IVF Outcome With High Level of AMH: Focus on Pcos Versus Non-pcos

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Research Article

Keywords: IVF, AMH level, PCOS patients, PCOS VERSUS NON-PCOS

Posted Date: September 23rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-850619/v1>

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Abstract

Background: To investigate whether high AMH level in PCOS patients resulted in different IVF outcomes compared to non-PCOS patients.

Methods: A cross-sectional observational study involving 238 women undergoing IVF who have AMH level > 4 ng/ml. Participants then grouped into PCOS and non-PCOS cohorts.

Results: The median AMH was significantly higher in the PCOS group (7.59 [4.61] ng/ml vs 5.91 [2.22] ng/ml) ($U = 4087$, $p < 0.001$). The PCOS group required less gonadotropin but yielded more oocytes after stimulation. Significantly more participants from PCOS group (41.5% [n=39]) developed hyper-response to ovarian stimulation compared to the non-PCOS group (26.4% [n=38]) (OR = 1.978, 95% CI: 1.138 – 3.488; $p = 0.015$).

Conclusion: Women with PCOS and high AMH levels have a higher risk of hyper-response after ovarian stimulation than their non-PCOS cohorts.

Introduction

Using anti-Mullerian hormone (AMH) analyses to predict ovarian response to stimulation has been widely spread. A high level of AMH, above 4–5 ng/ml, has been associated with a higher risk of hyper response and ovarian hyperstimulation syndrome (OHSS) occurrence (1). Hyper response is characterized by the number of oocytes retrieved ≥ 20 , development of OHSS, cycle cancellation due to hyper response, or combination of those conditions (2).

The AMH is secreted from pre-antral and small antral follicles. It also has been found to be associated with hyperandrogenism (3). Due to those natures, polycystic ovary syndrome (PCOS) patients tend to have higher AMH levels. Wiweko et al. reported that patients with mean AMH level ≥ 4.45 ng/ml have 9.35 times higher possibility to develop PCOS (4). However, high levels of AMH have also been found in non-PCOS patients. Studies that try to observe the difference of high AMH state in PCOS and non-PCOS patients and its effect on IVF outcomes are still very limited.

Therefore, this study aims to determine whether high AMH levels in PCOS and non-PCOS patients might result in differences in their IVF outcomes.

Methods

Study population

We conducted a retrospective cohort study of eligible women who underwent an IVF cycle in our fertility clinic between January 2013 - June 2020. The data were obtained from medical records. Approval for this study was obtained from Health Research Ethics Committee - University of Indonesia and Cipto

Mangunkusumo Hospital. Inclusion criteria used were serum AMH level > 4 ng/ml and underwent GnRH antagonist protocol. Exclusion criteria used were: 1) frozen embryo transfer (FET) cycle, 2) cycles canceled prior to oocyte pick up, 3) incomplete data.

The study population was divided into two groups, PCOS group vs. non-PCOS group. The diagnosis of PCOS was established based on the Rotterdam Criteria, when at least 2 of the following three criteria existed: oligomenorrhea or amenorrhea, clinical hyperandrogenism and/or hyperandrogenemia, and polycystic ovaries.

Clinical & laboratory protocols

Serum AMH levels were measured prior to initiating controlled ovarian stimulation (COS) protocol in each patient. All AMH measurements were performed at our clinic's laboratory.

Initial gonadotropin dosages were based on patient age, weight, antral follicle count, and previous response to stimulation, if any. COS was achieved with gonadotropins (Gonal F; EMD-Serono, Switzerland). Ovulation was suppressed using 0.25 mg Cetrotide (Cetrorelix; EMD-Serono, Switzerland).

rhCG (Ovidrel; EMD-Serono, Switzerland) was used as the ovulation trigger. The rhCG trigger was generally given when the two lead follicles attained a mean diameter > 18 mm. Oocyte retrieval was performed under conscious sedation and transvaginal ultrasound (TVUS) guidance with a 30 cm 16 G oocyte aspiration needle 35–36 h after the ovulatory trigger. Luteal support with 50 mg of intravaginal progesterone (Crinone; Merck Serono, Denmark) daily was begun the day after retrieval.

Fertilization of oocytes was performed with conventional insemination or intracytoplasmic sperm injection (ICSI) based on the couple's history and the male partner's semen analysis. All embryos were cultured using in-house culture media. Cleavage embryos were assessed on day 2 (44–46 h after insemination or sperm injection) and day 3 (66–72 h insemination or sperm injection). Embryos were graded based on the criteria described by Veeck [19]. Embryo transfers were performed with Wallace catheters (Smiths Medical, OH, USA) at approximately 1 cm less than the uterine depth identified at prior trial transfer.

Outcome variables

Demographic characteristics included AMH level, age, BMI, type of infertility, duration of infertility, and infertility diagnosis. Primary IVF outcomes measured were biochemical pregnancy (BP) rate, clinical pregnancy (CP) rate, ongoing pregnancy (OP) rate, and OHSS occurrence.

Secondary outcomes measured COS parameters and outcomes, including total days of COS, total gonadotropin administered, endometrial thickness, total number of oocytes retrieved, mature oocytes rate, and fertilization rate. Fertilization rate is defined as the percentage of transformation of inseminated oocytes into two pronuclei (2PN) embryos.

Embryo evaluation was performed according to the Graduated Embryo Score (GES) criteria (Fisch et al., 2001). Patients who have had at least three embryos with scores equal to or greater than eighty points according to the criteria on day three were considered eligible to undergo 5 days of embryo culture. On day 3 evaluation, embryos that meet these criteria: 1) less than 25% fragmentation, 2) stage-specific cell size for the majority of cells, and 3) no multinucleation, are considered as good quality embryos. The Gardner blastocyst scoring system was used to evaluate the quality of blastocyst on day 5 of embryo culture.

Statistical analysis

All statistical analyses were performed using SPSS version 25 (IBM Corp; Armonk, NY, USA). Categorical variables are presented by the number of cases and corresponding percentages. Continuous variables are presented as mean $\pm$ SD. Mean differences between 2 groups were compared using the Student t-test or Mann-Whitney test, as appropriate. A $p < 0.05$ was considered statistically significant.

Results

A total of 238 women aged 24–41 years old met the criteria and were enrolled in the study. The mean age of the non-PCOS group was found to be higher ($t(236) = 2.49$, $p = 0.01$). The median AMH was also significantly higher in the PCOS group ($U = 4087$, $p < 0.001$). No other characteristics showed a significant difference between the two groups (Table 1).

Table 1
Patient characteristics

Variables	PCOS (n = 94)	non-PCOS (n = 144)	p-value
Age (years)	32.73 ± 3.63	34.10 ± 4.45	0.010 ^b
BMI (kg/m ²)	23.21 (5.02)	23.62 (5.34)	0.784 ^a
Type of infertility (n [%])			
- Primary	80 (85.1%)	122 (84.7%)	
- Secondary	14 (14.9%)	22 (15.3%)	
Duration of infertility (years)	5 (4)	6 (6)	0.161 ^a
Infertility diagnosis (n [%])			
- Male factor	–	75 (52.1%)	
- Tubal		15 (10.4%)	
- Ovulatory		1 (0.7%)	
- Endometriosis		12 (8.3%)	
- Others		41 (28.5%)	
AMH (ng/ml)	7.59 (4.61)	5.91 (2.22)	< 0.001 ^a
^a Mann-Whitney test			
^b Independent t-test			

normally distributed continuous data are expressed as mean ± standard deviation, while non-normally distributed data are expressed as median (interquartile range); categorical data are expressed as n(%)

From the COS process, we found that the PCOS group required less gonadotropin ($U= 4733$, $p = 0.001$). However, despite less dosage of gonadotropin administered, more oocytes were retrieved in the PCOS group after stimulation ($U= 5152$, $p = 0.002$) (Table 2).

Table 2
COS parameters and outcomes

Variables	PCOS (n = 94)	non-PCOS (n = 144)	p-value
Total days of COS (days)	10 (2)	11 (2)	0.177 ^a
Total gonadotropin doses (IU)	2085 (938)	2550 (1706)	0.001 ^a
Endometrial thickness (mm)	11.7 (2.43)	12 (3.78)	0.131 ^a
Number of oocytes retrieved (n)	17 (12)	14 (10)	0.002 ^a
Mature oocytes rate (%)	83.30 (18.8)	83.65 (18.3)	0.142 ^a
Fertilization rate (%)	75.0 (23.3)	71.4 (25.7)	0.995 ^a
OHSS (n [%])	5 (5.3%)	5 (3.5%)	0.448*
Response (n [%])			0.077*
• Poor response	0 (0%)	2 (1.4%)	
• Normal response	55 (58.5%)	104 (72.2%)	
• Hyper response	39 (41.5%)	38 (26.4%)	
^a Mann-Whitney test			
*Chi-square test			

non-normally distributed continuous data are expressed as median (interquartile range); categorical data are expressed as n(%)

From the PCOS group, 2 patients had mild PCOS, and 3 had severe OHSS. Meanwhile, from the non-PCOS group, 4 patients had mild OHSS, and 1 had severe OHSS. Patients with severe OHSS must have their cycle canceled, and the embryo be frozen. Two other patients in the PCOS group also chose to have their embryos frozen due to non-clinical indications. Hyper response cases were found to be significantly higher in the PCOS group (41.5% vs 26.4%, $p = 0.015$) with OR = 1.978 (95% CI: 1.138–3.488) (Table 2).

We found no statistically significant difference between the two groups in embryo quality parameters (Table 3). Pregnancy rates also showed no significant difference between the two groups (Table 4).

Table 3
Embryo outcomes

Variables	PCOS (n = 89)	non-PCOS (n = 143)	p-value
Number of transferred embryo (n)	2 (1)	2 (1)	0.433 ^a
Good quality embryo (n [%])	82 (87.2%)	137 (95.1%)	0.237*
Day 5 Transfer (n [%])	43 (45.7%)	59 (41.0%)	0.292*
^a Mann-Whitney test			
*Chi-square test			

non-normally distributed continuous data are expressed as median (interquartile range); categorical data are expressed as n(%)

Table 4
IVF outcomes

Variables	PCOS (n = 89)	non-PCOS (n = 143)	p-value
Cumulative BP rate (%)	58 (61.7%)	80 (55.6%)	0.164*
Cumulative CP rate (%)	43 (45.7%)	55 (38.2%)	0.140*
Cumulative OP rate (%)	25 (26.6%)	49 (34.0%)	0.326*
*Chi-square test			

categorical data are expressed as n(%)

Additionally, we also tried to determine the cut-off value of AMH to predict the hyper response in both groups. Receiving Operator Curve (ROC) curve was plotted for each group, and the area under the curve (AUC) was recorded. AUC value of 0.626 and 0.617 (in PCOS and non-PCOS groups, respectively) indicates poor predictive quality (Table 5).

Table 5
AMH cut-off value for hyper response prediction

AMH	Cut-off value (ng/ml)	AUC	95% CI	Sensitivity (%)	Specificity (%)
PCOS	7.18	0.626	0.512–0.741	71.8%	52.7%
non-PCOS	5.57	0.617	0.515–0.720	71.1%	46.2%

Discussion

From the patient characteristics, we found a significant difference in the median age and AMH level between the two groups. These findings are similar to previous studies. For example, Lauritsen et al. (2014) reported a lower PCOS prevalence with increasing age (5). It might be caused by the decrease of ovarian reserve or antral follicle as a woman age (6).

Higher AMH levels in PCOS patients also has been reported by numerous studies (4, 7–10). It might be caused by the increased synthesis and secretion of AMH by pre-antral and small antral follicles (3). The level of AMH increases with antral follicle count (AFC) at a consistent rate of 0.2 ng/ml per follicle (11). In addition, granulosa cells in follicles of PCOS patients have been shown to produce 75-fold more AMH than normal (12).

From the IVF process, we found that the PCOS group received significantly less gonadotropin dose during COS. However, their number of oocytes retrieved was found to be higher. Moreover, the number of hyper responders in the PCOS was also significantly higher than the control group. Although all the study subjects received antagonist protocol, different doses may be given. Following our clinic's protocol, protocol and doses were decided based on age, BMI, AFC, and previous response to stimulation, if any.

Indeed, the major problem of gonadotropin stimulation is the increased risk of multiple pregnancy and OHSS. Therefore, low-gonadotropin stimulation has been introduced and lately has been accepted as best practice for PCOS patients, especially for those who have clomiphene citrate (CC)-resistant (13, 14). In the last few years, a more sophisticated method was introduced. The individualized COS (iCOS) warrants identifying high-risk patients through various biomarkers, with AMH and AFC seem to be promising variables (2, 15). Few studies have reported the use of iCOS led to significantly less OHSS occurrence (16, 17).

Our PCOS group showed significantly higher average numbers of retrieved oocytes despite lower gonadotropin doses administered. Various studies have well documented similar findings. Sahu et al. reported that despite a significantly lower total gonadotropin dose, PCOS and PCO-only groups yielded more oocytes compare to controls (18). However, they also found no differences in oocyte maturation, embryo quality, implantation, and pregnancy rate. We also found the same circumstances in our study. Swanton et al. also found significantly more oocytes retrieved in PCOS and PCO-only groups ($n = 14.2$ and $n = 16.2$, respectively) compared to control ($n = 10.5$). They also found significantly more severe OHSS cases in the PCOS and PCO-only groups, similar to ours.

Curiously, when we tried to determine a cut-off value of AMH levels for hyper-response prediction, it resulted in a poor predictive value. It is presumably the result of the heterogenicity of each antral follicle. While AMH production is high, each follicle's threshold to respond to ovarian stimulation may vary in women with PCOS. Kim et al. analyzed the correlation between AMH-MoM (AMH level multiples of median) and ovarian sensitivity to gonadotropin stimulation. They found that ovarian sensitivity was not correlated to the AMH-MoM value and even tended to decrease with an increasing AMH-MoM level (19). These findings may suggest that the use of basal serum AMH level as a predictor of ovarian response should be carefully applied, considering other factors in the patients.

Due to its retrospective design and a relatively small number of patients, our study resulted in low power. Moreover, various PCOS phenotypes were recruited in the study, which might play a role in PCOS patients' response to gonadotropin stimulations. More comprehensive factors and data must be included to further investigate the underlying causes associated with OHSS in PCOS patients.

Conclusion

Even though both groups have high AMH levels, women with PCOS still have a higher risk of hyper response after ovarian stimulation. Further investigation of its pathophysiology and other factors associated with it need to be done in the future.

Declarations

The study has been performed in accordance with the Declaration of Helsinki.

Ethics approval and consent to participate

Ethical approval for this study was obtained from Health Research Ethics Committee - University of Indonesia and Cipto Mangunkusumo Hospital (ID: 87/UN2F1/ETIK/2019). All participants have given written informed consent.

Consent for publication

All authors have given consent and obtained participant's consent for the publication of identifiable details, which can include photograph(s) and/or videos and/or case history and/or details within the text to be published in the BMC Women's Health journal.

Availability of data and material

The data that support the findings of this study are available upon request.

Competing interests

All authors have no competing interests to declare.

Funding

The study is self-funded by the authors.

Authors' contributions

RM, YDP, and AH designed the study. Acquisition of data was conducted by YDP and KAP. YDP, KAP, and MM contributed to the data analysis and interpretation, and manuscript writing. RM and AH supervised the overall process of the study.

Acknowledgements

Not applicable.

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