

Relationship between anti-mullerian hormone and assisted reproductive technique outcome in patients with polycystic ovary syndrome

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Abstract

Background: Anti-mullerian hormone (AMH) levels may represent the ovarian follicular pool and could be a useful marker of ovarian reserve. The clinical application of AMH measurement has been proposed in the prediction of quantitative and qualitative aspects in assisted reproductive technologies.

Objective: This study aimed to assess the relationship between the serum levels of AMH and results of assisted reproductive technique (ART) outcome in polycystic ovary syndrome (PCOS) patients versus control group.

Materials and Methods: This cohort study was conducted on 61 (PCOS) patients and 28 patients without PCOS (controls) candidates for assisted reproductive technique. Serum levels of AMH were measured on the 3rd day of menstrual cycle and all the patients underwent controlled ovarian hyper stimulation and ART. The relationship between AMH serum level with retrieved oocytes, mature oocytes and pregnancy rate were assessed.

Results: There was significant correlation between the AMH level with number of total retrieved oocytes and mature oocytes in patients with PCOS and controls ($p=0.001$). In PCOS and control groups AMH level in pregnant patients was higher, but it was not statistically significant ($p=0.65$, $p=0.46$, respectively). The major outcome of the study (pregnancy) did not differ significantly between two groups.

Conclusion: This study revealed that AMH level was higher in pregnant patients undergoing ART; but AMH may not be an accurate predictor for pregnancy in PCOS patients.

Key words: Anti-Mullerian hormone, Polycystic ovary syndrome, Assisted reproductive technique.

Introduction

Fertility clinicians have been faced with the challenge of determining the degree of ovarian reserve to better tailor assisted reproductive technology (ART) treatment. The costly drug regimens, the discomfort to patients and the significant risk of complications associated with ovarian stimulation, all justify the need for

obtaining clinically relevant information before commencement of treatment (1). A useful biomarker of ovarian response to controlled ovarian hyperstimulation in assisted reproduction is needed (2). Many tests have been developed to screen for diminished ovarian reserve. Traditional methods used to predict prospectively response to ovarian stimulation. These included mainly the measurement of baseline cycle day 3 serum concentrations of hormones such as FSH, estradiol and inhibins, or ultrasonographic tests such as pretreatment ovarian volume and the number of early antral follicles (3). Recently, anti-mullerian hormone (AMH), also referred to as Mullerian-inhibiting substance, has been proposed as a novel

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marker for predicting ovarian response to gonadotropin stimulation (2, 4). Antimüllerian hormone is a member of the transforming growth factor beta super family (5). In the women, it is solely produced by the granulosa cells of growing preantral and small antral ovarian follicles. Serum AMH levels may be used as a marker of ovarian reserve, representing the quantity and quality of the ovarian follicle pool (5-7). Women with PCOS are known to have elevated baseline AMH levels when compared with age-matched normoovulatory women (8, 9). As a marker for ovarian reserve, MIS/AMH correlates positively with ovarian response to COH in normoovulatory women, but this has not been assessed in women with polycystic ovary syndrome (PCOS) (10). Disturbed dominant follicle selection leading to an excess accumulation of preantral and small antral follicles in women with PCOS presumably causes the elevated MIS/AMH levels (10).

Although MIS has been shown to reflect antral follicle counts and to be elevated in PCOS, it is unclear how well measurements of MIS may reflect ovarian morphological parameters in PCOS, specifically ovarian size and blood flow (8). Searching available literature, we did not find any study comparing ART outcomes according to AMH levels between PCOS patients and non PCOS patients. To gain further insight about MIS/AMH as a predictor of ovarian response, we investigated the relationship between the serum levels of anti-müllerian hormone (AMH) and results of assisted reproductive techniques (ART) in PCOS patients versus non PCOS patients (Control group).

Material and methods

In this cohort study, 61 polycystic ovary syndrome (PCOS) patients and 28 non PCOS patients (controls), candidates for assisted reproductive technique entered the study. The study was conducted in the infertility department of Shariati Hospital, affiliated to Tehran University of Medical Sciences during 2008. The project was approved by the ethical committee of the infertility department of the university and was initiated after achieving written consents of the participants. PCOS diagnosis was according to Rotterdam criteria (9). According to the Rotterdam criteria, we accepted the presence of two of the three following characteristics for inclusion in the study: (1) oligomenorrhea/amenorrhea, (2) clinical (hirsutism) or biochemical finding of hyperandrogenism, and (3) polycystic ovaries on

transvaginal sonography. All the patients aged less than 35 years with normal prolactin and thyroid hormone levels and normal male spermogram in their spouse. In the control group, 28 patients candidates for COH and ART due to unexplained or/and tubal factor infertility were selected. Ages, body mass index (BMI), and duration of infertility were similar between two groups. In all patients the serum levels of anti-müllerian hormone (AMH) was measured on the 3rd day of menstrual cycle. Serum AMH was measured by enzyme-linked immunosorbent assay (ELISA) using the MIS/AMH ELISA kit (DRG instruments GmbH, Germany). The patients underwent controlled ovarian hyperstimulation (COH) with Gonadotropin/GnRH-agonist long protocol. All the participants received folic acid 1mg/day before initiating the induction cycle, low dose oral contraceptive pills on day 3 of the previous cycle and doxycycline 100 mg twice a day for the first 10 days of the previous cycle. Long term desensitization protocol using the GnRH agonist Buserelin 500 micrograms subcutaneously was started at the day 21 of the previous cycle. After complete desensitization, ovarian stimulation using Gonal F (Serono, Switzerland) was commenced on day 3 of the next cycle at a daily dose of 150 IU. It was replaced by HMG (Ferring, Germany) after the 7th day of the stimulation. Transvaginal ultrasound (Siemens, Sonoline G20) for follicular development was done every 3-5 days. Final oocyte maturation was triggered when at least 2 follicles with diameter of at least 17 mm was observed, with HCG (Ferring, Germany) 10000 IU administered as a single intramuscular injection. Oocytes were collected 36-38 hours later using transvaginal guided follicle aspiration under general anesthesia. After fertilization through intracytoplasmic sperm injection (ICSI), three good quality embryos were transferred transcervically 3 days later. Luteal phase support was started the day after ovum pick up by administration of progesterone suppository Cyclogest (Actavis, UK), 1200 mg daily. Chemical pregnancy was detected by serum beta-hCG analysis 14 days after embryo transfer and transvaginal ultrasound scan was scheduled 2 weeks later to detect the gestational sac of pregnancy.

Primary outcome measure was pregnancy and secondary outcome measures were stimulation cycle characteristics. Age, body mass index (BMI), infertility duration, number of oocytes retrieved, number of mature oocytes, number of gonadotropin injections, chemical pregnancy,

clinical pregnancy, ovarian hyperstimulation syndrome (OHSS) and cycle cancellation were considered. The relationship between serum level of measured hormonal markers with retrieved oocytes, mature oocytes and pregnancy rate were assessed.

Statistical analysis

For each participant a questionnaire was filled by the researchers. Data were collected from questionnaires, clinical, laboratory notes and ultrasound reports. SPSS16 software (SPSS Inc. Chicago IL.) was used for data collection and analysis. T-test, Mann-Whitney U-test for quantity data, χ^2 and fisher's exact test for quality variables, Pearson test for correlation were used. P value less than 0.05 was considered for statistical significance.

Results

The mean \pm SD age of patients in PCOS group was 29.25 \pm 5.16 years and in control group was 29.61 \pm 4.89 years ($p=0.655$). The duration of infertility was 6.2 \pm 3.12 years in PCOS group and 6.96 \pm 2.82 years in non PCOS group ($p=0.161$). Body mass index (BMI) of patients in PCOS group was 25.89 \pm 3.14 kg/m² and in control group was 25.64 \pm 3.14 kg/m² ($p=0.708$). The MIS level in

PCOS group was 5.24 \pm 3.33 ng/ml and in non PCOS group was 4.24 \pm 2.23 ng/ml ($p=0.251$) (Table I). In PCOS group total oocytes retrieved was 11.49 \pm 5.75 and in non PCOS group was 9.07 \pm 5.76 ($p=0.65$). The number of mature oocytes was 9.12 \pm 4.85 in PCOS group and 7.22 \pm 4.45 in control group ($p=0.64$). Fertilization rate was 64 \pm 26 percent in PCOS and 77 \pm 28 percent in control group ($p=0.59$). Twelve cases in PCOS group and six cases from non PCOS group had chemical pregnancy (positive beta HCG). Eleven cases in PCOS group and six cases in non PCOS group had clinical pregnancy (pregnancy sac on ultrasound scan). Severe ovarian hyper stimulation syndrome (OHSS) and cycle cancellation were not reported in any groups (Table I). Although AMH level was higher in pregnant group (in both PCOS and non PCOS groups) but there was no significant difference between AMH levels in both groups according to pregnancy (Table II).

There was a significant direct correlation between the serum MIS (AMH) level with number of total oocytes retrieved ($r=0.412$), and mature oocytes ($r=0.472$) in PCOS group and there was a significant direct correlation between MIS (AMH) level with number of total oocytes retrieved ($r=0.710$) and mature oocytes (0.744) in control group (Table III). There was not a significant difference between two groups.

Table I. Comparison of the PCOS and non PCOS groups.

	PCOS (N= 61)	Non-PCOS (N=28)	p-value
Age (year)	29.25 \pm 5.16	29.61 \pm 4.89	0.66
Duration of infertility (year)	6.2 \pm 3.12	6.96 \pm 2.82	0.16
BMI (kg/m ²)	25.89 \pm 3.14	25.64 \pm 3.14	0.71
FSH (IU/L)	8.07 \pm 2.86	8.73 \pm 1.86	0.51
AMH (ng/ml)	5.24 \pm 3.33	4.24 \pm 2.23	0.25
Total gonadotropins (n)	26.23 \pm 7	26.36 \pm 6.24	0.88
Total oocytes retrieved (n)	11.49 \pm 5.75	9.07 \pm 5.76	0.65
Mature oocytes (n)	9.12 \pm 4.85	7.22 \pm 4.45	0.64
Fertilization rate (%)	64 \pm 26	77 \pm 28	0.59
Chemical pregnancy	12 (19.67%)	6 (21.43%)	0.99
Clinical pregnancy	11 (18%)	6 (21.43%)	0.88
Severe OHSS	0	0	1
Cycle cancellation	0	0	1

Table II. AMH levels (ng/ml) in two groups according to pregnancy.

	PCOS	Non-PCOS	p-value
Pregnant	5.69±3.66	4.95±1.89	0.651
Non Pregnant	5.13±3.28	4.05±2.31	0.460

Table III. Correlation between AMH level and total oocytes retrieved, mature oocytes in two groups.

	PCOS	Non-PCOS	p-value
Total oocytes retrieved	0.412	0.710	0.001
Mature oocytes	0.472	0.744	0.001

Discussion

This study revealed that AMH is not a definite predictor for pregnancy and ART results in PCOS patients. Although, it is proven that AMH measurement prior to gonadotropin stimulation could provide useful information to direct the application of mild patient-friendly stimulation protocols in order to avoid OHSS (7).

Evaluation of patients who underwent Assisted Reproductive Technique (ART) is very important for both patients and physicians, because it is very expensive and time consuming and stressful for patients. It is important to predict who poor responder is before starting the procedure. Nowadays many tests such as FSH, estradiol, inhibins, antral follicle count (AFC), testosterone and free testosterone for predicting ovarian reserve are suggested.

Thus, despite the different validity of all these tests, there still remain patients who respond poorly to stimulation despite having normal tests of ovarian reserve. This supports the idea that ovarian reserve is not a simple static anatomic number and is not yet fully understood (11).

Participants in our study had the same age, though despite their same mean BMI they seemed somewhat overweight. Basal characteristics (including basal AMH) and cycle characteristics of the 89 patients were similar. In recent studies AMH has been proposed as a new method for predicting ovarian reserve. According to Aflatoonian *et al*, small AFC and AMH are equally

accurate predictors of high ovarian response to COH and allow us to identify the patients who are at increased risk of OHSS prior the commencement of stimulation, and help to determine the appropriate treatment protocols (12). AMH is the most significant predictor of embryo quality (AUC =0.728) (12). It has the ability to predict poor and excessive response to stimulation with exogenous gonadotropins. This biomarker is superior to basal FSH and AFC, and has the potential to be incorporated in to work-up protocols to predict patient's ovarian response (1).

On the other hand, serum level of AMH is a good predictor of the ovarian response to COH in normoovulatory women but not in PCOS (10). This study showed that, there was not a statistically significant difference between AMH level and pregnancy in PCOS in contrast to the control group. Wang *et al*, stating that baseline MIS/AMH is not a good predictor of the ovarian response to COH in PCOS women. The arrest of follicular growth and the heterogeneity of FSH sensitivity among MIS/AMH-secreting follicles in PCOS render baseline measures of MIS/AMH a poor predictor of the gonadotropin response in PCOS (10).

However, there are few published studies confirming the relation between AMH and ovarian response in COH in this group of patients exclusively. In a recent study to relate follicular fluid AMH and FSH levels in patients with PCOS, it was suggested that the granulosa cells from polycystic ovaries continue to produce elevated

levels of AMH, possibly because of impaired access of FSH to follicles. Such an excess in follicular fluid AMH may have harmful consequences on oocyte quality and final maturation (13).

Polycystic ovaries have an abnormally rich pool of growing follicles and a disturbance in the selection and subsequent maturation of a dominant follicle. Increased serum AMH is due to increased production per granulosa cell, suggesting an intrinsic granulosa cell dysregulation in PCOS. Not only is AMH expression increased, but it also might be protracted in polycystic ovary (PCO) follicles. The potential role of AMH on oocyte quality could be future field of reproduction research.

There was not a significant relationship between AMH levels and pregnancy rate in our study. This raises the hypothesis of a negative link between AMH and final oocyte maturation. In the four studies which evaluated the role of MIS/AMH in pregnancy prediction, two studies (14, 15) supported it and two studies did not (16, 17). On the other hand pregnancy rates after IVF are influenced by multiple (non-ovarian) factors including in vitro laboratory conditions, semen parameters, psychological stress and technique of embryo transfer.

Predicting response to gonadotropin treatment; nevertheless, remains an important aim in the evaluation of the couple struggling with infertility (18).

Our study, despite its limitations like small sample size, has the benefit of comparing PCOS patients with a control (non PCOS) group which recommends further well designed investigations in this regard.

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References

1. Nardo LG, Gelbaya TA, Wilkinson H, Roberts SA, Yates A, Pemberton P, et al. Circulating basal anti-Mullerian hormone levels as predictor of ovarian response in women undergoing ovarian stimulation for in vitro fertilization. *Fertil Steril* 2009; 92: 1586-1593.

2. Peñarrubia J, Fábregues F, Manau D, Creus M, Casals G, Casamitjana R, et al. Basal and stimulation day 5 anti-Mullerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist-gonadotropin treatment. *Hum Reprod* 2005; 20: 915-922.
3. Bukman A, Heineman MJ. Ovarian reserve testing and the use of prognostic models in patients with subfertility. *Hum Reprod Update* 2001; 7: 581-590.
4. Gruijters MJ, Visser JA, Durlinger AL, Themmen AP. Anti-Mullerian hormone and its role in ovarian function. *Mol Cell Endocrinol* 2003; 15: 211: 85-90.
5. Van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, et al. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod* 2002; 17: 3065-3071.
6. Van Rooij IA, Tonkelaar I, Broekmans FJ, Looman CW, Scheffer GJ, de Jong FH, et al. Anti-mullerian hormone is a promising predictor for the occurrence of the menopausal transition. *Menopause* 2004; 11: 601-606.
7. La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Carducci Arsenio A, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Hum Reprod Update* 2010; 16: 113-130.
8. Chu MC, Carmina E, Wang J, Lobo RA. Mullerian-inhibiting substance reflects ovarian findings in women with polycystic ovary syndrome better than does inhibin B. *Fertil Steril* 2005; 84: 1685-1688.
9. Piltonen T, Morin-Papunen L, Koivunen R, Perheentupa A, Ruokonen A, Tapanainen JS. Serum anti-Mullerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with polycystic ovary syndrome. *Hum Reprod* 2005; 20: 1820-1826.
10. Wang JG, Nakhuda GS, Guarnaccia MM, Sauer MV, Lobo RA. Mullerian inhibiting substance and disrupted folliculogenesis in polycystic ovary syndrome. *Am J Obstet Gynecol* 2007; 196: e1-5.
11. Laas A. Assessment of ovarian reserve, is there a role for ovarian biopsy? *Hum Reprod* 2001; 16: 1055-1057.
12. Aflatoonian A, Oskouian H, Ahmadi S, Oskouian L. Prediction of high ovarian response to controlled ovarian hyperstimulation: anti-Müllerian hormone versus small antral follicle count (2-6 mm). *J Assist Reprod Genet* 2009; 26: 319-325.
13. Desforges-Bullet V, Gallo C, Lefebvre C, Pigny P, Dewailly D, Catteau-Jonard S. Increased anti-mullerian hormone and decreased FSH levels in follicular fluid obtained in women with polycystic ovaries at the time of follicle puncture for in vitro fertilization. *Fertil Steril* 2009; 94: 198-204.
14. Hazout A, Bouchard P, Seifer DB, Aussage P, Junca AM, Cohen-Bacrie P. Serum antimullerian hormone/mullerian-inhibiting substance appears to be a more discriminatory marker of assisted reproductive technology outcome than follicle-stimulating hormone, inhibin B, or estradiol. *Fertil Steril* 2004; 82: 1323-1329.

15. Barriere P, Freour T, Mirallie S, Jean M, Bach-Ngohou K, Masson D. Anti-mullerian hormone (AMH): Proposition of a cut-off in assisted reproductive therapy (ART) for a promising new marker (measured with Beckman Coulter ELISA). *Fertil Steril* 2006; 86: S289.
16. Ficicioglu C, Kutlu T, Baglam E, Bakacak Z. Early follicular antimullerian hormone as an indicator of ovarian reserve. *Fertil Steril* 2006; 85:592-596.
17. Penarrubia J, Fabregues F, Manau D, Creus M, Casals G, Casamitjana R, et al. Basal and stimulation day 5 anti-Mullerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist-gonadotropin treatment. *Hum Reprod* 2005; 20: 915-922.
18. Sills E, Alper M, Walsh A. Ovarian reserve screening in infertility: Practical applications and theoretical directions for research. *Eur J Obstet Gynecol Reprod Biol* 2009; 146: 30-36.