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Antral follicle count and anti-Müllerian hormone level as predictors of ovarian hyperstimulation syndrome in women with polycystic ovarian syndrome undergoing controlled ovarian stimulation

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antral follicle count (AFC); polycystic ovarian syndrome (PCOS); ovarian hyperstimulation syndrome (OHSS)

Abstract

Aim: To compare the rate of ovarian hyperstimulation syndrome in women with and without polycystic ovarian syndrome, and to determine the cut-off for the antral follicle count and the anti-Müllerian hormone level predictive of ovarian hyperstimulation syndrome in both groups. **Methods:** This was a prospective cohort study conducted in women aged 20–35 years who were undergoing controlled ovarian stimulation. The women were divided into those with polycystic ovarian syndrome and the controls on the basis of the Rotterdam criteria. The outcome of stimulation was recorded, and the ovarian response markers were compared in both groups. **Results:** Among 689 women included in the study, 276 (40.1%) had polycystic ovarian syndrome, and 476 (59.9%) were used as the controls. Ovarian hyperstimulation syndrome occurred in 19.6% of the cases, and in 7.7% of the controls (p <0.001). The conception rate was greater in the group of cases (52.5% vs. 16.5%, p = 0.001). Among the cases, the sensitivity and specificity for the prediction of hyper-response were 94.4% and 97.3% for AFC, and 92.6% and 93.7% for the anti-Müllerian hormone, at the cut-off values of ≥18 and ≥6.425 ng/ml, respectively. Among the controls, the sensitivity and specificity for the prediction of hyper-response were 93.8% and 97.1% for the antral follicle count, and 93.6% and 94.5% for the anti-Müllerian hormone, at the cut-off values of ≥10 and ≥3.95 ng/ml, respectively. **Conclusion:** Group-specific values should be used to identify and counsel women undergoing controlled ovarian stimulation. In light of available evidence, gynaecologists should be trained to perform ultrasound evaluation, determine the antral follicle count of their patients, and offer them appropriate counselling.

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a serious complication encountered in some women undergoing controlled ovarian stimulation (COS)[(1)]. It is associated with an increased production of vasoactive substances such as angiotensin and vascular endothelial growth factor (VEGF), which leads to an increased capillary membrane permeability. The rise in permeability results in fluid accumulation in the third space and intravascular fluid depletion, thus making a woman susceptible to ascites, pleural effusion, and intravascular dehydration[(2)]. Mild OHSS occurs in 20–33% of women undergoing COS, moderate OHSS affects 3–6%, and only in 0.3% of all cases the syndrome is severe[(3)]. COS is commonly employed to increase the yield of ova available in order to enhance the success rate of artificial reproductive techniques.

The risk factors for OHSS include young age, polycystic ovarian syndrome (PCOS), decreased body mass index, and the presence of multiple follicles. The National Institute for Health and Care Excellence guideline on fertility predicts a likely high ovarian response to gonadotropin stimulation in an in vitro fertilisation (IVF) cycle and excessive ovarian response if any of the following is present: (i) a follicle-stimulating hormone (FSH) level of less than 4 IU/l, or (ii) an anti-Müllerian hormone (AMH) level greater than 25 pmol/l or (iii) an antral follicle count (AFC) greater than 16[4]. The problem with using these criteria – and specifically the AFC criterion – is that a woman is diagnosed with PCOS according to the Rotterdam criteria for PCOS[5]. As per the criteria; a woman is diagnosed when she has any two of the three features: 1) oligo- or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, or 3) polycystic ovaries; polycystic ovaries are defined as those found on the ultrasound to contain 12 or more follicles measuring 2 to 9 mm in diameter and/or have an increased volume of 10 mL or greater.

Only one ovary needs to meet these criteria for the definition of polycystic ovaries to be satisfied. Consequently, a woman with PCO may have 12 or more follicles on the initial scan and the criteria predictive of response may not fit in this particular scenario. This does not take away from the fact that PCOS is a high risk factor to begin with.

Now evidence is slowly emerging that these women may have a higher cut-off for the predictors as compared to the general population. A recent study showed that the cut-off for AMH in women with polycystic ovarian syndrome (PCOS) was higher. It has also been argued that the level of the anti-Müllerian hormone (AMH) is a better predictor of ovarian response than the antral follicle count (AFC)[6]. However, the data remains conflicting and studies continue to advocate both AFC and AMH as possible predictors of ovarian response[7]. Also, OHSS is more commonly seen in women with PCOS, but these women are also more likely to benefit from ovulation induction and have higher conception rates. We undertook this study to compare the rate of OHSS in women with PCOS undergoing ovulation induction and those without PCOS. We also set out to determine the cut-off of AMH and AFC for OHSS in women with PCOS and those without.

Methods

This was a prospective cohort study conducted at the Aziz Medical centre from the 1st July 2017 until the 31st December 2019. Women aged 20–39 years who were undergoing ovulation induction with clomiphene citrate were recruited. Women having polyps, thyroid dysfunctions, hyperprolactinaemia, premature ovarian failure, and ovulation induction with only gonadotrophins were excluded. Also excluded were women whose male partners were being treated for male factor infertility.

After obtaining informed consent, a brief history was taken from the women to ensure that they satisfied the study inclusion criteria. The women were explained the study protocol and follow-up. The patients undergoing induction were divided into those with PCOS and those without on the basis of the Rotterdam criteria. After that, they had a day 2 ultrasound to determine the antral follicle count. A Mindray DP-2200 scanner with a 5 to 7.5 MHz frequency endovaginal transducer was used for all the procedures. All scans were performed by the lead author who is a specialist in ultrasound and fertility, and has 10 years of professional experience in the field. For the estimation of serum AMH levels, all blood samples of selected subjects were drawn by venipuncture in serum separator tubes. Blood samples were taken for AMH levels on any day of the menstrual cycle. The serum AMH levels were determined by enzyme-linked immunosorbent assay (ELISA), using human AMH Elisa kit (CDN-E 1350) at the reference lab.

The women were induced with clomiphene citrate 50 mg from menstrual cycle day 2 to day 6, for a total of 5 days. The women were followed up with a scan after every 48 hours to assess the size of follicles. Follicular monitoring was done, and ovulation was triggered with human chorionic gonadotrophin when the lead follicle reached a size of 18 mm. Sexual contact was encouraged, and the women were counselled about the signs of OHSS. They were requested to report back if they become pregnant, when a transvaginal scan was done to confirm a foetal heartbeat.

OHSS was classified according to the signs and symptoms tabulated in the RCOG guideline on OHSS[8]. The cases of OHSS were classified as mild, moderate or severe. A standard protocol was followed.

Clinical pregnancy was defined as foetal heartbeat present on a transvaginal scan after the women had a beta-hCG positive value in the serum.

For the estimation of sample size, we searched the literature and identified a study that compared OHSS in women with PCOS undergoing an IVF cycle and women with tubal factor infertility[9] Using that study as a reference, and assuming similar proportions for the women with PCOS and those without, the sample size for the study came out to be $n = 128$ patients in each group. The sample size was calculated using the WHO software, where alpha = 5%, power of the test 1-beta = 80%, anticipated population proportion 1 = 6.3%, and anticipated population proportion 2 = 18.8%. We used the two group test (two-sided test) of equivalence in proportion to calculate the sample size (Sample Size Determination in Health Studies, Version 2.00, Copyright (c) 1996–98, World Health Organization). To compensate for protocol deviation, the sample size was inflated by 5%, so that minimum 135 women were needed in each study group

A proforma was used to collect the data. The demographic data of the women included age, height and weight. Reproductive history including the duration and type of infertility was also noted. The antral follicle count (AFC) on the initial scan, and the AMH level were also recorded in the proforma.

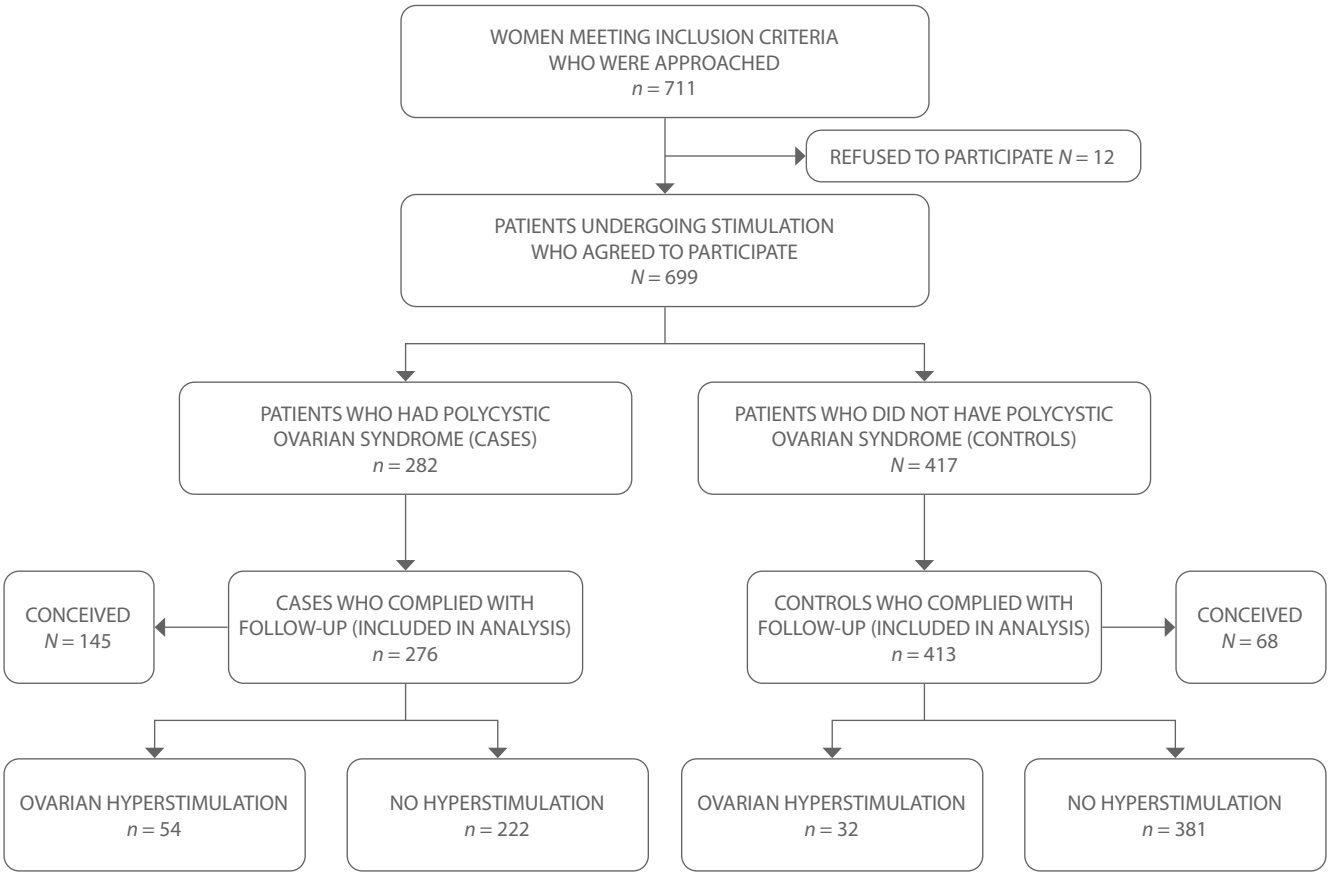

Fig. 1. *Profile of the study*

The primary outcome measure in this study was to assess to compare the rate of OHSS in women with and without PCOS. The secondary outcome measure was to assess the cut-off of AFC and AMH for OHSS in both groups.

All the participants provided their informed consent. In lieu of a formal ethics committee or formal institutional review board approval, the Declaration of Helsinki was followed. No subjects were harmed, confidentiality was maintained, and no patients were enrolled in the study without their formal informed consent.

Statistical analysis

Data was entered into the SPSS version 15. The Shapiro-Wilk test was used to assess the normality of data distribution. Quantitative variables were represented as means and standard deviation, and qualitative variables were represented as frequencies and percentages. Chi-square test and Fischer's exact test were used to compare these variables at the $p < 0.05$ level of significance.

An ROC curve was used to assess the sensitivity and specificity of both AFC and AMH levels for the prediction of OHSS in both groups. The SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Over the study period, a total of 711 women satisfied the inclusion criteria and were approached for participation. Of these, 12 women refused to participate and were excluded. Of these 699 women, a total of 282 had PCOS, and 417 were the controls. However, 6 cases and 4 controls did not comply with the follow-up and were excluded. Therefore, we included 689 women in the final analysis (Fig. 1).

Of these 689 women, 276 (40.1%) had PCOS, and 413 (59.9%) were the controls. The mean age of the study participants was 31.32 ± 3.82 years, and the mean BMI was 22.64 ± 1.71 kg/m^2. The mean duration of infertility was 3.19 ± 0.93 years, and the majority of the women (591, 85.8%) had primary infertility. The pregnancy rate of the study participants was 213 (30.1%), and OHSS occurred in 12.5% of them. Table 1 summarises the study population's characteristics.

The cases and controls were not significantly different in terms of their age ($p = 0.175$) or duration of infertility ($p = 0.245$), but there was a significant difference in BMI (23.27 ± 1.94 vs. 22.21 ± 1.41, $p < 0.001$) and type of infertility ($p < 0.001$). OHSS occurred in 19.6% of the cases, and 7.7% of the controls ($p < 0.001$). The mean antral follicle count among the cases was 14.32 ± 3.69 vs. 6.46 ± 2.47 ($p < 0.001$), in the controls. The mean AMH was also significantly different in both groups (6.36

Tab. 1. *Basic characteristics of the study population*

Characteristics	Mean ± SD or count (%)
Age (years)	31.32 ± 3.82
Height (m)	1.57 ± 0.04
Weight (kg)	55.66 ± 3.45
BMI (kg/m²)	22.64 ± 1.71
Duration of fertility (years)	3.19 ± 0.93
Type of infertility	
Primary	591 (85.8%)
Secondary	98 (14.2%)
Group	
PCO	276 (40.1%)
Control	413 (59.9%)
Pregnancy	
Yes	213 (30.1%)
No	476 (69.1%)
OHSS	
Yes	86 (12.5%)
No	603 (87.5%)

Tab. 2. *Comparison of cases and controls*

Characteristics	Group		*P* value
	PCO	Control	
	Mean ± SD	Mean ± SD	
	Count N %	Count N (%)	
Age	31.09 ±3.81	31.53 ± 3.82	0.175
BMI	23.27 ± 1.94	22.21 ± 1.41	0.001
Duration infertility	3.29 ± 0.91	3.13 ± 0.94	0.245
Type of infertility			0.001
Primary	214 (77.5%)	377 (91.3%)	
Secondary	62 (22.5%)	36 (8.7%)	
AFC	14.32 ± 3.69	6.4 ± 2.47	0.001
AMH	6.36±1.10	3.71 ± 0.45	0.001
OHSS			0.006
Yes	54 (19.6%)	32 (7.7%)	
No	222 (80.4%)	381 (92.3%)	
Pregnancy			0.001
Yes	145 (52.5%)	68 (16.5%)	
No	131 (47.5%)	345 (83.5%)	

Tab. 3. *AUC for AFC and AMH to predict hyper-response*

AFC	CUT-OFF	AUC	95 (CI)	Sensitivity	Specificity
PCO	18	0.969	(0.939–0.992)	94.4%	97.3%
Control	10	0.972	(0.975–0.997)	93.8%	97.1%
AMH					
PCO	6.425 ng/ml	0.972	(0.946–0.990)	92.6%	93.7%
Control	3.95 ng/ml	0.974	(0.956–0.993)	93.6%	94.5%

± 1.10 ng/ml among the cases vs. 3.71 ± 0.45 in the controls, p <0.001) (Tab. 2).

A receiving operator curve (ROC) was used to predict hyper-response; Table 3 shows the area under the curve and 95%CI both for the cases and controls.

In women with PCOS, the sensitivity and specificity for the prediction of hyper-response were 94.4 % and 97.3 % for AFC and 92.6% and 93.7% for AMH, at the cut-off values of ≥18 and ≥6.425ng/ml, respectively. In the controls, the sensitivity and specificity for the prediction of hyper-response were 93.8 % and 97.1% for AFC and 93.8% and 94.5% for AMH, at the cut-off values of ≥10 and ≥3.95 ng/ml, respectively.

Discussion

Main findings

Our study shows that OHSS occurred more commonly among the cases than the controls (19.6% vs. 7.7%, p <0.001). Also, the conception rate was greater among the cases (52.5% vs. 16.5%, p = 0.001).

In the group of cases, the cut-off values of ≥18 and ≥6.425 ng/ml, for AFC and AMH respectively, had higher specificity and sensitivity, while the same values for the controls were lower.

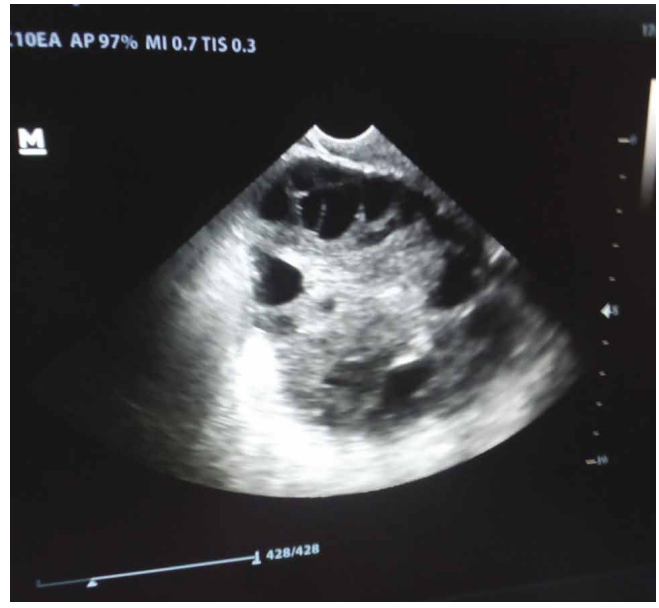

Fig. 2. *Polycystic ovary*

Strengths and limitations

Our study shows that women with PCOS have higher cut-offs than women without PCOS. To our knowledge and based on a literature review, this is the first study ever to predict the cut-off for AFC as a predictive factor of hyper-response both in women with PCOS and those without. The major limitation is the fact that the antral follicle count is a sophisticated technique and requires prior training to ensure that women receive a proper count.

Interpretation

PCOS affects 10% of women of reproductive age, and they often require ovulation induction, with

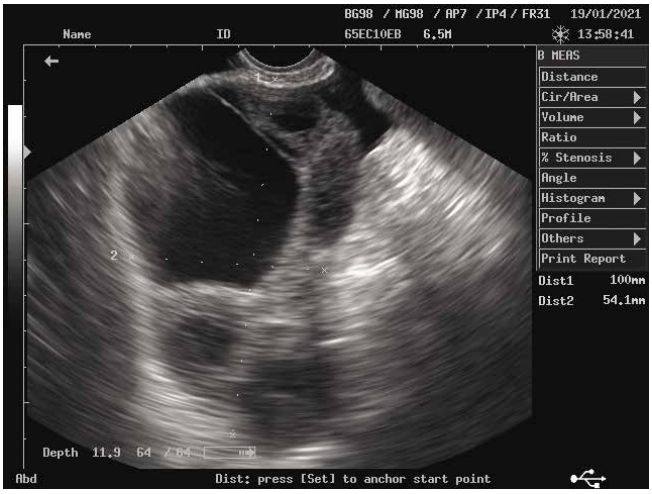

Fig. 3. *Hyperstimulated ovary*

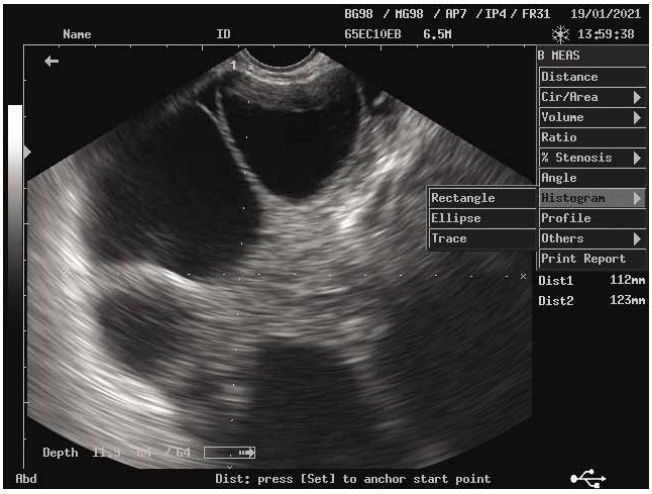

Fig. 4. *Ovarian hyperstimulation syndrome. Patient presented with abdominal pain and nausea after stimulation*

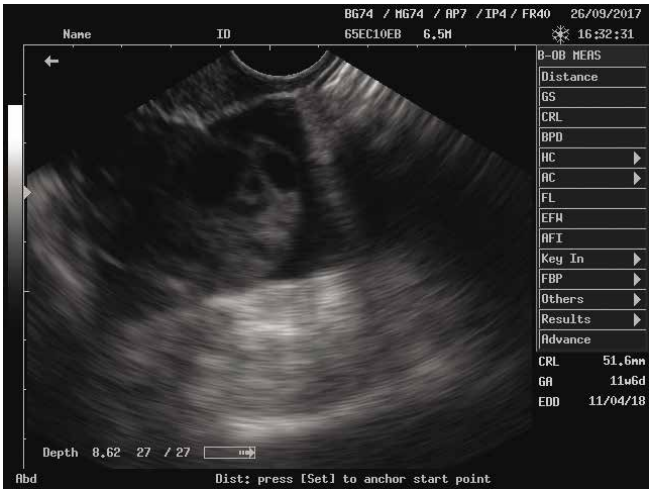

Fig. 5. *Resolving hyperstimulation, free fluid still seen in pouch of Douglas*

hyper-response to induction being a potential serious setback (Fig. 2). The prediction of hyper-response can help counsel these women and prevent failed cycles and complications[10].

Both AMH and AFC have some merit to their use as predictors of hyper-response[11,12]. However, one strict cut-off should not be used for both groups. Women with PCOS have a greater reserve and produce more AMH, and have more AFC. In our study, the women with PCOS had higher AFC and AMH levels than the controls. A study from India reported a cut-off of 6.85 ng/ml for AMH, which is almost similar to our study[6]. The study, however, did not measure AFC, and furthermore did not give a cut-off for AFC. The authors mentioned that the measurement of AFC requires special training and equipment which is not freely available. We would argue that all gynaecologists working in fertility clinics should be trained to perform transvaginal ultrasounds themselves, and fertility clinics should be well-equipped. There may be slight differences in measurements but gynaecologists should follow and track their patients.

A patient is said to have ovarian hyperstimulation if the ovaries are greater in area than 8 cm^2. However, RCOG proposes that ovarian size is not the sole measure of severity because of the effect of follicular aspiration. Although the clinical features are of paramount importance, the look of ovaries helps to counsel women about the prognosis and may be used to aid decisions on admission (Fig. 3, Fig. 4).

AMH levels are determined via blood tests and are invasive. AFC can be measured at the time of appointment and has been shown to have a good value as a predictor of hyper-response. Women who are being stimulated should have follicle tracking, and the decision to give further doses should be based on the response of the ovaries. Although the intricate balance of hormones is disrupted and the ovary may respond later, and hyperstimulation not seen initially may manifest later; this can also be controlled, and further injectables can be stopped or symptomatic treatment started if the patient is being monitored. The hyperstimulation resolves in most cases (Fig. 5).

Measurements need to be taken in the early phase of the cycle. The addition of AFC to AMH has been shown to improve ovarian reserve evaluation[13].

RCOG advises that 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 centres 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 gynaecologist and the woman to ensure that care is not compromised and no cases are missed.

In our study, the women with PCOS had better conception rates than the women without PCOS. The pregnancy rate in women with PCOS could be explained by their better reserve and the fact that the primary problem i.e. anovulation, was rectified by induction. Women with PCOS are the

best candidates for induction and proper prediction, and counselling is of paramount importance in these cases[14]. There are no studies that directly compare the AFC cut-offs for women with PCOS than those without. A few studies, however, have reported cut-offs of AMH to predict hyper-response. Our study, therefore, adds to those findings and provides cut-offs for AFC as well. The AFC values in women without PCOS are lower than the commonly used standard. Women without PCOS had OHSS at AFC greater than 10, while those with PCOS had it at a value greater than 18, which is higher than the accepted standard of 16.

These values are crucial for understanding the concept of counselling for hyper-response. Women should be counselled properly, so that they are compliant with their follow-up and aware of the risks of COS. Gynaecologists should be trained to perform ultrasound evaluation and determine the AFC of their patients, and counsel them in light of available evidence. Additionally, all sonologists should cooperate with the gynaecologist who requests follicle tracking to ensure that no cases of hyperstimulation are missed.

Conclusions

The cut-off value for AFC and AMH in women with PCOS are 18 and 6.425 ng/ml, respectively, but the values for non-PCOS women are lower, 10 and 3.95 ng/ml, respectively. The findings show that group-specific values should be used to identify and counsel women undergoing COS.

Conflict of interest

Authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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