Research Article ISSN 2639-9342

Gynecology & Reproductive Health

Could Anti-Mullerian Hormone Levels Predict Clomiphene Citrate Response in PCOS? A Cross-Section Study

Ayman S. Dawood[1*], Hashem A. Lotfy[2] and Mona K. Omar[1]

[1]*Assistant professor of Obstetrics and Gynecology, Tanta University, Tanta, Egypt.*

[2]*Lecturer of Obstetrics and Gynecology, Tanta University, Tanta, Egypt.*

[*]Correspondence:
Ayman Shehata Dwood, Department of Obstetrics and Gynecology, Tanta University, Tanta, 31111, Egypt, Tel: +201020972067.

Received: 02 July 2021; **Accepted:** 07 August 2021

Citation: Dawood AS, Lotfy HA, Omar MK. Could Anti-Mullerian Hormone Levels Predict Clomiphene Citrate Response in PCOS? A Cross-Section Study. Gynecol Reprod Health. 2021; 5(4): 1-7.

ABSTRACT

Background: *Clomiphene citrate (CC) is first choice as an ovulation-stimulating drug in polycystic ovarian syndrome. Anovulation problems could occur in some patients presenting with CC resistance. In PCOS patients, very high levels of AMH were observed. The role of anti-Müllerian hormone (AMH) in the prediction of ovarian response to CC in women with the polycystic ovarian syndrome (PCOS) is investigated in this study.*

Objective: *To assess the predictive value of Anti Mullerian hormone (AMH) in Clomiphene citrate response in patients with polycystic ovary syndrome.*

Methods*: This cross-sectional study was conducted at Tanta University Hospitals, Egypt. The study included 120 anovulatory PCOS women who underwent ovarian stimulation with clomiphene citrate. Day 3 measurement of AMH concentrations was done.*

Results: *Cycles with poor response had significantly ($p<0.0001$) higher basal serum AMH concentration compared to that of cycles with normal response. AMH area under receiver operating characteristic curve (ROC_{AUC}) 0.88; ($p<0.001$) and 0.81; ($p<0.007$) respectively. Using a cut-off level of 6.3 ng/ml, the good response rate was significantly ($p < .001$) higher in cycles with lower AMH (<6.3 ng/ml) compared to that in those with AMH (> = 6.3) ng/ml.*

Conclusion: *AMH levels could predict the ovarian response to clomiphene citrate in PCO women.*

Keywords

Clomiphene citrate, Polycystic ovary, AMH, Ovulation induction, Ovarian response

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder diagnosed based on the Rotterdam criteria with two of three features: anovulation, polycystic ovarian morphology on ultrasound and hyperandrogenism (HA) (clinical or biochemical) [1]. Rotterdam criteria of the polycystic ovary proposed the presence of >12 follicles of 2–9 mm diameter and/or increased ovarian volume (>10 cm^3).

The principles of therapy in the anovulatory PCO infertile women are first to optimize health before commencing treatment. In obese patients, weight loss greatly improves the endocrine profile. The aim is then to induce regular unifollicular ovulation, with minimal risks of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy. Multiple drugs are used like clomiphene citrate (CC) or the aromatase inhibitor (AI) letrozole, with second-line therapy being parenteral gonadotropin therapy or laparoscopic ovarian diathermy (LOD) (drilling). In some cases, there may be a role for the insulin sensitizer, metformin [2].

Clomiphene Citrate (CC) was introduced into clinical medicine in

the 1960's. Clomiphene citrate is considered a breakthrough in the medical management of anovulation as it is cheap, and effective in inducing ovulation with minimal side-effects [3,4]. Clomiphene citrate acts by antiestrogenic effect through binding to estrogen receptors on the hypothalamus leading to gonadotropin secretion from the anterior pituitary gland [5,6].

Clomiphene citrate resistance is defined when there is no ovulation for three cycles despite stimulation with the maximum dose (250 mg/d)". Resistance to CC is common and was recorded in approximately in 20-25% in women with PCOS. Multiple studies tried to identify the clinical predictors of the response to CC. Although high Body Mass Index, LH, LH/FSH ratio, testosterone, and the Insulin Resistance were reported to be associated with CC resistance. These factors avert ovaries from responding to raised endogenous FSH levels following CC therapy [7,8].

Anti-Müllerian hormone (AMH) is produced from granulosa cells of the preantral and small antral follicles. AMH inhibits recruitment of small resting follicles and hence regulates folliculogenesis and help in selection of the dominant follicle. AMH levels correlate with the number of antral follicles so it can serve as a molecular biomarker for the ovarian reserve and ovarian dysfunction, such as in women with Polycystic Ovary Syndrome [9]. AMH levels are increased in PCOS with subsequent reduction in follicle sensitivity to FSH, preventing follicle selection, and resulting in follicle arrest and failure of dominance [10]. Moreover, AMH inhibits aromatase enzyme activity leading to reduction of estradiol (E2) production [11].

Identifying factors that determine the response of women with PCOS to CC will help selecting patients who are likely to benefit from this treatment, thus improving success rates. So we designed this study to investigate whether serum AMH has a role in predicting ovarian response to CC treatment in women with PCOS. The sensitivity and specificity of AMH were tested.

Patients and Methods

Study design and settings

This study was a cross-sectional study conducted at Tanta University hospitals in the period from January 2017 till September 2020.

Patients

Hundred and twenty anovulatory women with PCOS were recruited from fertility outpatient clinics. All patients were diagnosed as polycystic ovary syndrome by Rotterdam's criteria [1], with presence of at least two of three criteria: olig/anovulation, hyper-androgenemia, and sonographic appearance of polycystic ovaries.

The inclusion criteria were: (i) BMI<30 (ii) Age<40 years, (iii) Normal other finding on transvaginal ultrasound scan and (iv) Normal semen profile. The exclusion criteria were: (i) Previous history of ovarian surgery, (ii) Exposure to cytotoxic drugs or pelvic radiation therapy, (iii) Other causes of anovulation such as thyroid dysfunction and hyperprolactinaemia. (iv) Other causes of hyper-androgenism as congenital adrenal hyperplasia and Cushing syndrome.

Methods

All patients' demographic data were registered. Measurement of FSH and AMH were obtained on Day 3 of the menstrual cycle. On the same day of the blood tests, transvaginal ultrasound scan was done to assess the total number of antral follicles (AFC) and the ovarian volume and to exclude other pelvic pathologies. The volume of each ovary was calculated by measuring the ovarian diameters (D) in three perpendicular directions (D1 × D2 × D3 × 0.5236.) For AFC, we calculated follicles with a diameter 2: 9 mm [12].

Hormone assays

Plasma for assay of AMH was separated within 2 hours from blood collection and frozen in aliquots at 70°C until thawed and assayed in batches. Measurements of AMH were determined using the ultra-sensitive enzyme-linked immunosorbent assay (ELISA). The functional sensitivity of the assay was typically > 0.046 ng/ml with a detection range of 0.156-10 ng/ml.

Clomiphene citrate treatment

At the day 3 of the cycle, women received an initial dose of 100 mg/d CC for 5 days till day 7. Monitoring was achieved by serial trans-vaginal ultrasound scanning every other day starting from cycle day 9. Size and number of follicles were recorded. When the leading follicle reach diameter ≥18 mm, a single dose of 10,000 IU human chorionic gonadotrophin was given and subsequent follicle rupture was assisted. Ovulation was confirmed by disappearance of the leading follicle and by midluteal serum progesterone level (levels >10 ng/ml indicating ovulation).

According to response, patients were categorized into 3 groups. Group [1] Normal Responders: patients who yielded less than three follicles >= 17mm in diameter and the estradiol level was less than 5000 nmol/l. Group [2] Hyper-Responders: patients who yielded three or more follicles≥ 17 mm and/or E2 levels > 5000 nmol/l. Group [3] Poor Responders: patients who yielded no follicular growth after 14 days of stimulation or when follicular growth became arrested after an initial response.

Ethical issues

The study was approved by local institutional review board of Tanta University hospital. Ethical committee code is 34543. All participants provided informed written consent.

Statistical methods

Difference between normal, excessive and poor responders were tested using the Anova-test, nonparametric test (Mann–Whitney U) and Chi square as appropriate. $P < 0.05$ was considered statistically significant. Using the results of the Receiver operating characteristic (ROC) curves we defined an appropriate threshold level for AMH and determined the sensitivity and specificity of

that threshold. Spearman's correlation also done to diagnose the value of serum AMH and other study variables for the prediction of ovarian responsiveness to CC stimulation.

Results

120 PCO patients were included in the study all met the inclusion criteria and had their baseline FSH, AMH, and AFC determined. According to their response to ovarian stimulation with clomophine citrate, 10 women (8.3%) were classified as high responders, 16 women (13.3%) had poor response and the remaining 94 women (78.3%) had normal response to stimulation. Out of the 94 subjects with normal response 28 women (29.7%) got pregnant (fetal heart activity visible on ultrasound scan at 6 weeks of gestation) as shown in figure 1.

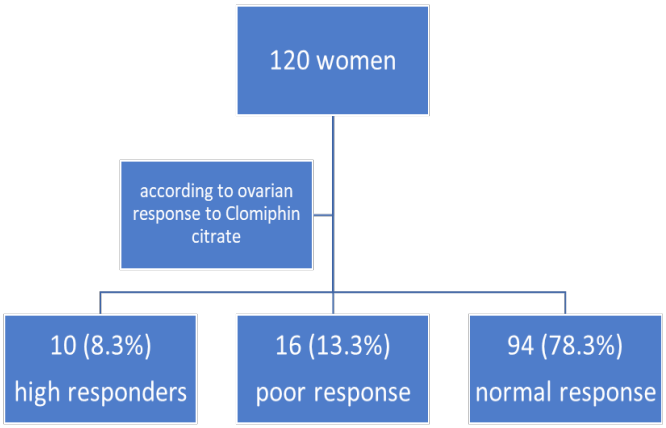

Figure 1: Show the group of the patient according to their response to CC treatment.

Patients' demographics characters in the three study groups are presented in table 1. Patient with poor response were significantly older compared with those in the other two groups difference p< 0.001. Patient with poor response had significant higher BMI compared with those in the other groups p< 0.04. The other symptoms related to PCO were the same in the three groups. The basal FSH, AMH, AFC, ovarian volume in the three groups were presented in table 2. P value consider significant (*) if (P< 0.05).

Table 1: Demographic criteria in the three study groups

Characters	Group 1 normal responder (n=94)	Group 2: poor responder (n=16)	Group 3: high responder (n=10)	P value
Age	25.7 ± 5.5	30.1 ± 7.5	26.2 ± 4.2	0.001*
BMI (Kg/m^2)	25.3 ± 6.3	29.7 ± 7.2	23.5 ± 5.1	0.04*
Menstrual cycle Regular Oligomenorrhoea Amenorrhoea	 16 (20%) 50 (62%) 14 (17%)	 4 (25%) 8 (50%) 4 (25%)	 0 (0%) 4 (40%) 6 (60%)	Ns
Hirsutism Yes No	 46 (57%) 34 (42%)	 8 (50%) 8 (50%)	 6 (60%) 4 (40%)	NS
Acne Yes No	 44 (55%) 36 (45%)	 10 (62%) 6 (38%)	 2 (20%) 8 (80%)	NS

The tests used were mean, SD, ANOVA and percentage

Table 2: The basal FSH, AMH, AFC, ovarian volume in the three groups.

Characters	Group 1 normal responder (n=94)	Group 2: poor responder (n=16)	Group 3: high responder (n=10)	P value
FSH (IU/L)	6.6 ± 1.02	7.1 ± 0.7	6.3 ± 0.7	0.11
AMH (ng/mL)	4.08 ± 0.86	6.63 ±1.01	5.2 ± 0.7	<0.0001*
AFC (n)	18.07± 1.9	20.6 ± 2.7	18.8 ± 2.8	<0.001*
Ovarian volume (ml)	10.9 ± 3.4	13.1 ± 2.5.	11.9 ± 2.8	<0.018*

The test used is mean, SD and ANOVA test.

Regarding the basal FSH level, table 2, figures 2 & 3 show that serum FSH level was reversely related to ovarian response but insignificantly (P<0.11), so high serum FSH level associated with poor ovarian response. The same result was found in AFC and ovarian volume but they show significant negative correlation (P<0.001, 0.01). Serum AMH levels were significantly raised in the poor responder's group and decreased in the normal responder's group (P<0.0001). An interesting finding, on comparing the AMH in patients who conceived (n=28) with the normal responder (n=94), we found that pregnant cases had a significantly lower serum AMH concentrations compared to that of non-conceived cases (4.08 ± 2.7 vs. 5.89 ± 1.95 ng/ml, P < 0.01).

Using the ROC curve, Figure 4, presents the sensitivity and specificity of the AMH, at different levels in predicting risk of excessive and poor response. AMH was found to be a useful predictor of poor response to ovarian stimulation–AMH area under receiver operating characteristic curve (ROCAUC) 0.88; (p<0.001). Also, AMH shows a ROCAUC of 0.81; (p<0.007) for over response, indicating a useful potential for predicting excessive stimulation response. Different cut-offs of AMH levels in predicting response to CC stimulation with the corresponding sensitivity and specificity was detected. In our study, we found that cut-off AMH of 6.3 ng/mL showed the most compromise level between 80.0% sensitivity and 77.8% specificity

The relation between AMH and other parameters of PCOS women with the cut-off level of AMH (6.3 ng/ml) is explained in table 3. AMH level was significantly positive correlated with (AFC) and ovarian volume (r=.52 p<0.001) (r=0.47, p<0.01) respectively. On the other hand, there was a weak negative correlation between serum AMH and serum FSH (r= 0.24, P < 0.1) but failed to reach a statistical significance.

The outcomes of CC ovarian stimulation between cycles with high AMH (>6.3 ng/ml) versus low AMH levels (<6.3 ng /ml) were shown in table 4. Patients with AMH levels less than 6.3 ng/ml had significantly higher ovulation rates than those with AMH of 6.3 ng/ml or greater (p<0.001).

Discussion

Accurate prediction of CC response has significant clinical value in optimization of stimulation strategies and in pretreatment counselling for women at increased risk of poor response. Despite many advances in the field of human assisted reproduction, the risk of extremes of response following CC is still a considerable problem in many programs [10].

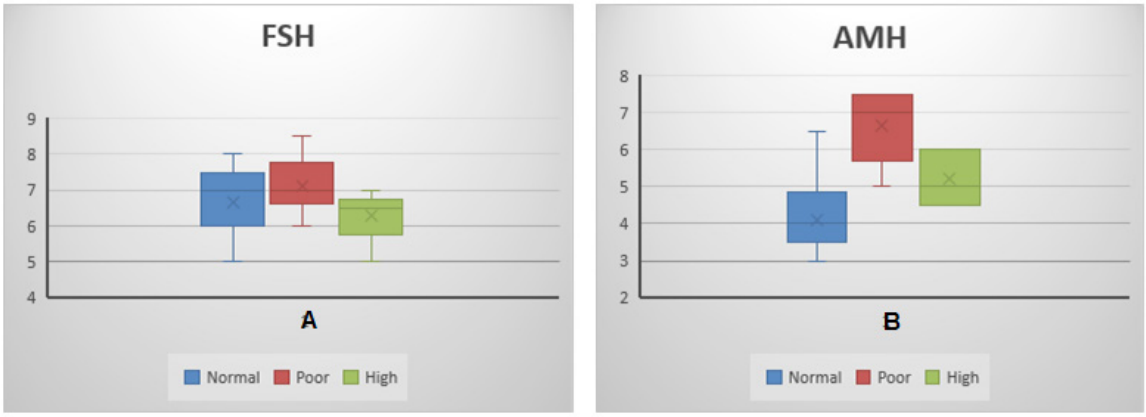

Figure 2: (A) Box-and-whisker plots for baseline FSH in poor, normal, and high-responder PCO women. (B) Box-and-whisker plots for baseline AMH in poor, normal, and high-responder PCO women.

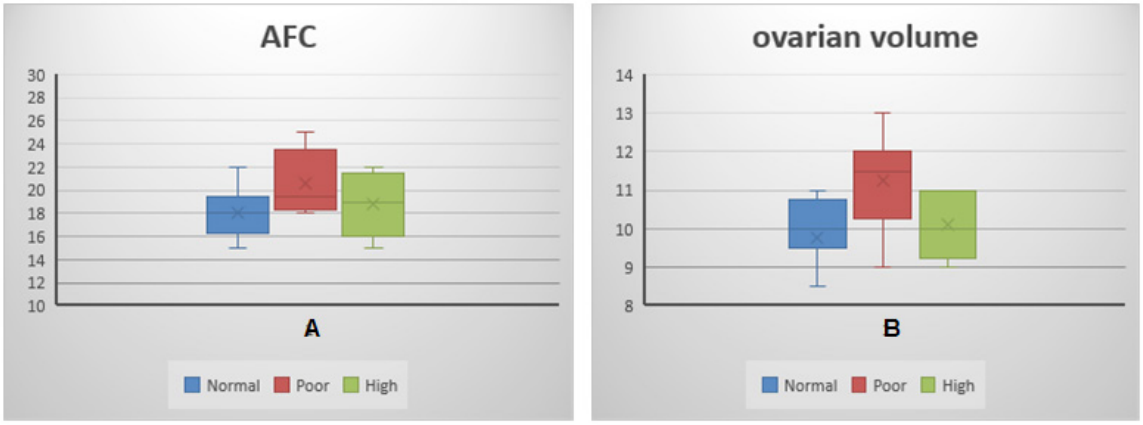

Figure 3: (A) Box-and-whisker plots for baseline AFC in poor, normal, and high-responder PCO women. (B) Box-and-whisker plots ovarian volume in poor, normal, and high-responder PCO women.

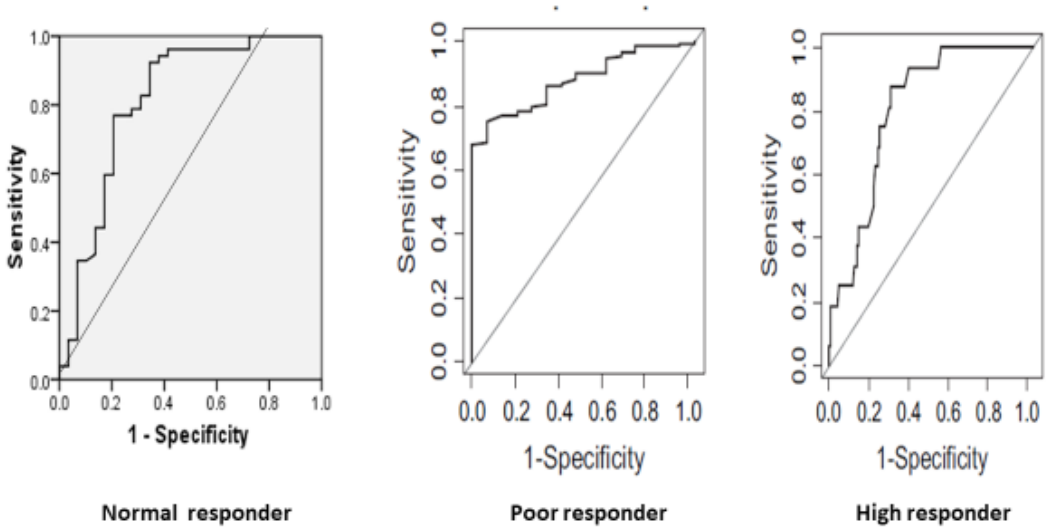

Figure 4: Show ROC for the sensitivity and specificity of AMH at different level in prediction of normal, poor and high response to CC.

Table 3: Comparison of PCOS women with high vs low AMH using a cutoff value of 6.3 ng/ml.

	AMH <6.3ng/dl (n=90)	AMH >6.3 ng/dl (n=30)	Pearson correlation (r)	P value
FSH (IU/L)	6.73 ± 1.16	6.6 ± 1.29	-0.2	<0.1
Ovarian volume (ml)	10.72 ± 1.31	12.96 ± 1.72	0.47	<0.01*
AFC (n)	17.24 ± 3.06	19.45 ± 4.31	0.52	<0.001*

The tests used are mean, SD, and Pearson correlation test.

Table 4: Outcomes of cc ovarian stimulation in cycles with high AMH (> 6.3 ng/ml) vs. cycles with low AMH.

	AMH <6.3ng/dl (n =90)	AMH >6.3 ng/dl (n=30)	P value
Good response (n, %).	(36) 80%	2 (13.3%)	<0.001*
Over response (n, %)	4 (8.8%)	1 (6.6%)	<0.1
Pregnancy (n, %)	13 (28.8%)	1 (6.6%)	<0.2

The tests used are Spearman correlation test.

In the current study, patients with poor response had significantly higher age and BMI compared to those in the other two groups at p<0.001, p<0.04 respectively. These results are in agreement with other studies that show the high BMI is associated with CC resistance [13-15]. Other study found no difference in these factors between groups [16]. These can be explained by the profound effect of the age on follicular growth dynamics [17] and decline of ovarian reserve with age [18].

In agreement with other researchers, our findings suggest that AMH is a strong predictor of both excessive and poor response to CC [19-21]. The current study demonstrates that AMH contributes strongly to extremes of ovarian response (P<0.0001). Furthermore, we measured the AFC, ovarian volume and FSH and compared their performance to AMH in prediction of CC response. Our analysis showed that AMH is a superior predictor of response and enables to identify women at risk of poor, OHSS and good response better than FSH (p<0.11), AFC(P<0.001) and OV (P<0.01).

Recently Pigny et al observed similar prediction accuracy for AMH and AFC to CC response [8]. Our findings seem to contradict the previous study by Lie Fong and co-workers [22] who suggested that serum AMH is not an accurate marker of ovarian response. But in that study, low dose gonadotrophin was used for ovulation induction in PCOS patients which might be the cause of the different findings.

AMH level is significantly higher in PCOS women [23] and is explained by the increase of the preantral follicle in PCO women [24]. Our study shows a negative influence of high AMH levels on ovarian responsiveness to CC. These findings suggest that high circulating AMH is associated with more ovarian resistance to CC stimulation, because AMH has negative effect on the sensitivity of growing antral follicles to the administered CC preventing folliculogenesis [10,25].

Other interesting studies on the impact of circulating AMH on the outcome of laparoscopic ovarian drilling and ovarian responsiveness to gonadotrophin therapy [25]. All of them hypothesis that PCOS women with relatively high serum levels of AMH seem to be resistant to all methods of ovarian stimulation [11].

Interestingly and in contrast to the above, AMH concentrations are known to positively predict ovarian response to gonadotrophin stimulation during IVF. Meanwhile, low AMH levels are indicative of a diminished ovarian reserve, and so is associated with poor response [26].

In the current study, high AMH levels were linked to poor response to CC treatment. Furthermore, we have identified a cut-off level of serum AMH concentration (6.3 ng/ml), above which the chances of good ovarian response were markedly reduced. The present data indicate that for poor response a cutoff of 6.3 ng/mL would have 80% sensitivity and 77.8% specificity. This is different from the findings of Amer et al. who identified a cut-off level of serum AMH concentration (4.7 ng/ml), [11] above which the chances of good ovarian response to hMG were markedly reduced from 100% (in women with lower AMH) to 35%. The reason for that difference in these AMH cutoff is t Amer et al. used hMG for induction not the CC. Our cut-off is greater than those of previously reported by Mahran A et al. [27] who reported that (3.4 ng/ml). Xi et al. (2016) also show different Cut off level of (7.77 ng/ml) [7].

In the current study, Serum AMH is to be positively correlated with AFC and mean ovarian volume. There is a statistical difference between both groups of PCOS women with high vs low AMH using a cutoff value of 6.3 ng/ml as regard to the AFC and OV (p<0.001, p<0.01) respectively. These correlation results were consistent with earlier findings [9,24,28]. In the present study, we established a weak negative insignificant correlation between serum AMH levels and FSH (p<0.1). On the other hand, another study by Mahran show there was a statistically significant negative correlation [29].

In current study, pregnancy rate increase with AMH level below 6.3 ng/ml but it fails to reach the significant value (P<0.2). In study done by Xi, the AMH levels were significantly higher in non-pregnancy compared with pregnancy group [7]. However, this result is due to inclusion of the women with CC-resistant in the analyses. This may be due to the fact that most CC resistant patients in this study had relatively higher AMH were excluded from the non-pregnancy group.

Regarding OHSS, current study showed that women with AMH<6.3 had insignificant higher rate of OHSS than those with AMH>6.3 (p<0.1). On the other hand, it is not the role in the normal IVF cycle, where the patients with elevated AMH (>3.5 ng/mL) should be considered at high risk of OHSS [25]. But at high AMH, less ovarian response to CC and subsequently less OHSS risk is expected.

The main strength of this study is its prospective design with

inclusion of consecutive patients fulfilling the study inclusion criteria. The main limitation of this study is the relatively small number of patients included. However, serum AMH levels are known to be generally stable with minimal variation allowing small studies to show significant differences. Another limitation is the CC dose used in this study as we use 100mg /day. FDA does not approve the doses that excess of 100 mg per day which may induce ovulation in CC non-responder in our study.

Conclusion

AMH could help in counseling women with PCOS regarding the chance of success and the risks of over response with CC therapy. In addition, pre-treatment measurement of serum AMH levels could help in determining the protocol of the ovarian stimulation wither CC or gonadotrophin. Further studies are required to look into the benefit of adjustment of the doses of CC or gonadotrophin according to the level of circulating AMH.

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