Brief Report

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Higher serum AMH level is associated with better pregnancy outcomes of IVF/ICSI assisted pregnancy in infertile patients under 35 years old

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SUMMARY This study aimed to investigate the effect of anti-Mullerian hormone (AMH) on the pregnancy outcome of infertility assisted by IVF/Micro-Insemination/Embryo Transfer Infertility Assistance (IVF/ICSI-ET). A total of 324 patients under the age of 35 who received IVF/ICSI-ET assistance in our center were included in this analysis. AMH levels of these patients were measured by chemiluminescence method and divided into clinical pregnancy group (175 cases) and non-pregnancy group (149 cases) according to the final pregnancy outcome. The relationship between the two groups' pregnancy outcomes and AMH levels was analyzed. The above association was re-evaluated after excluding patients with polycystic ovary syndrome. There was no significant difference in age, body mass index (BMI), follicle-stimulating hormone (FSH), and 2 pronucleus (PN) between clinical and non-clinical pregnancy groups. Compared with the clinical pregnancy group, the level of AMH in the non-pregnancy group was significantly lower ($p < 0.05$). A higher AMH level was closely related to better IVF/ICSI-ET assisted pregnancy outcome *in vitro*. After excluding AMH abnormalities, the AMH level was still significantly associated with pregnancy outcomes of *in vitro* IVF/ICSI-ET-assisted pregnancy. Our results show a correlation between AMH level and pregnancy outcome of *in vitro* IVF/ICSI-ET assisted pregnancy. For women under age 35, lower AMH levels may be one of the predictors of adverse pregnancy outcomes. For patients with low AMH level, it is suggested to strengthen monitoring to ensure the safety and smoothness of the pregnancy process.

Keywords infertility, *in vitro* fertilization-embryo transfer, pregnancy outcome, AMH

1. Introduction

"Infertility" refers to a couple's failed pregnancy after 12 months of unprotected sexual intercourse and pregnancy attempts. Infertility is increasing yearly, affecting about 15-20% of couples worldwide (*1*). About 85% of cases have a clear cause, such as ovulatory dysfunction, male infertility, or fallopian tube lesions. However, there are unexplained infertility cases where the cause is unknown.

It is important to note that lifestyle and environmental factors like smoking and obesity can negatively affect fertility (*2*). Aggravation of environmental pollution has particularly contributed to the occurrence of infertility. Exposure to perfluoroalkyl substances (PFAS) may be associated with decreased fertility. Higher PFAS exposure may lead to a decline in female fertility (*3*). Exposure to ambient air pollutants (such as sulfur dioxide) may potentially adversely affect male semen quality (*4*). In addition, ovarian reserve was negatively correlated with women's exposure to air pollutants (*5*).

For infertile patients, assisted reproductive technology (ART) has become their hope of becoming parents (*6*). ART, or *in vitro* fertilization and embryo transfer (IVF-ET), is an advanced reproductive technique that helps infertile individuals achieve pregnancy and treat fertility issues by facilitating the birth of healthy babies. The ART program includes processing eggs or embryos *in vitro* to promote fertilization and embryonic

development. The current ART process covers IVF and possible intracytoplasmic sperm injection (IVF/ICSI) (*7*).

Unfortunately, even with the help of IVF-ET technology, not all infertile people can achieve clinical pregnancy results for various reasons (*8*). Existing studies have shown that infertility is associated with adverse pregnancy outcomes, with or without fertility treatment (*9*). Certain lifestyle factors have also been found to impress the pregnancy outcomes of IVF/ICSI treatment. For example, women drinking > 84 grams/week were related to a lower pregnancy rate, while men drinking > 84 grams/week were linked to a lower live birth rate after IVF/ICSI treatment (*10*). The study also found that after age adjustment, the live birth rate decreased significantly as the duration of infertility increased from 1 year to 12 years. Fertility (that is, the possibility of becoming pregnant during a menstrual cycle) begins to decline significantly in the early thirties and even faster a few years later (around the age of 37) (*11,12*). However, our understanding of the factors affecting the success rate of *in vitro* pregnancy is still limited, which limits the optimization of IVF/ICSI treatment to improve the outcome of assisted pregnancy in infertile patients.

Anti-Mullerian hormone (AMH), a hormone produced by follicular granulosa cells, reflects the continuity of ovarian functional reserve, which can predict the response of ovary to gonadotropin stimulation and be used in individualized treatment strategy to improve the efficacy and safety of treatment (*13*). AMH is supposed to indicate the likelihood of success in controlled ovarian stimulation (COS) (*14*). AMH levels are utilized to evaluate ovarian reserves and aid in ovulation induction programs for women undergoing IVF cycles. However, the impact of AMH levels on pregnancy outcomes in IVF/ICSI is not fully understood. To address this, we conducted a retrospective analysis of the correlation between pre-pregnancy AMH levels and pregnancy outcomes in women less than 35 years of age who received IVF/ICSI-assisted pregnancy.

2. Materials and Methods

2.1. Study population

Three hundred seventy assisted pregnancy patients who met the indications of IVF-ET from January 2021 to December 2022 were screened, of which 324 were less than 35 years old. Patients were divided into two groups according to pregnancy outcome: clinical pregnancy group (confirmed to be pregnant by ultrasound); non-clinical pregnancy group (serum or urine examination indicated increased progesterone or human chorionic gonadotropin, but pregnancy was not confirmed by ultrasound; or patients who were not pregnant by biochemistry and ultrasound). The study was approved by the Institutional Review Board of Zhoushan Maternal and Child Health Hospital and followed the ethical guidelines of the 2000 Declaration of Helsinki. All the participants signed the informed consent document. Figure 1 is a flowchart of the study.

2.2. Data collection

AMH was detected on a model iFlash 3000 (Yahuilong Company, Shenzhen, Guangdong, China) using the chemiluminescence method, following the manufacturer's instructions. Follicle-stimulating hormone (FSH) and 2 pronucleus (PN) were extracted from medical records. Body mass index (BMI) was calculated as weight (kg)/ height $(m)^2$.

2.3. Determination of pregnancy

Clinical pregnancy was determined according to the results of ultrasound, which was performed using the GE Voluson E11 system (General Electric Medical Systems, Milwaukee, WI, USA). If the ultrasonic examination did not detect a pregnancy, and combined with human chorionic gonadotropin (hCG) < 7 U/mL, then it was judged as not a clinical pregnancy. hCG was determined using the hCG diagnostic reagent kits (Roche Diagnostic Reagent Co., Ltd. Marburg, Germany), according to the manufacturer's instructions. The detection method of hCG is electrochemiluminescence, using the Cobas e601 type electrochemiluminescence instrument (Roche Diagnostics, Indianapolis, IN, USA).

2.4. Statistical analyses

To compare the difference between the two groups, mean ± standard deviation (SD) was presented for normally distributed continuous variables, and *t*-test was used. Data were expressed as numbers and percentages for categorical variables, and the chi-square test was used. SPSS version 25.0 was utilized for the statistical analysis, with a significance threshold set at two-sided $p < 0.05$.

3. Results and Discussion

The BMI, FSH, and 2PN indexes remained indistinguishable between the clinical and non-clinical pregnancy group (Table 1). As shown in Figure 2, the average AMH level of the clinical pregnancy group (4.795 ± 3.12 ng/mL) notably increased compared to the non-pregnancy group (3.802 ± 2.13 ng/mL) ($p = 0.0025$).

As one of leading causes for female infertility worldwide, about 10% of patients with polycystic ovary syndrome (PCOS) seek ART (*15*).PCOS is an endocrine disease with metabolic abnormalities, and one of the characteristics of the disease is elevated levels of AMH (*16*). To rule out whether the increase of AMH in the two groups of our results is caused by subsequent diagnosis of PCOS patients, we performed subgroup analyses in non-PCOS patients.

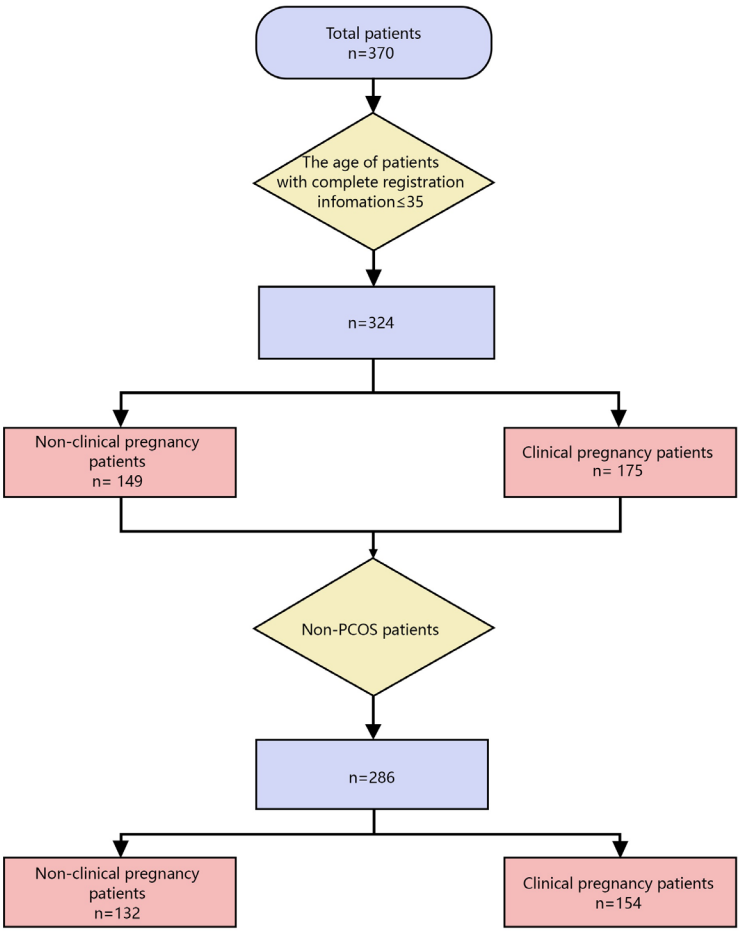

Figure 1. Study flowchart.

Table 1. Characteristics of non-clinical pregnancy *vs.* clinical pregnancy group

	Non-clinical pregnancy (n = 149)	Clinical pregnancy (n = 175)	p
Age	30.08 ± 2.80	30.70 ± 2.80	0.99
BMI	21.99 ± 3.30	22.32 ± 3.11	0.35
FSH	6.55 ± 2.06	6.41 ± 1.85	0.53
2PN	6.02 ± 3.24	5.36 ± 3.02	0.06
Years of infertility	3.31 ± 2.16	3.39 ± 2.48	0.92

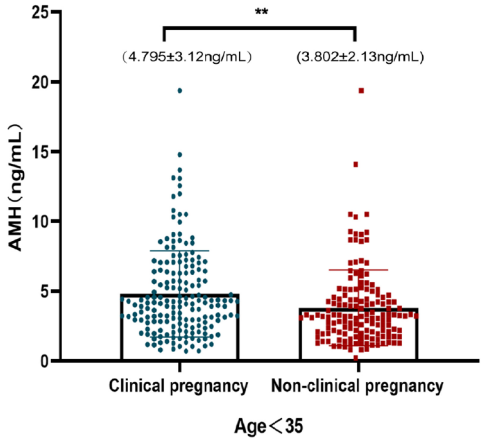

Figure 2. The level of AMH in patients under 35 years old, clinical pregnancy group (4.795 ± 3.12 ng/mL) *vs.* non-pregnancy group (3.802 ± 2.13 ng/mL). Each point represents a patient. The unit of AMH is ng/mL (**, p = 0.0025).

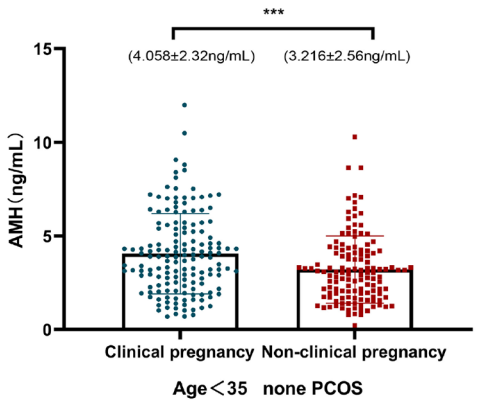

Figure 3. The level of AMH in patients under 35 years old without PCOS, clinical pregnancy group (4.058 ± 2.32 ng/mL) *vs.* non-pregnancy group (3.216 ± 2.56 ng/mL). Each point represents a patient. The unit of AMH is ng/mL (***, p = 0.0004).

In our study, 37 (11.4%) patients were later diagnosed with PCOS. After excluding these patients, the remaining 286 non-PCOS assisted pregnancies were used for subgroup analysis. As shown in Figure 3, the average AMH level of the clinically pregnant group (4.058 ± 2.32 ng/mL) was markedly higher than that of the non-pregnant group (3.216 ± 2.56 ng/mL) ($p < 0.05$).

The research on the pregnancy outcome of women using IVF-ET is still very limited. Therefore, it is of significant value to identify variables associated with the

pregnancy outcome and closely monitor them to improve the safety and smoothness of the pregnancy process. The current study focused on patients under 35 years of age receiving IVF/ICSI. The results showed obvious differences in AMH between the clinical pregnancy group with successful *in vitro* assisted pregnancy and those who failed to reach the clinical pregnancy stage. Specifically, AMH levels were positively correlated with successful clinical pregnancy assisted by IVF/ICSI. After excluding subsequent diagnosed PCOS patients, the high level of AMH was still positively related to the successful clinical pregnancy outcom*es* in *in vitro* assisted pregnancy, indicating that AMH may be a predictive index.

Recently, the research of AMH has made important progress in reproductive medicine. A retrospective cohort study found that serum AMH levels influenced pregnancy outcomes treated with gonadotropin-releasing hormone antagonists, indicating the positive role of AMH levels in predicting ovarian response and pregnancy outcomes (*17*). Another study revealed that AMH and antral follicle count (AFC) could forecast the final obtained egg number and the effect of embryo freezing during IVF cycles; high AMH and AFC were positively correlated with the number of collected oocytes (*18*). Furthermore, a cohort study found that young women with high AMH levels were more likely to experience an early miscarriage during their first IVF/ICSI procedure compared with women with intermediate AMH levels, suggesting that high AMH levels may be a risk factor of early miscarriage (*19*). A study discovered that young women with elevated AMH levels were more prone to early miscarriage during their initial IVF/ICSI treatment than those with moderate AMH levels. In young women, serum AMH levels were independently linked to the risk of early abortion after IVF-ET treatment (*20*). The results based on follicular fluid have shown that the AMH of follicular fluid matched by oocytes is a valuable index for predicting live birth after fresh single embryo transfer (*21*). However, whether there is any relationship between serum AMH and IVF/ICSI pregnancy outcome in infertile patients has not been fully evaluated.

In women, the production of AMH begins in the later stage of the fetus and continues into adulthood, then decreases steadily throughout the reproductive years until a severe decline during menopause, and finally to a level that cannot be detected (*22*). AMH is crucial in regulating various stages of follicular development and functions as a neuroendocrine hormone. Additionally, serum AMH levels are widely accepted as a dependable indicator of ovarian reserve, predicting the occurrence of PCOS and primary ovarian failure (*23*). Our study results suggest that the IVF/ICSI treatment regimen adjusted according to the level of AMH may improve the success rate of pregnancy and reduce the time and cost of treatment. For example, in women with lower levels of AMH, higher doses of ovulation-inducing drugs can stimulate follicular development to obtain more eggs and improve the pregnancy success rate. The relationship between the level of AMH as an indicator of ovarian reserve function and the clinical pregnancy rate of *in vitro* pregnancy is not fully understood. Further understanding of the mechanism of AMH in *in vitro* pregnancy outcomes can help adjust targeted assisted pregnancy program based on AMH levels to improve the success rate of out-of-vitro pregnancy in infertile patients.

Our research provides preliminary information for understanding AMH and *in vitro* pregnancy outcomes. However, considering our results are based on retrospective analysis from a single center, there may be biases and confounding factors influencing the study results. Due to the nature of the study design, we can only evaluate the association but not causal relationship. Studies are needed to determine the underlying mechanism to improve the clinical intervention of patients with low AMH and improve the *in vitro* pregnancy outcomes. In addition, future research could explore whether it can effectively improve the pregnancy success rate of low-AMH patients under the age of 35 by optimizing interventions for this population, such as supplementation with AMH (*24*).

To conclude, our results show that AMH levels were positively associated with clinical pregnancy outcomes of *in vitro* assisted pregnancy. To enhance the clinical pregnancy success rate of patients with low AMH assisted pregnancy and ensure the safety and smoothness of the pregnancy process, we need to carry out closer monitoring and adjust the intervention measures for low-AMH assisted pregnancy.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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