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Anti-Mullerian hormone as an examination for ovulatory dysfunction of female.

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Abstract

Objective: To investigate the relationship between anti-mullerian hormone (AMH) and ovulatory dysfunction.
Methods: The enzyme-linked immunosorbent assay (ELISA) was used to detect the serum level of AMH, the estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), the progestin (P), and the testosterone (T) in 106 participates with infertility (observation group)and carried on comparing with 102 women with pre-pregnancy physical examination (control group)Data were statistically analyzed.
Results: The serum hormone levels of AMH and LH in the observation group were higher than those in the control group (P<0.05). The FSH level of observation group was obviously lower than that in the control group (P<0.05). Pearson correlation analysis showed positive relationship between serum AMH and LH (P<0.05) as well as T (P<0.05). Serum AMH level in the observation group was negatively collated with FSH (P<0.05). No significant relationships were found between serum AMH and E2 level.
Conclusion: This study suggests that serum AMH level was associated with abnormal follicular development and would an effective measurement for ovulatory dysfunction of sterile female.

Keywords: Infertility, Anti-mullerian hormone(AMH), Ovulatory dysfunction, Anovulation.

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Introduction

As the higher incidence of infertility found in Chinese couples, there are more incidence of ovulatory dysfunction were found in women under reproductive age [1,2]. The diagnosis of clinical infertility should be examined by physicians in a range of medical specialties, including family medicine, paediatrics [3]. Thus one reliable, validated diagnostic examination is desired for the worldwide.

Anti-Mullerian Hormone (AMH, also named Müllerian inhibiting substance, MIS), a glycoprotein produced in the granulosa cells of the ovary [3], regulates early follicular recruitment, secreted by preantral follicle and small antral follicle, is known as the most precise predictor of ovarian reserve in women [4]. It is produced by the granulosa cells of small antral and preantral follicles and reflects the size of the pool of these follicles [5]. AMH declines with age but remains stable during the menstrual cycle allowing its determination at random [4,6].

The purpose of this study was to obtain AMH levels in infertility females to categorize them as ovulation after treatment of ovulation stimulants [7]. Through identification of treatment variables that increase the probability of ovulation, ovarian function in infertility females can be monitored more closely and if necessary prompt early referral, even in adolescence, to reproductive specialists. These patients might benefit from detailed discussions regarding reproductive potential and perhaps interventions for fertility preservation [8,9].

Materials and Methods

Subjects

All subjects were obtained between December 2013 and December 2015 from the department of obstetrics and gynecology, Renmin Hospital of Wuhan University (Wuhan, China). The study obtained all informed written consent from all subjects and the study was approved by Ethics Committee of the Renmin Hospital of Wuhan University.

Infertile women with ovulatory dysfunction were recruited as the observation group, aged 22-40 years, inclusive with two patent fallopian tubes, two functional ovaries and a body mass index <37.0 kg/m^2. The patient's usual cycle length had to be either <21 or >35 days with six or more menses per year or >35 days with fewer than six menses per year and a positive response to a progestin challenge within the last 6 months. The subjects received 2 years' follow-up. Women with normal ovulation who taking pre-pregnancy physical examination for pregnancy preparation as control group, aged 22-40 years, inclusive with two patent fallopian tubes, two functional ovaries and a body mass index <37.0 kg/m^2. The patient's

usual cycle length between 21 and 35 days with six or more menses per year and the biphasic basal body temperature within the last 6 months. The subjects received long-term follow-up until pregnancy.

Blood samples for serum AMH and hormonal levels were obtained at the third day of the menstrual cycle. Transvaginal ultrasound was also performed on each subject at the same time. Transvaginal ultrasound was performed as routine and recorded the data of antral follicle count (AFC), peak systolic velocity (PSV), plasticity index (PI), and resistant index (RI) of ovary.

AMH and Hormone Assays Serum samples were collected in a 5 mL serum separator tube and processed within 4 h to avoid blood cell lysis. Samples not processed the same day were stored at 4°C. Samples were centrifuged for 10 minutes at 1500 rpm at room temperaturethen transferred into 1 mL Eppendorf tube and stored at 80°C. AMH levels were determined using the enzymatically amplified two-site AMH-Gen-II ELISA (Beckman Coulter, Immunotech, Webster, Texas, USA) according to the manufacturer's specifications [10]. Serum samples were dispensed into the wells which were coated with anti-AMH antibody, followed by the addition of the anti-AMH detection antibody labelled with biotin, 100 µl of the streptavidin-horseradish peroxidase (HRP) was added after washing, followed by the addition of 100 µl of substrate solution containing TMB for 8 to 12 min. Using an automatic ELISA reader (Bio- Rad, Hercules, CA) the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 nm and between 600 and 630 nm. The absorbance measured was directly proportional to the AMH concentration in the samples which was calculated from the calibration curve. The results were expressed in ng/ml. Concentrations below 0.08 ng/ml were considered undetectable. Hormone levels were determined using ELISA [11], as done by routine.

Statistical analysis

Data are presented as mean ± SD, t-test was used to determine significant difference between two groups. Data were performed by one-way analysis-of-variance (ANOVA), followed by a post hoc Bonferroni's multiple comparison test when appropriate. Pearson's test was used to establish whether there was a linear relationship between two characteristics. P value of <0.05 was considered as significant difference. All data were analyzed by SPSS 22.

Results

A total of 208 women enrolled in the study, of which 202 women completed it. Six women form the control group quitted the study. A third sterile female were found to be diagnosed with polycystic ovary syndrome (PCOS) which affect the development of dominant follicle and hormone secretion especially FSH, LH and T [12,13]. According to the results of trans-vaginal ultrasound, all women diagnosed with PCOS had polycystic ovaries [14]. The AMH levels of women diagnosed with PCOS ranged from 5.3 to 12.6 ng/mL, while those of women who were not diagnostic with PCOS ranged from 4.6 to 8.2 ng/mL.

Table 1. *The general condition of subjects.*

	Observation group	Control group	P
Age	30.94 ± 6.27	29.24 ± 5.34	>0.05
AFC	5.5 ± 2.3	3.2 ± 1.3	<0.05
PSV (cm/s)	13.6 ± 11.41	13.2 ± 12.01	>0.05
PI	0.63 ± 0.31	0.64 ± 0.43	>0.05
RI	0.52 ± 0.03	0.51 ± 0.04	>0.05
AMH (ng/ml)	9.59 ± 4.41	4.45 ± 2.31	<0.05
Hormone levels			
E2 (pg/ml)	44.59 ± 17.41	33.45 ± 9.31	<0.05
P (ng/ml)	4.05 ± 3.28	3.45 ± 2.52	>0.05
FSH (mIU/ml)	8.45 ± 6.66	9.72 ± 3.45	<0.05
LH (mIU/ml)	7.32 ± 0.27	5.47 ± 2.63	<0.05
T (ng/ml)	0.84 ± 6.98	0.46 ± 5.38	<0.05

Compared the general condition of the observation group with control group in Table 1, there were significant difference on hormone levels except progestogen (p<0.05). It expressed lower AMH level, E2, LH and T, but less FSH level (p<0.05) [15]. There was significant difference on AFC between the sterile female and the controls (p<0.05). While there was no difference on PSV, PI or RI of ovary (p>0.05).

There are 64 women of the observation group were pregnancy within the follow-up time (60.37%), 87 women of the control group were pregnancy in the 2 years (90.62%), none spontaneous abortion occured. We further analyzed the correlation between possibilities of pregnancy in sterile female and the serum hormone level (Table 2). The women with success pregnancy were significantly younger than the women with non-pregnancy (p<0.05). It expressed lower AMH level in the women with non-success pregnancy (p<0.05). There was less AFC in the women with success pregnancy (p<0.05).

Table 2. *The correlation between probabilities of pregnancy and the AMH level in the sterile female.*

	Pregnancy women	Non-pregnancy women	P
Age	27.94 ± 4.57	30.12 ± 3.54	<0.05
AFC	3.1 ± 1.3	5.3 ± 2.3	<0.05
PSV(cm/s)	12.6 ± 10.91	13.4 ± 12.51	>0.05
PI	0.59 ± 0.34	0.62 ± 0.34	>0.05
RI	0.53 ± 0.02	0.50 ± 0.03	>0.05
AMH (ng/ml)	4.49 ± 3.31	3.35 ± 6.31	<0.05
Hormone levels			

E2 (pg/ml)	42.29 ± 10.41	39.45 ± 9.91	>0.05
P (ng/ml)	4.05 ± 3.28	3.45 ± 2.52	>0.05
FSH(mIU/ml)	8.45 ± 4.06	8.42 ± 3.45	>0.05
LH(mIU/ml)	5.38 ± 0.27	5.42 ± 0.33	>0.05
T(ng/ml)	0.54 ± 5.38	0.52 ± 4.98	>0.05

Discussion

In this report we describe the effectiveness of an AMH measurement as a diagnostic for ovarian reserve in sterile female. In this study it showed that there was a significant correlation between circulating AMH concentration and AFC. Many reports have mentioned that AMH is good correlated with ovarian follicle count [16,17]. Precious reports have mentioned that serum AMH would be an adjunct examination in the diagnosis of PCOS [18-20], we took a hypothesis that AMH would evaluated the function of ovarian and even regular ovulatory through detecting AMH [21,22]. However, little is known about the performance of AMH in predicting the number of retrieved oocytes and the follicular development even the success of live birth [23,24].

The probability of pregnancy is the most important issue for infertile couples and still remains a challenge for clinicians [25]. One rapid and effective measurement of ovulatory dysfunction for women would be important for deciding the therapeutic regimen. In the study, it showed that AMH, LH and T could be a substantial cross-predictor of premature ovulatory dysfunction and specific ranges of AMH would be an independent predictor of successful pregnancy [16]. The findings show patients with an AMH concentration below 4 ng/mL in the blood plasma are unlikely to achieve with successful pregnancy, whereas the blood plasma AMH concentration above 7 ng/mL is a substantial independent predictor of ovulation failure with PCOS as many studies have mentioned [26,27].

As more women are willing to delay the giving birth time, an increasing number of women seek the possibility of pregnancy when they appeared with ovulation failure [28]. Woman age is a substantial independent predictor which would be the first factor taken into consideration by clinicians as the increasing risk of ovulation failure with age [29,30]. As previous reports have mentioned that AMH concentration were associated with age [31,32], in this study we limited the age range of all subjects and taking a general examination of the function of ovary to avoid confounding factors. While the results have showed that the women with success pregnancy were significantly younger than the women with non-pregnancy. Age may be an insurmountable factor for infertile couples [33,34].

In conclusion, in this study the results suggest that serum AMH testing could represent a useful and practical test to screen the general risk of ovulation failure for the masses and a potential independent predictor of dominant follicle ovulation and successful pregnancy. We will launch larger multicenter studies for better validate these preliminary results.

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