Correlation of serum Anti-Müllerian hormone concentrations on day 3 of the in vitro fertilization stimulation cycle with assisted reproduction outcome in polycystic ovary syndrome patients

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Abstract

Purpose To investigate whether serum Anti-Müllerian hormone (AMH) on day 3 could predict controlled ovarian stimulation and reproductive outcomes in women with polycystic ovary syndrome.

Methods A total of 164 PCOS patients undergoing their first IVF treatment cycle were prospectively included. Serum AMH levels on cycle day 3 was measured. The controlled ovarian stimulation and clinical outcomes for the study population were divided according to the <25th, 25 to 75th, or >75th percentile of serum day-3 AMH.

Results Estradiol levels on hCG day and the number of retrieved oocytes significantly increased with increasing serum AMH levels, while total consumption of gonadotropin dose showed a significant decrease ($P<0.05$). Fertilization rate and the number of good quality embryos were comparable among the low, average and high groups ($P>0.05$). Embryo implantation rates in the high AMH group was significantly inferior

to those with low and average AMH concentration (27 versus 48.8 and 50%, $P<0.01$). Clinical pregnancy rates was lower in the high AMH group than that of the low and average group (45.9 versus 65 and 66.7%, $P=0.09$), but this difference was only close to statistical significance. In addition, ordinal regression analysis indicated that LH level was the only independent predictor of embryo implantation rates ($P=0.017$). **Conclusions** In PCOS women, AMH levels on day 3 of the IVF stimulation cycle positively predict ovarian response to gonadotrophins. However, the women with high AMH levels had a significantly decreased IR, which may be due to remarkably increased LH concentrations.

Keywords Anti-Müllerian hormone · Assisted reproduction · Polycystic ovary syndrome · Serum AMH

Capsule In PCOS women, high AMH levels on day 3 of the IVF stimulation cycle predict high ovarian response to gonadotrophins, but low embryo implantation rates.

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Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance (MIS), is produced specifically by granulosa cells of early developing pre-antral and small antral follicles in the ovary, and declines with advancing age. AMH has therefore been proposed as a novel clinical marker of ovarian reserve [1]. Another major reason for the interest in AMH is the fact that serum AMH levels are increased significantly in women with polycystic ovary syndrome (PCOS) when compared with normoovulatory women [2, 3].

PCOS is the most frequent cause of anovulatory infertility and hyperandrogenism in young women [4]. Women with this syndrome are characterized by an excessive number of small antral follicles (2–3 fold that of normal ovaries) [5]. As an exclusively granulosa cell product, it was initially proposed that the rise in serum AMH in PCOS was a consequence of the increased small antral follicle number in these ovaries [6]. The

same authors even suggested that serum AMH concentration could be a good surrogate marker for predicting the antral follicle count in women with PCOS [5]. However, the recent studies observed that the increase in AMH concentrations was more due to the increase in production of AMH by each follicle. Indeed, concentrations of AMH were found to be five times higher in follicular fluid from unstimulated follicles in women with PCOS than normoovulatory controls [7].

AMH levels appear to be related to the severity of PCOS, as represented by elevated luteinizing hormone (LH), testosterone or insulin levels or an increased follicle number and/or ovarian volume as established on ultrasound [8–11]. Moreover, AMH is higher in amenorrheic compared with oligomenorrheic women with PCOS, indicating higher AMH levels reflect more impaired disruption in folliculogenesis and granulosa cell function in the ovary [8].

Recently, several studies demonstrated that day 3 AMH concentrations accurately predicted ovarian response to gonadotrophins and the chance of pregnancy during in vitro fertilization (IVF) [12–16]. However, majority of these studies have not focused on women with PCOS. Since a relationship between AMH and the severity of PCOS has been proposed, we were interested in investigating whether the remarkably increased serum AMH in PCOS patients was associated with poor reproductive outcomes. For this purpose, the current study was designed to evaluate whether serum AMH measurement on day 3 of the stimulation cycle could predict ovarian response and outcome in women undergoing assisted reproductive technique.

Materials and methods

Materials

This study was approved by the Ethics Committee of Citic-xiangya reproductive and genetic hospital. All participants provided their informed consent before their involvement in this study. Subjects included 164 women with PCOS who were referred to our department for IVF because of previously unsuccessful conception with ovulation induction ($n=29$) or bilateral tubal blockage ($n=135$) at hysterosalpingography but without hydrosalpinges on transvaginal ultrasonography. The diagnosis of PCOS was based on the Rotterdam criteria, in which at least two of the following three criteria were met: oligomenorrhea or amenorrhea, clinical hyperandrogenism and/or hyperandrogenemia, and polycystic ovaries [17]. The inclusion criteria included: patients 35 years old or younger without previous IVF attempts and partners with normal semen parameters. No PCOS patient had evidence of hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia or androgen-secreting tumors. All patients underwent ovarian stimulation IVF cycles.

Methods

Ovarian stimulation

According to long down-regulation protocol, the women were injected intramuscularly once with Triptorelin Acetate 1.5 mg (Ferring Ltd, Herzliya, Israel) on the 21st day of treatment with contraceptive pills. Controlled ovarian hyperstimulation (COH) was performed by administration of recombinant FSH (Gonal-F; Serono, Geneva, Switzerland) after pituitary suppression was reached. The initial FSH dose was 112.5–150 IU daily. Follicle development was monitored with serial ultrasonographic examinations and serum estradiol measurements. Afterwards, 5,000–10,000 IU of hCG were administered when at least three follicles were 18 mm in diameter. Transvaginal ultrasound-guided follicular aspiration was performed 34–36 h later. Embryo transfers were performed on day 3. Two embryos were transferred. Good quality embryos were defined by their morphologic features and cleavage rate, and included embryos with less than 20% fragmentation and 6–10 cells at day 3. Embryos with the highest score were selected for transfer by using a Cook catheter. According to local criteria, in patients who were high risk for ovarian hyperstimulation syndrome (OHSS), all embryos were cryopreserved. The luteal phase support included vaginal micronized progesterone (600 mg daily), starting from the day of oocyte retrieval.

The implantation rate (IR) was calculated as the number of gestational sacs divided by the number of embryos transferred. Clinical pregnancy was defined as the presence of a gestational sac on ultrasound performed at 4 weeks after embryo transfer. The clinical pregnancy rate (CPR) was calculated as the number of clinical pregnancies divided by the number of embryo transfer procedures.

Hormone assays

Serum AMH was assessed by a second-generation ELISA (reference A16507; Immunotech Beckman Coulter Laboratories, Villepinte, France) on the day in which pituitary desensitization was confirmed, before starting gonadotrophins treatment (day 3). The analytical sensitivity of this assay is 0.14 ng/mL. Intra- and inter-assay coefficients of variation were $\leq 12.3\%$ and $\leq 14.2\%$, respectively. On cycle day 3 before the start of GnRH agonist medication, serum levels of follicle-stimulating hormone (FSH), LH, estradiol, testosterone and insulin were measured using electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). Insulin resistance, defined by the homeostasis model assessment insulin resistance index (HOMA-IR), was calculated using the following equation: $\text{HOMA-IR} = \text{fasting insulin (IU/ml)} \times \text{fasting glucose (mmol/L)} / 22.5$.

Statistical analysis

The AMH concentrations were classified as below the 25th percentile (low AMH), between the 25th and the 75th percentiles (average AMH), or above the 75th percentile (high AMH). The controlled ovarian stimulation and clinical outcomes for the study population were divided according to the <25th, 25 to 75th, or >75th percentile of serum day-3 AMH. Data analysis was performed by using Statistical Package for Social Sciences (SPSS) for Windows, version 13.0 (Chicago, IL). Wilcoxon Sign Rank test was applied for intra-group comparisons. While mean difference among groups were compared by one-way analysis of variance (ANOVA), medians were tested by Kruskal–Wallis test. When the P value from the one-way ANOVA or Kruskal–Wallis test statistics were statistically significant, post-hoc or non-parametric multiple comparison tests, where appropriate, were used to establish which groups differed from which others. The rate of fertilization, implantation and pregnancy were analyzed by the χ^2 -test. $P<0.05$ was considered statistically significant.

Results

Population characteristics in the low, average, and high serum AMH groups are summarized in Table 1. The mean ($\pm$ SD) value of day 3 AMH for all 164 patients was 7.12 ± 3.3 ng/ml. The range, however, was from

1.54 to 19.17 with a median of 6.66 ng/ml. As shown, antral follicle count and testosterone concentration increased significantly from the low to high group for serum AMH on day 3. LH levels were higher in the high AMH group than in the other groups. Women's age, body mass index (BMI), FSH and HOMA-IR were comparable in the low, average and high serum AMH groups. Estradiol levels on the day of HCG administration and the number of retrieved oocytes were significantly increased with increasing serum AMH, while total dose of the FSH showed a significant decrease (Table 1).

The outcome of assisted reproduction cycles in the low, average, and high serum AMH groups are presented in Table 2. Fertilization rates (FR) and the good quality embryos were not statistically different in the three sets of AMH groups. IR in the high AMH group was significantly inferior to those with low and average AMH concentration (27 versus 48.8 and 50%, $P<0.01$). CPR was lower in the high AMH group than that of the low and average group (45.9 versus 65 and 66.7%, $P=0.09$), but this difference was only close to statistical significance (Table 2). Embryo transfers cancelled due to OHSS risk from the low to high serum AMH groups were 1, 4 and 7 cases, respectively.

When ordinal regression analysis was used for prediction of embryo implantation, LH level was the only independent predictor of embryo implantation rates ($P=0.017$) (Table 3).

Table 1 Patient characteristics in the low, average, and high level of serum AMH groups

Variable	Day-3 serum AMH levels(ng/ml)		
	Low, ≤ 4.85 $n=41$	Average, 4.85–8.82 $n=82$	High, ≥ 8.82 $n=41$
Women's ages (yr)	28.63 ± 3.35	28.80 ± 3.71	28.07 ± 3.42
BMI (kg/m^2)	22.32 ± 2.74	21.96 ± 2.75	21.51 ± 2.59
2–9 mm antral follicle/ovary	21.32 ± 8.96	25.63 ± 9.61^a	35.17 ± 16.68^b
FSH(IU/L)	6.25 ± 1.28	6.42 ± 1.34	6.27 ± 1.05
LH(IU/L)	7.59 ± 3.73	6.67 ± 2.57	10.37 ± 4.72^c
T	0.4 ± 0.13	0.49 ± 0.18^a	0.69 ± 0.23^b
HOMA-IR	2.59 ± 1.37	3.06 ± 1.85	3.10 ± 2.21
Total FSH dose(IU)	1952.7 ± 708.1	1584.1 ± 485.2^a	1330.8 ± 256.7^b
Duration of FSH stimulation	11.95 ± 1.53	11.49 ± 1.86	11.05 ± 1.77
E ₂ on day of HCG	2672.51 ± 955.58	3332.59 ± 1316.92^a	3946.07 ± 1279.40^b
No. of oocytes retrieved	13.05 ± 3.35	16.17 ± 5.78^a	19.83 ± 6.58^b
No. of good-quality embryos	6.68 ± 3.09	7.93 ± 3.96	8.54 ± 4.27

Note: Data are expressed as mean $\pm$ standard deviation. $P<0.05$ was considered statistically significant. BMI = bodymass index; FSH = follicle-stimulating hormone; LH = luteinizing hormone; T = testosterone; HOMA-IR = homeostasis model assessment for insulin resistance index; E₂ = estradiol; hCG = human chorionic gonadotrophin;

^a Kruskal–Wallis multiple comparison test was used to determine which group differed from the others: $p<0.05$, average versus the other groups.

^b Kruskal–Wallis multiple comparison test was used to determine which group differed from the others: $p<0.01$, high versus low.

^c Kruskal–Wallis multiple comparison test was used to determine which group differed from the others: $p<0.01$, high versus the other groups.

Table 2 Outcomes of assisted reproduction cycles in the low, average, and high level of serum AMH groups

Variable	Day-3 serum AMH levels(ng/ml)		
	Low, ≤ 4.85	Average, 4.85–8.82	High, ≥ 8.82
No. of embryo transfer procedures	40	75	37
Fertilization rate, %	65.1(347/533)	64.9(852/1312)	60.7(498/820)
Implantation rate, %	48.8(39/80)	50 (75/150)	27(20/74) ^a
Clinical pregnancy rate, %	65(26/40)	66.7(50/75)	45.9(17/37)

Note: Data are expressed as mean $\pm$ standard deviation. $P < 0.05$ was considered statistically significant. Overall P values were determined by χ^2 test. FR = fertilization rate; IR = implantation rate; CPR = clinical pregnancy rate per embryo transfer;

^a $P < 0.01$ versus the other groups.

Neither AMH nor any of the other analyzed parameters were shown to be an independent predictor. In addition, binomial logistic regression analysis indicated that no parameters were significantly associated with clinical pregnancy (data not shown).

Discussion

The hitherto published studies regarding the association between increased serum AMH in PCOS women and their IVF outcome were rare. In the present study, for the first time, we found IR decreased significantly in the high AMH group.

In our study, estradiol levels on the day of HCG administration and the number of retrieved oocytes showed a gradual increase from the low to high AMH group, but the total dose of gonadotrophins was significantly decreased. Our report is in agreement with previous studies indicating that serum AMH levels on day 3 of the stimulation cycle positively predict ovarian responsiveness to gonadotrophins [18, 19]. But the result may seem paradoxical given that an inhibitory effect of AMH on FSH-stimulated aromatase

mRNA expression has been shown in human granulosa-luteal cell [20], suggesting the remarkably increased AMH may reduce follicle sensitivity to FSH and estradiol production. Indeed, a very recent study found an inverse relationship between serum AMH on day 3 during stimulation cycles and serum estradiol levels on the day of HCG administration in PCOS patients [21]. However, AMH has been found mainly synthesized by granulosa cells of preantral and small antral follicles, in follicles growing into dominance this production ceases [22, 23]. Hence, we speculate that the significantly decreased AMH in mature follicle may relieve the inhibition on aromatase expression and estradiol synthesis. Since antral follicles were significantly increased from the low to high AMH group in the present study, it might be anticipated that with more follicles developed during hyperstimulation, estradiol levels accordingly increased and greater numbers of oocytes were obtained in the higher AMH group.

Previously, a few studies showed a positive association between baseline circulating concentrations of AMH and fertilization rate, implantation rate and pregnancy outcome in IVF–ICSI cycles [14, 15]. Also, it has been reported that serum AMH levels on the day of HCG administration were linked to embryology score. The author concluded that AMH ≥ 2.7 ng/mL indicated good oocyte quality as reflected by a higher IR and CPR [24]. However, these studies have involved women with different or non specified ovarian morphology. In our study, women with PCOS has been specifically determined, we found IR decreased significantly in the high AMH group, the CPR also dropped in women with high AMH, but this difference was not significant.

Although the exact reasons for decreased IR in high AMH group in this study are unclear, one mechanism can be suggested. It has been demonstrated that typically increased LH in PCOS patients suppressed FSH function, promoting premature granulosa cells luteinization and follicular atresia in small antral follicles [25–27], which all impaired the quality of both oocyte and embryo [28, 29].

Table 3 Ordinal regression analysis of factors predictive of embryo implantation

Variable	95% CI	P
LH	−0.222– −0.021	0.017
BMI	−0.198–0.032	0.157
AMH	−0.268–0.013	0.074
T	−3.094–0.417	0.135
FSH	−0.397–0.101	0.243
2–9 mm antral follicle/ovary	0.047–0.012	0.243
HOMA-IR	−0.019–0.368	0.078
Women's ages	−0.082–0.091	0.921
E ₂ on day of HCG	0.000–0.000	0.462

Indeed, LH levels on day 3 before starting GnRH agonist medication were significantly increased in the high AMH group, and regression analysis indicated LH level was the only independent predictor of IR in our study, so the mostly elevated LH may be contribute to the decreased embryo developmental competence and consequently declined IR in the high AMH group. All patients in our study prepared for COH were administrated with GnRH agonists on the preceding cycle for pituitary desensitization, and stable suppression of LH levels during ovarian hyperstimulation was achieved. However, folliculogenesis encompasses the many stages involved in the transformation of a primordial follicle into a mature preovulatory graafian follicle, this phase spans a period of three ovarian cycles. Thus small antral follicles may be still exposed to high LH concentrations before pituitary desensitization. Therefore, we assume that the increased LH levels before GnRH agonists still have a detrimental effect on the follicle and embryo quality in PCOS patients, but more studies concerning this theory are needed.

Until now only one study has been published relating baseline serum AMH to the clinical outcome following IVF in women with PCOS [21]. Nevertheless, our findings were in contrast to those of kaya and colleagues [21], they reported FR, IR and CPR were statistically increased from low to high serum AMH levels on day 3 of the stimulations cycle in sixty PCOS patients. This discrepancy could be explained by differences in the studied populations. Since PCOS is a heterogeneous syndrome characterized by ovarian hyperandrogenism, polycystic ovaries, LH hypersecretion and insulin resistance. Consequently the variable phenotypic expression of reproductive and metabolic abnormalities in PCOS women leads to differences in oocyte developmental competence [30]. We observed the patients in the high AMH group with most elevated LH levels, which were proposed to be the cause of decreased IR. But Kaya did not depict the endocrine characteristics in their study population.

Conclusion

In conclusion, we observed a positive link between day-3 serum AMH levels during stimulation cycles and ovarian response to gonadotropin in PCOS patients. For the first time, however, we found the women with high AMH levels on day 3 had a significantly decreased IR, which may be due to significantly increased LH concentrations. However, PCOS is a heterogeneous syndrome, variable phenotype of PCOS would lead to different outcome in assisted reproduction. Therefore, further studies, specifically on PCOS patients with different phenotype, would be needed to elucidate the role of endocrine abnormality in assisted reproduction.

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