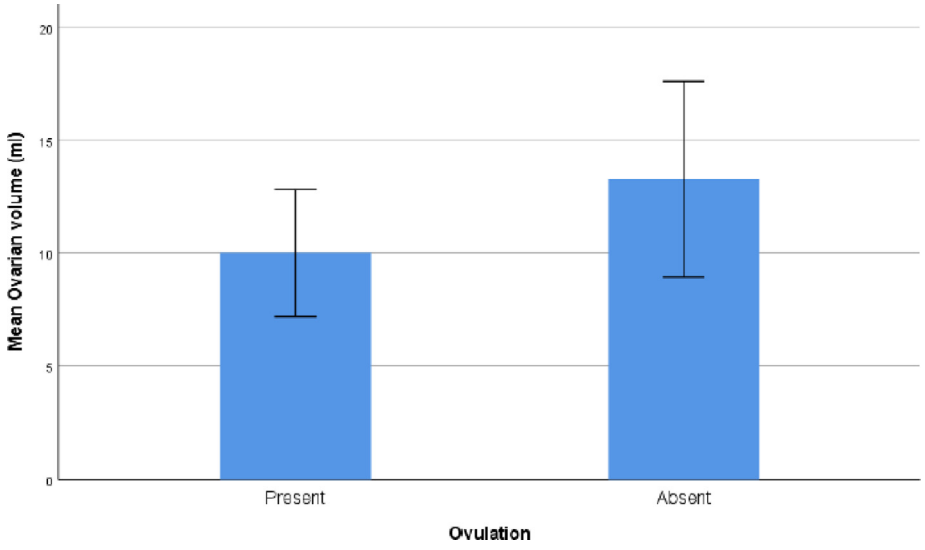

Fig. 6. Simple bar chart showing the relation between ovulation and ovarian volume.

The most optimal point at which serum AMH in estimation of ovarian response among the participants of the research studies is less than or equal to 6.5 ng/ml with area under curve 0.913, sensitivity 89.7 %, specificity 90.5 %, value positive in terms of prediction 92.9 %, 86.4 % having a negative predictive value, and overall precision 90 % (Table 8, Fig. 7).

4. Discussion

AMH levels are unaffected by the menstrual cycle or the use of oral contraceptives or gonadotropin-releasing hormone agonists. AMH levels are also not impacted by the use of gonadotropin-releasing hormone antagonists. As a result, the measurement of blood AMH has become increasingly popular in clinical practice for the purpose of assessing ovarian

Table 8. Performance of serum anti-Mullerian hormone in prediction of ovarian response among the studied patients.

Cutoff	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	*P*
≤6.5 ng/ml	0.913	89.7 %	90.5 %	92.9 %	86.4 %	90 %	<0.001[a]

AUC, area under curve; NPV, negative predictive value; PPV, positive predictive value.
[a] *P* value less than or equal to 0.001 is statistically highly significant.

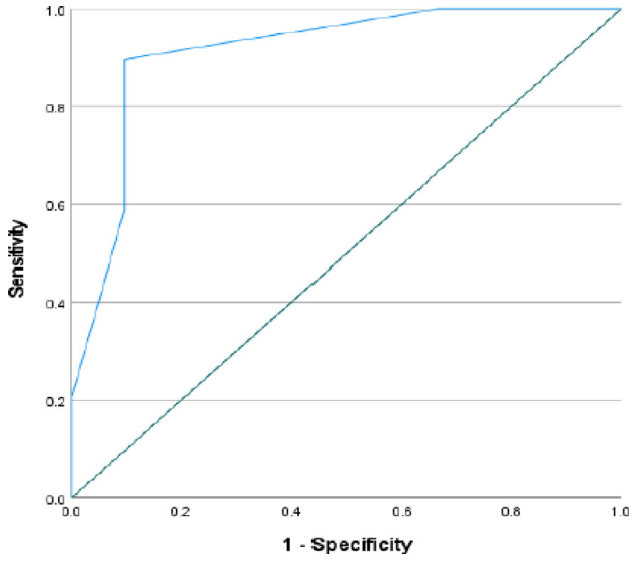

Fig. 7. ROC curve showing the efficiency of serum AMH in prediction of ovarian response among the studied patients. AMH, anti-Mullerian hormone; ROC, receiver operating characteristic.

reserve, as well as is evaluated routinely during the first phase of the diagnostic process for infertility.[8]

The expression of both the FSH receptor and ovarian aromatase is greatly reduced in response to AMH, resulting in follicular arrest. It is possible that 'follicular arrest' is caused by a failure to down-regulate AMHR2 expression in response to luteinizing hormone.[9]

In this work, age ranged from 18 to 29 years, with mean of 22.66 years. This result is comparable to those reported by Roy et al.[10] who reported that the mean age was 28.2 years. In addition, Sunj et al.[11] included 96 infertile PCOS women with a mean age of 29.3 ± 3.31 years. Also, Izhar et al.[12] determined the cutoff for the AFC and the AMH level predictive of ovarian hyperstimulation syndrome and found that the mean age of the people who took part in the study was 31.32 ± 3.82 years.

Our study showed mean BMI of 20.94 kg/m^2 with range from 17 to 32. Larger percentage of patients had normal BMI, while 14 % were overweight and obese. On the other hand, Munira et al.[13] recruited 46 infertile women with PCOS in the study and found that overweight and obese women were higher than the average prevalence in both groups than those with normal weight, while Izhar et al.[12] found that the mean BMI was 22.64 ± 1.71 kg/m^2.

A high FSH level is not a reliable indicator of future infertility.[14,15] However, repeated basal FSH tests might be effective as a short-term predictor.[3] In our study, serum FSH ranged from 4 to 8 with mean of 5.8.

In our study, serum AMH ranged from 3 to 9 with mean of 5.74. Moreover, Li et al.[16] conveyed a mean AMH level of 9.85 ng/ml, while Catteau-Jonard et al.[17] reported 6.59 ng/ml in patients with PCOS. In their study, Piltonen et al.[18] found that AMH levels in females with PCOS were consistently three times higher than those seen in healthy individuals among the ages of 16 and 44. In our study, there is statistically a significant relation among ovulation and AMH, as serum AMH was significantly higher in those with failed ovulation. Also, Munira et al.[13] serum AMH concentration was observed to be considerably lower for those who ovulated during the first CC cycle (50 mg/days) compared with those who did not. Women who achieved ovulation while using CC (50–150 mg/days) for as many as six cycles had considerably lower serum AMH concentrations than those who did not. Similarly, pregnant women had considerably lower serum AMH concentrations (3.0 ng/ml) compared with nonpregnant patients (4.4 ng/ml).

In our trial, the cutoff is bigger than that reported by Amer et al.[19] who demonstrated a cutoff value of AMH of 4.7 ng/ml as highly sensitive (100 %) as well as precise (58 %) for predicting a lackluster response to HMG stimulation. Also, Munira et al.[13] found that area under the curve of 0.809 for serum AMH levels in predicting no ovulation after CC ovulation inducement. To predict no ovulation, AMH showed a sensitivity of 73 % at a cutoff value of 3.4 ng/ml and a specificity of 78 %. Also, Sweed et al.[20] demonstrated that AMH for efficacious ovulation was less than or equal to 3.6 ng/ml (sensitivity 97.2 % and specificity 82.1 %).

But, the cutoff of our study is lesser than that reported by Sweed et al.[20] who confirmed a threshold of 7.7 ng/ml with 78 % sensitivity and also 76 % specificity, Amer et al.[21] used the cutoff level of 8 ng/ml, and Xi et al.[22] investigated whether serum AMH has a part in forecasting ovarian response to CC treatment in a large cohort of females unable to conceive with PCOS, and found the cutoff level of AMH as 7.77 ng/ml in their study, including 81 anovulatory women with PCOS, demonstrating that the plasma AMH can predict ovarian response to CC treatment. Specificity was 65 %, while sensitivity was 92 %. Therefore, it may be helpful to predict the outcome of CC administration by measuring blood AMH levels in pretreatment anovulatory women with PCOS. Our findings were supported by these other investigations, demonstrating the importance of AMH in predicting follicular development failure.

4.1. Conclusion

Evaluation of serum AMH concentration before ovulation induction in women with PCOS may be a valuable technique for predicting the success of

ovulation induction and may make ovulation-induction procedures more patient-tailored and cost-effective. This may also be a good technique for predicting the result of ovulation induction in women without PCOS.

4.2. Conclusion and recommendations

We observed that higher AMH-level women had poorer response to ovulation induction compared with women with low AMH level. AMH was also positively correlated with ovarian volume, age, and AFC. AMH can be used as a predictor of ovarian response and hormonal status of PCOS patients.

Evaluation of serum AMH concentration for PCOS women prior ovulation induction may be a useful tool in predicting the outcome of ovulation induction and may render the ovulation-induction protocols more patient-tailored and more cost-effective.

However, the results of the present work should be taken cautiously due to small sample size. Cohort studies with large sample size and involving multiple centers are recommended to determine the cutoff level of AMH for ovarian response with the highest sensitivity and specificity. In addition, we recommend validation of the present work on a large series of patients in future work before globalization of their results.

A proper protocol should be in place for women who are undergoing stimulation. All clinicians involved in care should be aware of this condition, so that women do not face any adverse outcomes due to the lack of coordination between the centers providing fertility treatments and other emergency departments where they may present in case of hyperstimulation. All sonologists should be able to pick the signs on ultrasound and cooperate with the gynecologist and the woman to ensure that care is not compromised and no cases are missed.

Ethical statement

The ethical statement was approved by faculty counsel.

Conflict of interest

There are no conflicts of interest.

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