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Predicting the outcome of different protocols of *in vitro* fertilization with anti-Muüllerian hormone levels in patients with polycystic ovary syndrome

Ya Chen[1], Bilv Ye[1], Xiaojing Yang[2], Jiujia Zheng[1], Jinju Lin[1] and Junzhao Zhao[1]

Abstract

Objective: This study evaluated associations of basal serum and follicular fluid (FF) anti-Muüllerian hormone (AMH) levels with *in vitro* fertilization (IVF) outcomes in polycystic ovary syndrome (PCOS) patients.

Methods: This prospective study included 179 consecutive women undergoing IVF, including 59 with PCOS and non-PCOS controls. Thirty PCOS cases had long gona-dotrophin-releasing hormone agonist (GnRH-a) and 29 had antagonist (GnRH-ant) protocols. Controls underwent conventional GnRH-a. Associations of basal serum and FF AMH levels with IVF outcomes were assessed.

Results: Median serum and FF AMH levels, antral follicle count (AFC), oestradiol human chorionic gonadotropin injection day (peak E2), and retrieved oocyte numbers were higher in PCOS patients than in controls (all $P < 0.01$). Oocyte maturation and high-quality embryo rates were lower in PCOS patients than in controls ($P < 0.01$), but both groups had similar fertilization, implantation, clinical pregnancy, and newborn rates. Peak E2 was higher in GnRH-ant than in GnRH-a protocols (16.5 nmol/L vs. 12.1 nmol/L, $P < 0.05$). AMH levels were correlated with AFC in PCOS patients ($P < 0.01$). Peak E2 and FF AMH levels were independent predictors of oocyte number. Peak E2 predicted the fertilization rate.

Conclusion: Serum basal AMH levels are predictive of oocyte quantity, but not oocyte quality or IVF outcomes. Serum AMH, FF AMH, and outcomes are similar among protocols.

[1]Reproductive Center, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China
[2]Gynaecology and Obstetrics Department, The First People's Hospital of Hangzhou, Hangzhou, Zhejiang, China

Corresponding author:
Ya Chen, Reproductive Center, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China.
Email: chenyakelly@163.com

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder causing ovulation failure in women of reproductive age. PCOS has an incidence ranging from 8.7% to 17.8%, depending upon the criteria used.[1–4] PCOS is characterized by an excessive number of growing ovarian follicles of up to 2 to 5 mm in size.[5] When *in vitro* fertilization (IVF) is considered, PCOS is a well-known risk factor for development of ovarian hyperstimulation syndrome (OHSS).[6] Therefore, carefully monitoring for OHSS during controlled ovarian hyperstimulation (COH) is important.[7]

Anti-Muüllerian hormone (AMH) is a dimeric glycoprotein of the transforming growth factor-β (TGF-β) family. AMH is produced in the ovary by granulosa cells of pre-antral and small antral follicles of less than 4 mm diameter. Similar to established predictors, such as maternal age, basal serum follicle-stimulating hormone (FSH) levels, and antral follicle count (AFC),[8,9] AMH is considered a useful marker of ovarian reserve, and even clinical outcome of IVF.[10–14] However, a recent meta-analysis suggested that there were weak associations between AMH and implantation and clinical pregnancy rates, while in women with diminished ovarian reserve, AMH had a poor predictive ability of IVF outcome.[15] The main advantage of measurement of AMH levels in IVF may stem from their low inter- and intra-cycle variability. Therefore, AMH levels could be used as a menstrual cycle-independent marker of ovarian response to COH.[16] AMH is superior to age and FSH levels for estimation of ovarian reserve and adjusting the stimulation protocol in infertile women. However, the value of AMH levels for predicting pregnancy outcomes is still uncertain.[13,14]

AMH levels in women with PCOS are two- to three-fold higher than those obtained in patients without PCOS.[17] However, whether AMH levels in women with PCOS provide useful information on IVF outcomes is controversial because they may not be an accurate predictor of outcome in these patients.[18,19] This study aimed to evaluate whether basal and follicular fluid (FF) AMH levels affect IVF outcome in patients with PCOS. Our data suggest that serum basal AMH levels are a significant predictor of oocyte quantity, but not oocyte quality or IVF outcomes.

Methods

Subjects and study design

This prospective, observational study involved 179 consecutive patients who were referred to the Reproductive Center Department of the First Hospital of Wenzhou Medical University, China, from January 2012 to January 2014. The inclusion criteria were patients with PCOS, fallopian tube problems without PCOS, or treatment due to male infertility without PCOS. Exclusion criteria were as follows: >38 years, serum FSH levels >12 IU/L, a history of ovarian surgery, ovarian cyst or tumour, hydrosalpinx, endometriosis, and endocrine or systemic illnesses. In case of a positive Chlamydia test, women received routine antibiotics treatment. IVF was performed once the test became negative.

There was some difference in protocols among the women who were included in this study because we found that women with

PCOS who were treated with the agonist protocol were at high risk of OHSS. Therefore, women with PCOS treated afterwards received the antagonist protocol. Consequently, 59 women with PCOS were randomly assigned to two groups according to their admission time There were 30 and 29 cases in the agonist and antagonist treatment groups, respectively. A total of 120 non-PCOS patients with regular menstrual cycles were assigned to the control group and underwent a conventional long agonist protocol. PCOS was diagnosed according to the revised Rotterdam criteria.[20]

The study was approved by the hospital's Ethics Committee (Approval ID: [2013] 27). The protocol was explained to the patients before enrolment, and informed consent was obtained from each couple.

Protocol

Women with PCOS in the agonist group were initiated with an oral contraceptive pill containing ethinyloestradiol and cyproterone acetate (Diane-35; Schering AG, Berlin, Germany) on cycle days 3–5 for 14 days. This treatment was performed to synchronize their cycle and subsequent follicle development. This was followed by pituitary downregulation via administration of a gonadotrophin-releasing hormone agonist (GnRH-a) consisting of 0.4 to 0.8 mg of triptorelin acetate (Decapeptyl; Ipsen Pharmaceuticals, France). After 14 days, ovarian stimulation was started with recombinant FSH (Gonal-F; Merck Serono, Switzerland) injection. Women with PCOS in the antagonist group were pretreated with Diane-35 for 21 days and received Gonal-F injection on days 3–5 of the subsequent cycle. A total of 0.25 mg of cetrorelix (Cetrotide; Merck Serono, Switzerland) was administered subcutaneously daily from the moment the leading follicle reached 14 mm in diameter until human chorionic gonadotrophin (hCG) injection. Infertile women in the control group were provided GnRH-a (triptorelin acetate) in the mid-luteal phase (day 21) of the cycle at the same dosage as that used for patients with PCOS in the agonist group. Gonal-F was administrated after downregulation of the pituitary. The patients were provided 5000–10,000 IU of hCG when the leading follicle reached 18 mm in diameter with at least three follicles >16 mm in diameter. The oocytes were collected 36 h after hCG administration. After IVF or intracytoplasmic sperm injection (ICSI), embryos with equally or slightly unequally sized blastomeres and <20% anucleate fragments were regarded as good-quality embryos. Two or three good-quality embryos were transferred 3 days after collection of oocytes. Luteal support was provided by injecting 60 mg of progesterone once daily and 10 mg of dydrogesterone twice daily from the day of retrieval of oocytes. When oestradiol levels were greater than 15,000 pmol/L on the day of hCG injection, or more than 15 oocytes were retrieved, the risk of OHSS was explained to the patients. A suggestion was then made to freeze the embryos. In these cases, the women received frozen thawed embryo transfer (FET) at a later date.

Collection of specimens

A total of 5 ml of venous blood was collected during a natural cycle on days 2–5, 1 week before starting the IVF procedure. The women with PCOS who were included in this study all had menstrual cycles for this measurement. FF was obtained from only the first and largest follicle (>16 mm) to avoid bleeding in the specimen. Blood and FF samples were allowed to clot in collection tubes for 30–60 min, and were centrifuged at 1000 rpm for 10 min. The resulting supernatants were stored at −80°C until use. Serum AMH (defined as bAMH) and FF AMH levels were determined by enzyme-linked immunosorbent sensitive assays according to the manufacturer's instructions

(DSL, Webster, TX, USA). Hormone monitoring, including estradiol (E2) and β-hCG concentrations, was performed by chemiluminescence methods (Beckman Coulter, Inc., Fullerton, USA).

The AFC was determined at days 2–5 of the natural menstrual period. Follicles from bilateral ovaries with diameters between 4 and 6 mm were counted by ultrasound imaging. Serum E2 levels on the day of hCG injection were defined as peak E2. The oocyte maturity rate was calculated as the ratio of oocytes at metaphase II (MII) to the total oocytes retrieved. The fertilization rate was calculated as the ratio of two-pronuclear zygote (2PN) oocytes to MII. The good-quality embryo rate was calculated as the number of good-quality embryos multiplied by that of 2PN oocytes. The clinical pregnancy rate was calculated as the number of gestational sacs multiplied by that of transferred fresh embryos. The abortion rate was calculated as the ratio of spontaneous abortion cases to clinical pregnancy cases. The take-home newborn rate was calculated as the birth number divided by the number of fresh embryos transferred. The implantation rate was calculated as the number of gestational sacs multiplied by that of transferred embryos.

Follow-up and observational indices

The follow-up examinations included serum levels of hCG 14 days after transplantation to determine the success of pregnancy. Subsequently, transvaginal ultrasound was performed to determine intrauterine pregnancy, gestational sac number, and embryo heart beats 14 days later. The patients were then followed up by telephone interviews until 3, 5, and 8 months of pregnancy when ultrasound examinations were conducted to assess foetal development. Data regarding the mode of delivery, number of neonates, body weight, sex, and deformities at birth were also collected.

Statistical analysis

Statistical analyses were performed with SPSS 17.0 (IBM, Armonk, NY, USA). Normally distributed variables are presented as mean ± standard deviation (SD), and were assessed by the two independent samples *t*-test. Frequencies were compared with the chi-square test. Correlations between AMH and the other indices were evaluated with Pearson's correlation analysis. Multivariate analysis was performed to evaluate whether there were relationships between bAMH or FF AMH levels and demographic variables with IVF outcome. $P < 0.05$ was considered statistically significant.

Results

Overall population characteristics

The mean age of patients in the PCOS and control groups was 29.1 ± 3.5 and 30.3 ± 3.9 years old, respectively ($P > 0.05$). Mean body mass index (BMI) was 22.2 ± 3.2 and $21.4 \pm 2.9\,kg/m^2$, respectively ($P > 0.05$). AFC, peak E2, bAMH levels, and FF AMH levels in the PCOS group were significantly higher than those in the control group (all $P < 0.01$).

COH and IVF results

Oocyte maturation and good-quality embryo rates in the PCOS group were markedly lower than those in the control group (all $P < 0.01$). However, there were no significant differences in fertilization, implantation, abortion, and take-home newborn rates between the two groups (all $P > 0.05$, Table 1).

In the PCOS group, women who were considered at risk of OHSS later received FET, but 46 women received fresh embryo transfer (including 23 women in each subgroup), of whom 22 women achieved a clinical pregnancy. All 120 controls received

Table 1. Laboratory and clinical data in the control and PCOS groups.

	Control (n = 120)	PCOS (n = 59)	*P* value
AFC	15.9 ± 4.62	29.07 ± 9.86	<0.001
Days of Gn	10.71 ± 1.55	9.69 ± 2.09	<0.001
Doses of Gn (IU)	1614.96 ± 375.25	1234.11 ± 306.09	<0.001
Peak E2 (pmol/L)	8604.73 ± 4509.32	14,250.07 ± 8406.43	<0.001
bAMH (ng/ml)	3.33 ± 1.19	11.86 ± 4.79	<0.001
FF AMH (ng/ml)	6.17 ± 2.49	10.16 ± 6.26	<0.001
Oocyte number	11.98 ± 5.30	16.51 ± 10.33	<0.001
Maturation rate (%)	89.8 (1291/1437)	83.8 (816/974)	< 0.001
Fertilization rate (%)	74.3 (947/1291)	75.2 (614/816)	0.335
Good-quality embryo rate (%)	66.9 (634/947)	53.6 (329/614)	<0.001
Embryo implantation rate (%)	42.1 (106/252)	32.3 (32/99)	0.093
Clinical pregnancy rate (%)	60.8 (73/120)	47.8 (22/46)	0.130
Abortion rate (%)	16.4 (12/73)	9.1 (2/22)	0.394
Take-home newborn rate (%)	49.2 (59/120)	43.5 (20/46)	0.511

Table 2. Comparison of demographic and endocrine profiles of patients with PCOS by protocol.

	Agonist protocol (n = 30)	Antagonist protocol (n = 29)	*P* value
AFC	30.57 ± 12.34	29.9 ± 10.02	0.820
Days of Gn	10.67 ± 2.04	8.69 ± 1.63	0.000
Doses of Gn (IU)	1277.5 ± 318.36	1189.22 ± 291.54	0.272
Peak E2 (pmol/L)	12,084.97 ± 6948.58	16,489.83 ± 9282.17	0.043
bAMH (ng/ml)	11.94 ± 5.01	11.75 ± 4.57	0.887
FF AMH (ng/ml)	9.63 ± 6.04	10.75 ± 6.57	0.504
Oocyte number	15.87 ± 8.12	17.17 ± 12.32	0.631
Maturation rate (%)	81.9 (390/476)	85.5 (426/498)	0.127
Fertilization rate (%)	73.3 (286/390)	77 (328/426)	0.226
Good-quality embryo rate (%)	52.1 (149/286)	54.9 (180/328)	0.491
Embryo implantation rate (%)	40 (20/50)	24.5 (12/49)	0.099
Clinical pregnancy rate (%)	56.52 (13/23)	39.13 (9/23)	0.238
Take-home newborn rate (%)	52.17 (12/23)	34.78 (8/23)	0.234

fresh embryos, including 73 who were successfully impregnated and two with ectopic pregnancies. According to the 2009 guidelines by Golan and Weissman concerning OHSS,[21,22] because women at risk received FET, there were no moderate or severe OHSS cases.

In patients with PCOS, no differences were found in indices, including IVF outcome measures (Table 2), compared with the antagonist subgroup. An exception was peak E2 values, which were lower in the agonist subgroup than in the antagonist subgroup ($P < 0.05$).

Associations of AMH levels and IVF outcome

In the control group, positive correlations were observed between bAMH or FF AMH levels and the AFC, oocyte number, fertilization, and good-quality embryo number

Table 3. Correlations between bAMH or FF AMH levels and demographic variables.

	Control (n = 120)		PCOS (n = 59)	
	r	*P* value	r	*P* value
bAMH, AFC	0.259	0.007	0.600	<0.001
bAMH, number of oocytes	0.535	<0.001	−0.059	0.685
bAMH, number of fertilizations	0.395	<0.001	−0.041	0.776
bAMH, number of good-quality embryos	0.250	0.009	−0.097	0.502
FF AMH, AFC	0.253	0.008	0.368	0.005
FF AMH, number of oocytes	0.490	<0.001	−0.203	0.131
FF AMH, number of fertilizations	0.527	<0.001	−0.110	0.417
FF AMH, number of good-quality embryos	0.427	<0.001	−0.063	0.639

(all $P < 0.01$). In the PCOS group, positive correlations were found between bAMH or FF AMH levels and the antral follicle count (AFC) ($P < 0.01$) (Table 3).

Predictors of oocyte number and fertilization rate

Several parameters were assessed for their predictive value of oocyte number and fertilization rate, including age, BMI, bFSH, peak E2, gonadotrophin dose, bAMH, FF AMH, and AFC. As shown in Table 4, peak E2 ($P < 0.001$) and FF AMH levels ($P = 0.040$) were independent predictors of oocyte number. Peak E2 had a significant predictive value for the fertilization rate ($P < 0.001$). The remaining parameters were not significant independent predictors of oocyte number or the fertilization rate ($P > 0.05$) (Table 4).

Discussion

This study aimed to assess the possible associations of bAMH or FF AMH levels with IVF outcome in patients with PCOS. We also evaluated two protocols that were designed for IVF in patients with PCOS for their effects on IVF outcome. No significant differences were found in fertilization, implantation, clinical pregnancy, and take-home newborn rates among the groups. Serum AMH levels correlate well with ovarian reserve.[13,14] In our study, positive correlations were observed between bAMH or FF AMH levels and the AFC, oocyte number, fertilization, and good-quality embryos in control women without PCOS. However, in the PCOS group, a positive correlation was only observed between bAMH or FF AMH levels and the AFC. These findings suggest that AMH levels are a good predictor of IVF outcome in women without PCOS undergoing fertility treatment.[10,12,23] Our findings are in contrast to a previous study[9] and a recent meta-analysis.[15] Notably, our results are in agreement with previous reports, which showed that AMH levels were not a reliable predictor of IVF outcome in women with PCOS.[18,19] Therefore, AMH levels have different predictive values with various patient populations, and should be used with caution as a measure of IVF outcome.

Measurement of ovarian reserve includes the quantity and quality of oocytes growing in follicles that can mature and eventually be fertilized. Regrettably, there are no non-invasive methods available for assessment of ovarian reserve, and oocytes cannot be seen by the naked eye. Therefore, the AFC has become a surrogate for oocyte quantity. In our study, bAMH levels were highly and

Table 4. Multivariate analysis of various parameters for their predictive value of oocyte number and fertilization rate.

	B	Standard error	Unstandardized coefficients	*t*	*P* value
Age	−0.035	0.326	−0.013	−0.108	0.915
BMI	−0.171	0.422	−0.055	−0.406	0.687
bFSH	−0.191	0.845	−0.030	−0.226	0.822
Peak E2	0.001	0.000	0.656	5.467	<0.001
Gn dose	0.001	0.004	0.039	0.319	0.751
bAMH	0.140	0.321	0.067	0.435	0.666
FF AMH	−0.467	0.220	−0.285	−2.123	0.040
AFC initiate	−0.008	0.130	−0.008	−0.061	0.952
Age	0.065	0.228	0.035	0.287	0.776
BMI	0.060	0.295	0.029	0.205	0.839
bFSH	0.060	0.295	0.029	0.205	0.839
Peak E2	0.001	0.000	0.649	5.099	<0.001
Gn dose	−0.001	0.003	−0.025	−0.194	0.847
bAMH	0.077	0.224	0.056	0.344	0.732
FF AMH	−0.216	0.154	−0.200	−1.403	0.168
AFC initiate	−0.010	0.091	−0.016	−0.106	0.916

Independent variables: age, body mass index (BMI), basal follicle stimulation hormone (bFSH), peak estradiol (peak E2), gonatropin dose (Gn dose), bAMH, FF AMH, and AFC initiate.

positively associated with the AFC in both patient groups, as well as with the number of oocytes retrieved after ovarian stimulation in control patients. FF AMH and bAMH levels in patients with PCOS were significantly higher than those in non-PCOS patients. Additionally, the AFC and the number of oocytes retrieved from patients with PCOS were significantly higher than those obtained from control patients. These results suggest that bAMH values reflect the quantity of oocytes in ovaries to a certain extent. Therefore, in terms of the quantitative aspect of ovarian reserve, the higher the serum AMH level, the greater the number of antral follicles and oocytes. Therefore, individualized COH protocols based on serum AMH levels might eliminate serious OHSS and decrease cancellation of IVF cycles. Because no valid method is available for assessment of oocyte quality, indirect indices are used, including oocyte maturation and clinical pregnancy rates. We obtained discrepant data on the predictive value of AMH levels for oocyte quality. Maturation and good-quality embryo rates in the PCOS group were significantly lower than those in the control group. However, no differences were found in fertilization, embryo implantation, clinical pregnancy, abortion, and take-home newborn rates between the two groups. Additionally, FF AMH levels were an independent predictor of oocyte number, but not of the fertilization rate. Therefore, we speculate that serum AMH levels do not necessarily reflect the quality of oocytes.

When women with PCOS also have fallopian tube or male factor fertility problems, IVF becomes an important method of treatment. The standard long agonist protocol is the conventional and most common method of COH during IVF. However, the antagonist protocol is increasingly being

contemplated because of its shorter therapy time and lower gonadotrophin dosage.[24] Indeed, the incidence of OHSS can be reduced by the antagonist protocol, which is important for patients with PCOS, particularly owing to their risk of severe complications involving OHSS.[24] In this study, no cases of moderate or severe OHSS occurred. This may be due, at least in part, to the seven and six patients who chose to freeze good-quality embryos in the agonist and antagonist subgroups, respectively. This finding might have also resulted from administration of Voluven and prednisone for 3 days from the day of ovum collection to prevent the women from having OHSS when a high risk of OHSS was expected. No significant differences in in bAMH or FF AMH levels were found between patients undergoing the long and antagonist protocols. This finding is in agreement with a previous report[24] in which FF AMH levels were not mentioned. In our study, there were no significant differences in the number of oocytes retrieved, as well as maturation, fertilization, good-quality embryo, implantation, clinical pregnancy, and take-home newborn rates, between protocols. These results suggest that the antagonist protocol has no advantage of improving clinical outcomes in patients with PCOS undergoing IVF.

Favourable oocyte growth and normal embryo development after fertilization are greatly affected by the constituents of the FF, which directly surrounds the oocytes. AMH, which is secreted by granulosa cells, not only plays a role in blood circulation, but also plays a part in the autocrine and paracrine pathways.[25] FF AMH values might effectively predict the outcomes in women with PCOS or those without PCOS after IVF therapy.[23,26,27] However, discrepant data were obtained in this study, with significant positive correlations between FF AMH levels and maturation, fertilization, and good-quality embryos in the control group, but not in the PCOS group. These data are consistent with Mashiach's study.[28] Taken together, these findings suggest that the value of FF AMH levels in predicting pregnancy outcomes in patients with PCOS or those without PCOS after IVF is inconsistent. Recently, a report showed that in normal ovulatory women, secretion and protein expression of FF AMH by small follicles are greatly increased compared with those in medium or big follicles.[29] This phenomenon is not observed in women with PCOS. FF AMH levels in follicles that were unstimulated by exogenous gonadotrophin were five times higher in patients with PCOS compared with controls.[29,30] These studies suggest a pathological imbalance in the expression and secretion of AMH in patients with PCOS, which may have a correlation with abnormal follicular recruitment and development of PCOS.

In conclusion, use of AMH as a reliable indicator of ovarian reserve is important because it represents the numbers of preantral and small antral follicles. For patients with PCOS who have a high risk of OHSS, AMH levels should be assessed to predict and help prevent OHSS. Whether the conventional long protocol can be replaced by the antagonist protocol in IVF for patients with PCOS needs to be confirmed by further studies.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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