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Efficacy of Anti-Müllerian Hormone in Predicting the Levels of Sex Hormones and Pregnancy Outcomes in Patients with Polycystic Ovary Syndrome Undergoing IVF-ET

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JD designed the research study. YC performed the research. JD and YC provided help and advice on the experiments. JD and YC analyzed the data. JD and YC wrote the manuscript. All authors contributed to editorial changes in the manuscript.

Key words
Anti-Mullerian hormone, Polycystic ovary syndrome, *In vitro* fertilization-embryo transfer, Sex hormone levels, Pregnancy outcome

ABSTRACT

Our study aimed to explore the value of Anti-Mullerian hormone (AMH) level in sex hormone level and pregnancy outcome in patients with the polycystic ovarian syndrome (PCOS) who underwent Invitro fertilization-embryo transfer (IVF-ET). This study selected 200 patients with PCOS who took IVF-ET in our hospital from January 2016 to January 2020 as subjects. The study subjects were divided into group A (low-level group, n=90) and group B (high-level group, n=110) according to serum AMH levels; and group C (successful group, n= 88) and group D (failure group, n=112) according to whether they completed clinical pregnancy. These groups were used to compare the differences in sex hormone, pregnancy outcomes, and ROC curve analysis of serum AMH levels in the predictive value of IVF-ET clinical pregnancy in PCOS patients. Subjects in Group A showed significantly lower FSH, LH, and T levels (P<0.05) than Group B. Compared to patients in Group B, patients in Group A also showed significantly higher rate in implantation rate, high-quality embryo rate, biochemical pregnancy rate as well as clinical pregnancy rate (P<0.05), while the incidence of early miscarriage, pregnancy-induced hypertension, and gestational diabetes of patients in Group A is significantly lower (P<0.05). AMH level showed high specificity (77.52%) and sensitivity (81.46%) in predicting IVF-ET clinical pregnancy in PCOS patients. PCOS patients with lower AMH levels showed better sex hormone levels and clinical pregnancy outcomes in IVF-ET. Serum AMH level is valuable (of good value) in predicting IVF-ET clinical pregnancy in PCOS patients. In clinical practice, interventions can be taken in advance for patients with higher serum AMH levels to improve pregnancy outcomes.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a relatively common endocrine and metabolic disease in women of gestational age, which has symptoms such as menstrual abnormalities, hirsutism, infertility, and acne (Fu *et al*., 2020). The clinical incidence of PCOS is relatively high. According to statistics, its incidence in women of gestational age is about 5% to 10% (Katsigianni *et al*., 2019). At present, the pathogenesis of PCOS is not completely clear in clinical practice, and there is no radical cure plan. Its clinical treatment is mainly symptomatic, such as improving symptoms, completing fertility, promoting health, and improving quality of life (Tso *et al*., 2020). The infertility symptoms of PCOS patients are mainly due to their hyperandrogenemia, hyperinsulinemia and other diseases. The treatment is mainly oral anti-androgen active contraceptives or insulin sensitizers (Chehin *et al*., 2020). However, some patients have drug ineffectiveness and long-term infertility, and in vitro fertilization-embryo transfer (IVF-ET) is needed for treatment (Sterling *et al*., 2016). IVF-ET mainly achieves conception through controlled superovulation, in vitro fertilization and transplantation after egg retrieval, but some patients have poor pregnancy outcomes (Chen *et al*., 2017). At present, there is still a lack of clinically useful items to evaluate the pregnancy status and pregnancy outcome of patients with PCOS after IVF-ET. Anti-mullerian hormone (AMH) belongs to the TGF-β superfamily, and its expression level can reflect ovarian reserve and female reproductive ability

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Abbreviations
PCOS, Polycystic ovary syndrome; IVF-ET, in vitro fertilization-embryo transfer; AMH, Anti-müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; P, progesterone; T, testosterone; PRL, prolactin.

to a certain extent, which is, the higher the expression of AMH, the more egg stock (Aghadavod *et al*., 2015; Moini *et al*., 2019). Studies have shown that the expression of AMH in PCOS is about five times that of healthy ovulating women (Chiofalo *et al*., 2017). Another study found that AMH can also evaluate the patient's response to controlled ovarian stimulation during assisted reproduction therapy and predict the number of oocytes (Köninger *et al*., 2018). This study investigated 200 PCOS patients undergoing IVF-ET in our hospital, aiming to evaluate the serum AMH level in PCOS patients with IVF-ET sex hormone levels and the evaluation value of pregnancy outcome.

MATERIALS AND METHODS

Patient clinical data

Two hundred PCOS patients who underwent IVF-ET in our hospital from January 2016 to January 2020 were selected as the research objects. The patients were 25 to 36 years old and the average age was (29.47±2.15) years. According to their AMH level, subjects were divided into two groups: group A (n=100) and group B (n=100). The Medical Ethics Committee has approved this study, and all patients have signed an informed consent form.

Inclusion and exclusion criteria

Inclusion criteria: Patients were included based on: (1) All patients were clinically diagnosed with polycystic ovary syndrome; (2) All patients were undergoing in vitro fertilization-embryo transfer (IVF-ET) in our hospital; (3) The patients had complete clinical data and cooperated with treatment and follow-up; (4) The serum AMH of group A patients was less than 6.99 ng/ml, and the serum AMH of group B patients was greater or equal to 6.99 ng/ml; (5) Within 4 to 6 weeks after transplantation, patients in group C B-ultrasound showed intrauterine gestational sacs or pathologically confirmed villi after surgery, conversely for patients in group D.

Exclusion criteria: Patients were excluded from this study based on: (1) patients with congenital immunodeficiency, severe infectious diseases; (2) patients with mental diseases; patients with poor mental status; (3) patients with other malignant tumors or severe cardiovascular and cerebrovascular diseases; (4) Patients with severe abnormalities in heart, liver, kidney and hematopoietic functions.

AMH level detection

Three mL of fasting venous blood was drawn from two groups of patients during the menstrual cycle. The whole blood after coagulation was centrifuge at about 1000-2000g for 10 min at 4°C, separating serum which was used for the determination of AMH levels using ELISA. Blank wells, standard wells, and control wells were setup as internal controls. Briefly, we diluted the standard with 0.05M PH9 carbonate coating buffer to make final protein concentration of 5 μg/mL, added 0.1 mL of it to each reaction well, and incubated at 4°C overnight. On the next day, the solution was discarded, and washed 3 times with washing buffer for 3 minutes each time. Then, 0.1ml of diluted sample was added to the above-mentioned coated reaction well, and incubate at 37°C for 1 h followed by washing. Later, 0.1ml of freshly diluted enzyme-labeled antibody was added, and incubated at 37°C for 1 h. Then after washing, 0.1ml of temporarily prepared TMB substrate solution was added, and incubated at 37°C for 30 min. Subsequently, 0.05ml of 2M sulfuric acid was added to each well. Finally, the ELISA reader was adjusted to zero at 450nm for the blank control well, and the O•D value of each well was measured to calculate the concentration of AML. All ELISA test reagents were purchased from Shanghai Enzyme-Linked Biology.

Sex hormone detection method

Five mL of fasting venous blood was drawn from all patients during their menstrual cycle to determine the level of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2), progesterone (P), testosterone (T) and prolactin (PRL) using ORG 300 automatic immunoassay analyzer (Germany ORGENTEC company). The operation was carried out in strict accordance with the instrument instructions.

IVF-ET treatment method

Three weeks after the patient takes oral contraceptives, triptorelin (FerringGmbH, X20010072) pituitary downregulation, gonadotropin is used to stimulate ovulation, when there are 3 dominant follicles> 18mm, HCG injection on the same day (Ma'anshan Fengyuan Pharmaceutical, H34023361) 6k~1w U, 36 h later, puncture egg retrieval, 72 h after egg retrieval, embryo transfer.

Observation indicators

The following observation indicators were used:

(1) Sex hormone levels of the two groups of patients such as FSH, LH, E2, P, T and PRL; (2) Pregnancy outcomes of the two groups of patients such as fertilization rate, cleavage rate, implantation rate, and high-quality embryo rate, biochemical pregnancy rate and clinical pregnancy rate, high-quality embryos start from normal fertilization, and the number of embryos three days after fertilization is 6 to 10 embryos with a fragmentation degree of <15%; biochemical pregnancy is blood HCG>25U/L or urine

The HCG test was positive, but the ultrasound showed no gestational sac, and the pregnancy was terminated. (3) Early miscarriage rate and pregnancy complications occurred in the two groups: early miscarriage is the spontaneous abortion in the first 12 weeks of pregnancy. (4) The predictive value of AMH level in IVF-ET clinical pregnancy in patients with PCOS: ROC curve is used for analysis.

Statistical analysis

The SPSS 25.0 software package was used for statistical analysis, in which the measurement data was expressed as mean ± standard deviation, and two independent samples were used for nonparametric test for analysis between groups; count data was recorded in the form of rate (%), Chi-square test analysis was used for comparison between the two groups, and ROC curve was used to analyze the predictive value of AMH level in IVF-ET clinical pregnancy in PCOS patients. $P<0.05$ indicated that the difference was statistically significant. Use the Gradpad Prism 7.0 software package for mapping.

RESULTS

Demographic data

A total of 200 patients were enrolled in the study (Table I). They were divided into A and B groups according to their AMH levels, and into C and D groups according to their clinical pregnancy outcomes. A total of 200 patients were enrolled in the study. They were divided into A and B groups according to their AMH levels, and into C and D groups according to their clinical pregnancy outcomes. There were no statistically significant differences in general information such as age, BMI, age at menarche, and infertility time between group A and group B, group C and group D ($P<0.05$), and they were comparable. The patients were followed up for survival. There were 2 and 3 lost cases in groups A and B, respectively (Fig. 1).

Sex hormone levels

Compared with patients in group B, the levels of FSH, LH and T in group A were significantly lower and the difference was statistically significant ($P<0.05$), while the levels of E2, P and PRL in group A were not significantly different ($P> 0.05$). The ovarian function of patients in the low-level AMH group was significantly better than that in the high-level AMH group (Fig. 2, Table II).

Pregnancy outcome

Compared with patients in group B, the implantation rate, high-quality embryo rate, biochemical pregnancy rate, and clinical pregnancy rate in group A were significantly higher and the difference was statistically significant ($P<0.05$). However, there were no significant differences in the fertilization rate and cleavage rate of patients in group A ($P>0.05$). The pregnancy outcomes of patients in the low-level AMH group were significantly better than those in the high-level AMH group (Fig. 3, Table III).

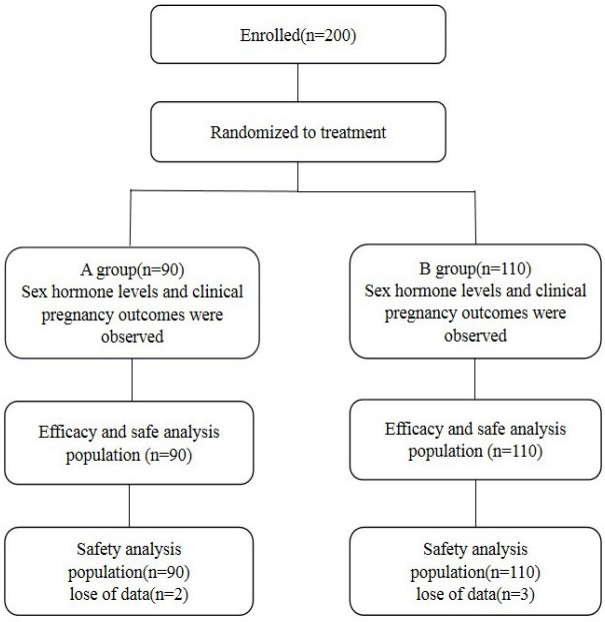

Fig. 1. A combined diagram of the process.

Table I. Comparison of general information of patients in the two groups.

Groups	Group A (n=90)	Group B (n=110)	Group C (n=88)	Group D (n=112)
Age (year)	28.74±2.07	29.83±2.26[a]	28.85±2.03	29.74±2.23[b]
BMI (kg/m^2)	22.43±1.35	22.68±1.42[a]	22.38±1.33	22.71±1.39[b]
Menarche time (year)	14.16±1.58	14.62±1.89[a]	14.28±1.73	14.59±1.85[b]
Infertility time (year)	6.72±2.31	7.15±2.36[a,]	6.58±2.17	7.29±2.38[b]

Note: compared with group A, [a]$P<0.05$; compared with group C, [b]$P<0.05$.

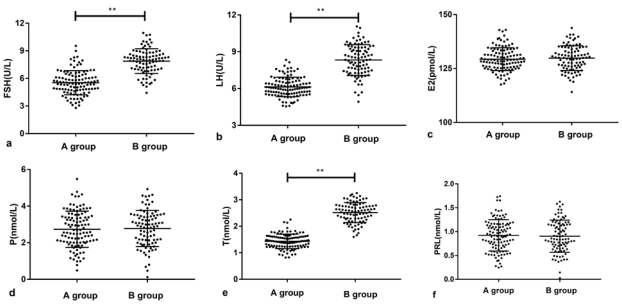

Fig. 2. Comparison of sex hormone levels of patients in two groups. a is follicle-stimulating hormone (FSH); b is luteinizing hormone (LH); c is estradiol (E2); d is progesterone (P); e is testosterone (T); f is prolactin (PRL); ** means $P<0.001$.

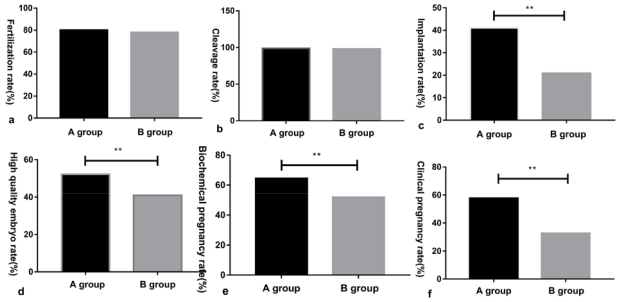

Fig. 3. Comparison of pregnancy outcomes of patients between the two groups. a is Fertilization rate; b is Cleavage rate; c is Implantation rate; d is High quality embryo rate; e is Biochemical pregnancy rate; f is Clinical pregnancy rate; ** means $P<0.001$.

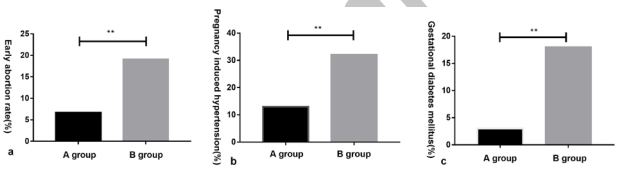

Fig. 4. Comparison of Early abortion rate and pregnancy complications of patients in the two groups. a is Early abortion rate; b is Pregnancy-induced hypertension; c Gestational diabetes mellitus; **means $P<0.001$.

Comparison of early miscarriage rate and pregnancy complications

Compared with patients in group B, the incidence of early miscarriage, gestational hypertension and gestational diabetes in group A were significantly lower and the difference was statistically significant (P<0.05). The rate of early miscarriage and pregnancy complications in the low-level AMH group was significantly lower than that in the high-level AMH group (Fig. 4, Table IV).

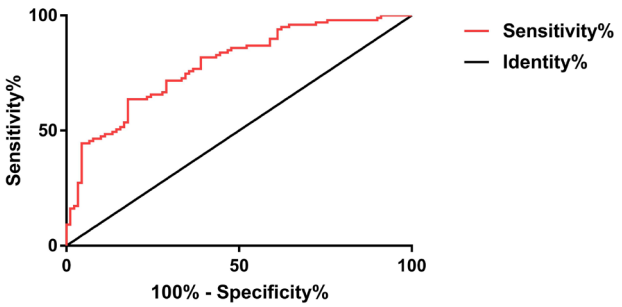

Fig. 5. ROC curve of AMH levels predicting IVF-ET in clinical pregnancy in PCOS patients.
ROC curve analysis shows that the area below the curve is 0.783, 95% of CI is 0.586 to 0.826, the specificity is 77.52%, the sensitivity is 81.46%, the Youden index is 53.26, and the optimal cut-point is 7.149.

Table II. Comparison of sex hormone levels between the two groups($\bar{x}\pm s$).

Groups	Group A(n=90)	Group B(n=110)	T value	P value
FSH(U/L)	5.53±1.26	7.93±1.35	6.425	0.001
LH(U/L)	6.14±0.83	8.21±1.27	6.372	0.001
E2(pmol/L)	128.73±5.42	130.26±5.49	0.586	0.524
P(nmol/L)	2.65±1.04	2.81±1.18	0.539	0.516
T(nmol/L)	1.47±0.28	2.53±0.36	6.197	0.001
PRL(nmol/L)	0.92±0.35	0.86±0.31	0.632	0.423

For abbreviations, see Figure 2.

The predictive value of AMH level in IVF-ET clinical pregnancy in PCOS patients

In this study, the serum AMH level of patients in group C was (6.52±1.04) ng/ml, and the serum AMH level of patients in group D was (7.49±1.12) ng/ml. The ROC curve was used to analyze the predictive value of serum AMH level in IVF-ET clinical pregnancy in PCOS patients, and the results showed that it has high specificity (77.52%) and sensitivity (81.46%), as shown in Figure 5.

DISCUSSION

As a complex disease caused by metabolic and endocrine disorders, PCOS mainly affects women of childbearing age (Al-Gareeb *et al*., 2016). PCOS patients often have hyperandrogenemia, hyperinsulinemia, ovulation dysfunction, or even anovulation, so patients are often accompanied by infertility symptoms (Kim *et al*., 2016). IVF-ET therapy is often used clinically for infertile patients with PCOS who are ineffective in drug therapy. However, there are specific differences in the pregnancy outcomes of PCOS infertile

Table III. Comparison of pregnancy outcomes of patients between the two groups (n, %).

Groups	Group A (n=90)	Group B (n=110)	X^2 value	P value
Fertilization rate(%)	80.00(73/90)	78.18(86/110)	0.574	0.526
Cleavage rate(%)	98.89(89/90)	98.18(108/110)	0.639	0.438
Implantation rate(%)	41.11(37/90)	20.91(23/110)	6.541	0.001
High quality embryo rate(%)	52.22(47/90)	40.91(45/110)	6.325	0.001
Biochemical pregnancy rate(%)	64.44(58/90)	51.82(57/110)	6.293	0.001
Clinical pregnancy rate(%)	57.78(52/90)	32.73(36/110)	6.741	0.001

Table IV. Comparison of early abortion rates and pregnancy complications (n, %).

Groups	Group A (n=90)	Group B (n=110)	X^2 value	P value
Early abortion rate(%)	6.67(6/90)	19.09(21/110)	6.315	0.001
Pregnancy induced hypertension(%)	13.33(12/90)	31.82(35/110)	6.762	0.001
Gestational diabetes mellitus(%)	3.33(3/90)	17.27(19/110)	6.439	0.001

patients with different conditions after IVF-ET treatment (Buyuk *et al*., 2011). Both AMH and sex hormone levels can effectively assess ovarian reserve in women of gestational age, and its expression changes are of great significance in some infertility diseases (Simões-Pereira *et al*., 2018).

AMH is one of the important members of the TGF-β superfamily, and it mainly plays a physiological role through AMH receptor II (Kocaay *et al*., 2018). Serum AMH is one of the important indicators for diagnosing PCOS patients. Studies have found that the expression of serum AMH in PCOS patients is about 2 to 3 times that of normal ovulating women, and the higher its expression, the higher the diagnosis rate of PCOS (Amer *et al*., 2013). In addition, the serum AMH level can also be used in the assessment of ovarian reserve. A poor ovarian reserve may lead to menopause and infertility in women of gestational age (Mahajan and Kaur, 2019). In this study, compared with patients in group B, patients in group A had significantly lower levels of FSH, LH and T. FSH, LH, and T are the main items of sex hormone examination. The pathological increase of their levels can indicate to a certain extent that women's ovarian function is poor, ovarian regression, etc., and can have a negative impact on normal pregnancy in women of gestational age. Scholars such as Ishii R found in research that high expression levels of T can promote the secretion of AMH and cause incomplete follicular development (Ishii *et al*., 2019). According to the research of (Cai *et al*., 2019), the high expression of AMH, FSH, and LH is one of the important mechanisms leading to hyperandrogenemia in the body, and can lead to further damage to the body's follicular function. Therefore, in this study, the FSH, LH, and T levels of patients in the high-level serum AMH group were also significantly higher, which can indicate to a certain extent that patients in this group have poor frontal ovarian function. In this study, there was no significant difference in E2, P, and PRL levels between groups A and B, which may be related to the limited number of samples in this study.

In terms of the pregnancy outcome of the two groups of patients, compared with the B group, the implantation rate, high-quality embryo rate, biochemical pregnancy rate, and clinical pregnancy rate of group A were significantly better. Research by Bedenk J and other scholars showed that the pathological increase of serum AMH level can not only affect the ovarian function of pregnant women, but also damage the normal ovarian environment, resulting in poor oocyte quality and ultimately the quality of embryos is reduced, affecting normal pregnancy (Bedenk *et al*., 2020). According to the research of (Pigny *et al*., 2003) the pathological expression of serum AMH and FSH, LH, and T may also affect the expression of cytokines related to endometrial morphology and endometrial receptivity, resulting in endometrial volume. Receptivity is reduced, affecting normal pregnancy. Therefore, in this study, the pregnancy and embryo quality of group A patients are closely related to their lower FSH, LH, T and serum AMH expression, better ovarian function and internal environment, and more complete endometrial morphology and function (Pigny *et al*., 2003).

Studies have shown that high androgen status in PCOS patients can affect the insulin metabolism of related cytokines in the uterus, and the high androgen status also has a certain impact on the normal development of the placenta, causing the hormones secreted by the placenta to enhance insulin resistance. Therefore, PCOS patients

are prone to have diabetes during pregnancy (Cree-Green *et al*., 2018; Li *et al*., 2018). According to another study, PCOS patients with high androgen status will lead to a large increase in the secretion of vascular smooth muscle cells, resulting in narrowing of the vascular lumen, increased blood flow resistance, poor vascular endothelial function, and eventually the development of pregnancy-induced hypertension (Pergialiotis *et al*., 2018). In this study, compared with patients in group B, the incidence of gestational diabetes and hypertension in pregnancy in group A was significantly lower, which may be due to the low serum AMH level improving the high androgen status of PCOS patients. Regarding the early abortion rate of the two groups of patients, the patients in group A were also significantly better, which was more wholly related to their endometrial morphology and function. Therefore, this study found that low serum AMH levels can improve the high androgen status and intrauterine environment of PCOS patients, and reduce the rate of early pregnancy miscarriage and pregnancy complications during IVF-ET. In this study, the ROC curve was used to analyze the predictive value of serum AMH levels in IVF-ET clinical pregnancy in PCOS patients. The results showed that it has high specificity and sensitivity, which is consistent with the research results Melado VL and other scholars (Melado Vidales *et al*., 2017). Clinical interventions can be taken in advance for PCOS patients with high serum AMH levels when undergoing IVF-ET to improve their pregnancy outcomes.

CONCLUSION

In summary, PCOS patients with lower serum AMH levels have better sex hormone levels and clinical pregnancy outcomes during IVF-ET. Serum AMH levels have a higher value in predicting IVF-ET clinical pregnancy in PCOS patients. Patients with high levels of AMH should take intervention measures in advance to improve pregnancy outcomes. There are still some shortcomings in this study. For example, the study only investigated the clinical pregnancy rate and early miscarriage rate of patients, and did not discuss whether the patient's late pregnancy process was smooth or the newborn's health. The trial period should be extended in future studies. To comprehensively explore the relationship between AMH level and the whole process of IVF-ET pregnancy in PCOS patients.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Statement of conflict of interest

The authors have declared no conflict of interest.

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