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Review Article

Effects of anti-mullerian hormone and basal antral follicle count on IVF/ICSI-ET outcomes in older adults

Hairu Sun[1], Lijuan Ma[2], Yanmei Miao[1], Yangyang Wu[3], Yan Jiao[1], Hui Su[1]

[1]*Department of Reproductive Medicine, Hengshui People's Hospital, Hengshui City, Hebei Province, China;* [2]*Department of Production Inheritance, Hengshui Second People's Hospital, Hengshui City, Hebei Province, China;* [3]*Department of Gynaecology, Hengshui People's Hospital, Hengshui City, Hebei Province, China*

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Abstract: To explore the correlation of anti-Mullerian hormone (AMH) and basal antral follicle counts (bAFC) with *in-vitro* fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) outcomes in older adults. A total of 135 patients who underwent IVF/ICSI-ET assisted pregnancy in Hengshui People's Hospital from February 2014 to May 2016 were collected and were divided into the clinical pregnancy group (n=81) and the non-clinical pregnancy group (n=54) according to whether the patients had a clinical pregnancy. The AMH and bAFC were compared between the two groups. The diagnostic value of AMH, bAFC and pregnancy outcomes were detected by receiver operating characteristic (ROC) curve, and logistic regression analysis was used to analyze the clinical risk factors of IVF/ICSI-ET in patients with non-clinical pregnancy. Compared with the non-clinical pregnancy group, the AMH and bAFC in the clinical pregnancy group were notably increased. The ROC curve showed that the area under the curve (AUC) of AMH and bAFC was 0.888 and 0.730, respectively. Older age was an independent risk factor for the inability to get pregnant in patients with IVF/ICSI-ET, while higher AMH, bAFC, and number of high-quality embryos were protective factors. AMH and bAFC can well predict the outcome of IVF/ICSI-ET in patients. Older age is an independent risk factor for patients who cannot become pregnant, and higher AMH, bAFC, and number of high-quality embryos are protective factors.

Keywords: Anti-Mullerian hormone, basal antral follicle counts, IVF/ICSI-ET

Introduction

Currently, there is a high incidence of infertility in China, with an estimated prevalence of approximately 25%, which often threatens the family harmony of patients [1, 2]. Today, *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) procedures have been widely used to treat infertility, which is also a common and effective treatment used in the clinic [3]. However, not all patients with IVF/ICSI-ET have a better pregnancy outcome. Age is a factor that affects IVF/ICSI-ET pregnancy outcomes, generally patients over 35 years of age often have lower pregnancy outcomes than patients under 35 years of age [4]. Currently, there are some methods that can predict the pregnancy outcome of IVF/ICSI-ET, such as ultrasound images, and others are also being explored, clinically. Since the interpretation of results are often prone to subjective factors, as well as the peristalsis of surrounding organs may also affect the accuracy of observation [5, 6], we need more precise predictive methods.

Anti-mullerian hormone (AMH) belongs to the TGFβ family, which participates in the formation of follicles and is an effective indicator of ovarian function [7]. Serum AMH concentration can predict a woman's follicular density and ovarian reserve [8], so it has also become a fertility-related predictor. The study of Kostrzewa et al. [9] showed that serum AMH may be a predictor of early miscarriage in young women. At present, it is generally believed that a reduced AMH level may affect the therapeutic results of assisted reproductive technology [10]. Evidence has shown that AMH is significantly related to the age of patients, and it will gradually decrease with age [11]. However, it is not clear whether the AMH level of older patients can be used as a reference for their IVF/ICSI-ET pregnancy outcomes.

Basal antral follicle count (bAFC) refers to the number of antral follicles of 2-10 mm on the second day of menstruation, which reflects the patient's ovarian reserve. By detecting bAFC, the ovarian reserve can be monitored, and the number of eggs harvested and the success rate of *in vitro* fertilization can be predicted [12]. Many studies have also indicated that since AMH is involved in the recruitment of antral follicles, AMH can predict bAFC in patients [13].

This study observed and compared AMH and bAFC levels in IVF/ICSI-ET patients with and without clinical pregnancy to explore their predictive value in pregnancy outcomes.

Materials and methods

Patient collection

A total of 135 patients who underwent IVF/ICSI-ET in Hengshui People's Hospital from March 2016 to August 2017 were collected and divided into the clinical pregnancy group and non-clinical pregnancy group according to whether the outcome was pregnancy or no pregnancy. Among them, 81 patients were in the clinical pregnancy group with an average age of (38.4±2.4) years old, and 54 patients were in the non-clinical pregnancy group with an average age of (39.8±2.1) years old. The study was approved by the Medical Ethics Committee, and all patients were informed in advance and had signed an informed consent form.

Inclusion and exclusion criteria

Inclusion criteria: patients over 35 years old, who were infertile due to fallopian tube problems and willing to cooperate with treatment and follow-up, with a normal menstrual cycle, and complete clinical data.

Exclusion criteria: patients with ovarian insufficiency; patients with diabetes and hypertension; patients with severe endometriosis or adenomyosis; patients with chromosome abnormalities or uterine malformations; patients who withdrew from the study halfway.

Detection methods

Three days after menstruation, 4 mL venous blood was collected from patients, and placed in a coagulation tube and centrifuged (3000xg at 4°C for 10 min) to collect the serum. AMH was determined by the chemiluminescence quantitative method, and bAFC was recorded and collected by transvaginal ultrasonography with a vaginal ultrasound probe. High-quality embryo evaluation criteria: ≥4 blastomeres can be observed on the second day after normal oocyte fertilization, ≥6 blastomeres can be observed on the third day, and those with fragments <20% can be graded as high-quality embryos. Clinical Pregnancy: On the 28th day after embryo transfer, the presence of the gestational sac can be seen by ultrasound.

Outcome measures

Main outcome measures: AMH and bAFC levels in the clinical pregnancy group and non-clinical pregnancy group were observed, and the predictive value of the two in pregnancy outcomes was detected by receiver operating characteristic (ROC) curve. Secondary outcome measures: multivariate logistic regression was used to analyze the risk factors of non-clinical pregnancy in older adults with IVF/ICSI-ET.

Statistical methods

The collected data were statistically analyzed using SPSS 20.0 (SPSS Corp., Chicago, USA) and plotted by GraphPad Prism 7 (GraphPad Software, San Diego, USA). The counting data were described in percentage (%) and analyzed by a chi-square test (expressed by X^2). The measurement data, all normally distributed, were expressed as mean ± standard deviation (Mean ± SD), and the inter-group comparisons were performed by the independent t-test. The predictive value of AMH and bAFC in pregnancy outcomes was evaluated by ROC. $P<0.05$ indicated that the difference was statistically significant.

Results

Clinical data of patients

There was no significant difference in age, years of infertility, body mass index (BMI), Gn dosage, duration of Gn stimulation, type, residence and infertility factors between the two groups, indicating comparability between the two groups (**Table 1**).

AMH and bAFC in two groups

After comparison, it was found that AMH and bAFC levels in the clinical pregnancy group

Table 1. Clinical data of patients

	Clinical pregnancy group (n=81)	Non clinical pregnancy group (n=54)	t/X^2	P
Age (years old)	38.4±2.4	39.8±2.1	3.487	<0.001
Years of infertility (years)	8.5±2.0	9.1±1.8	1.776	0.078
BMI (kg/m^2)	21.57±1.86	22.18±2.14	1.757	0.081
Gn dosage (IU)	2428.15±724.16	2612.64±811.34	1.382	0.169
Duration of Gn stimulation (days)	11.56±2.43	12.03±2.76	1.042	0.299
Type			1.428	0.232
Primary	43 (53.09)	23 (42.59)		
Secondary	38 (46.91)	31 (57.41)		
Residence			0.278	0.598
Urban	66 (81.48)	42 (77.78)		
Rural	15 (18.52)	12 (22.22)		
Infertility factor			0.995	0.608
Tubal	25 (30.86)	13 (24.07)		
Ovulation disorders	10 (12.35)	9 (16.67)		
Others	46 (56.79)	32 (59.26)		

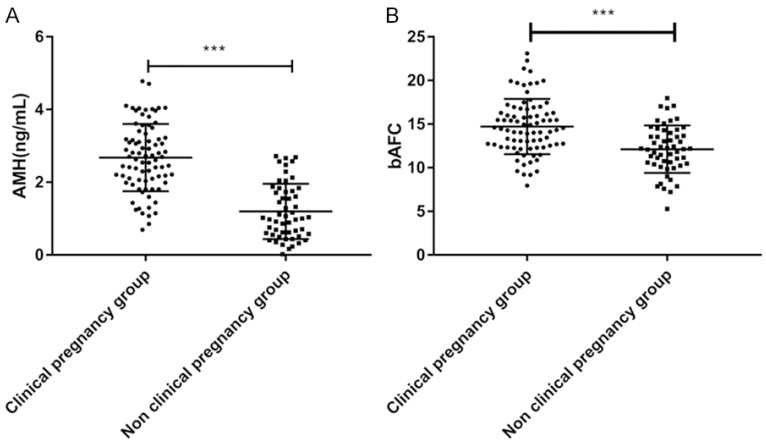

Figure 1. AMH and bAFC in the two groups. A: The AMH in the clinical pregnancy group was noticeably higher than that in the non-clinical pregnancy group (P<0.001). B: The BAFC in the clinical pregnancy group was notably higher than that in the non-clinical pregnancy group (P<0.001). *** indicated P<0.001.

were dramatically higher than those in the non-clinical pregnancy group (**Figure 1**).

Predictive value of AMH and bAFC on pregnancy outcomes

We utilized ROC curve to detect the predictive value of AMH and bAFC on pregnancy outcomes. It showed that the AUCs of AMH and bAFC in predicting pregnancy outcomes were 0.888 and 0.730, respectively. The specificity and sensitivity of AMH were 79.01%, 81.48%, and those of bAFC were 80.25% and 55.56%, respectively (**Figure 2**; **Table 2**).

Univariate analysis of non-clinical pregnancy

Patient's clinical data was collected for univariate analysis, and it was found that there were differences in age, E2, FSH, number of fertilized eggs, number of high-quality embryos, AMH and bAFC between the two groups (**Table 3**).

Multivariate analysis

We included the indicators with differences in univariate analysis into the assignment (see **Table 4** for assignment table), and then chose: LR for multivariate logistic regression analysis. The results revealed that age (OR: 3.435, 95% CI: 1.342-6.861) was an independent risk factor for failure of clinical pregnancy in older adults with IVF/ICSI-ET; AMH (OR: 0.310, 95% CI: 0.120-0.799), bAFC (OR: 0.315, 95% CI: 0.118-0.835) and number of high-quality embryos (OR: 0.309, 95% CI: 0.117-0.819) were protective factors (**Table 5**).

Discussion

Ovarian reserve function refers to a sufficient number of oocytes capable of developing into fertilized eggs [14-16]. At present, ovarian

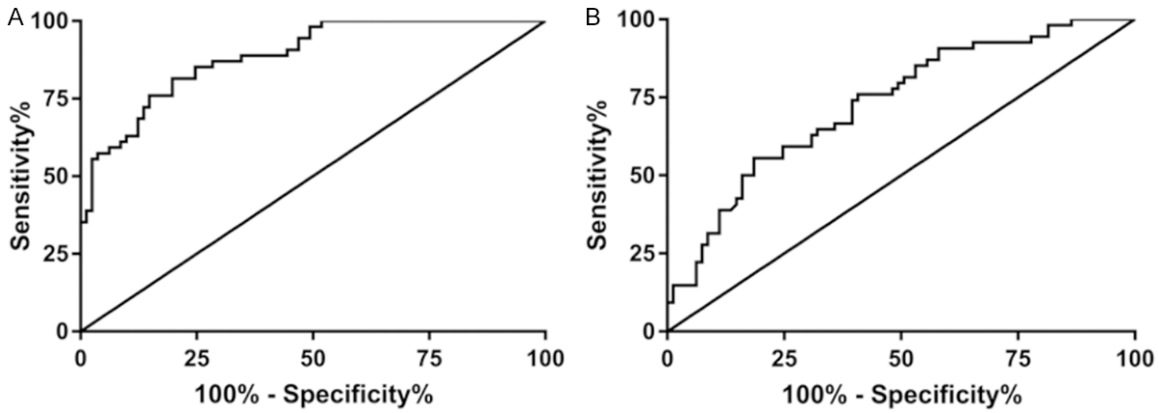

Figure 2. ROC curve of AMH and bAFC for prediction of pregnancy outcomes. A: ROC curve of AMH in predicting pregnancy outcomes. B: ROC curve of bAFC in predicting pregnancy outcomes.

Table 2. ROC data

	AUC	95% CI	Specificity	Sensitivity	Yordon index	Cut-off
AMH	0.888	0.834-0.941	79.01%	81.48%	60.49%	<1.959
bAFC	0.730	0.645-0.815	80.25%	55.56%	35.81%	<12.270

Table 3. Univariate analysis

	Clinical pregnancy group (n=81)	Non clinical pregnancy group (n=54)	t	P
Age (years old)	38.4±2.4	39.8±2.1	3.487	<0.001
Infertility (years)	8.5±2.0	9.1±1.8	1.776	0.078
BMI (kg/m^2)	21.57±1.86	22.18±2.14	1.757	0.081
E2 (pg/mL)	44.41±18.47	53.26±17.32	2.795	0.006
LH (IU/L)	4.89±1.62	4.50±1.42	0.202	0.841
FSH (U/L)	6.25±1.77	5.57±1.36	3.391	0.018
Gn dosage (IU)	2428.15±724.16	2612.64±811.34	1.382	0.169
Gn stimulation days (days)	11.56±2.43	12.03±2.76	1.042	0.299
Number of eggs (n)	10.3±3.6	9.4±2.1	1.657	0.100
Number of fertilized eggs (n)	9.5±2.6	8.2±2.2	3.022	0.003
Number of high-quality embryos (n)	2.4±1.1	1.6±0.7	4.740	<0.001
AMH (ng/mL)	2.57±0.93	1.14±0.97	8.603	<0.001
bAFC (n)	14.72±3.16	12.12±2.72	4.946	<0.001

Note: E2: estradiol; LH: luteinizing hormone; FSH: follicle-stimulating hormone; Gn: gonadotropin.

reserve function is an index widely used in the clinic to reflect the reproductive ability and reproductive potential of women [17-19]. Declined in ovarian reserve function means that the number of initial follicles stored and retained in the ovary tends to decrease or the oocytes cannot be fertilized successfully because the oocytes are not mature, leading to female fertility decline or clinical symptoms of menopause [20]. Therefore, if an indicator can be evaluated to predict ovarian reserve function, it can predict the possibility of successful pregnancy.

In the present study, we grouped all IVF/ICSI-ET patients according to their pregnancy outcomes and divided them into clinical pregnancy group and non-clinical pregnancy group. By comparing the AMH and bAFC in both groups, we found that AMH and bAFC levels in the clinical pregnancy group were remarkably higher than those in non-clinical pregnancy group, suggest-

Table 4. Assignment table

Factors	Assignment
Age	Raw data analysis for continuous variables.
E2	Raw data analysis for continuous variables.
FSH	Raw data analysis for continuous variables.
Number of fertilized eggs	Raw data analysis for continuous variables.
Number of high-quality embryos	Raw data analysis for continuous variables.
AMH	Raw data analysis for continuous variables.
bAFC	Raw data analysis for continuous variables.
Clinical pregnancy	No=1, yes=0

Table 5. Multivariate analysis

Factors	B	S.E,	Wals	Sig.	Exp (B)	95% C.I. for Exp (B)	
						Lower bound	Upper bound
Age	1.862	0.800	5.418	0.020	3.435	1.342	6.861
AMH	-1.171	0.483	5.876	0.015	0.310	0.120	0.799
bAFC	-1.156	0.498	5.387	0.020	0.315	0.118	0.835
Number of high-quality embryos	-1.173	0.497	5.575	0.018	0.309	0.117	0.819

ing that the two may be able to predict pregnancy outcomes in older IVF/ICSI-ET patients. Further, we evaluated their predictive value by ROC curve, and found that the AUC of AMH was 0.888, the specificity and sensitivity were 79.01% and 81.48%, while the AUC, specificity and sensitivity of bAFC were 0.730, 80.25% and 55.56% respectively, suggesting that the monitoring of AMH and bAFC can effectively predict the pregnancy outcome of older IVF/ICSI-ET patients. This indicates that we can implement a more personalized and more suitable regimen in the course of treatment by measuring AMH and bAFC levels in the clinic, so as to reduce the low response rate of the ovaries during ovulation induction and avoid the over-stimulation of the ovary. Previously, Amer et al. [21] tested the diagnostic value of AMH in female premature ovarian failure (POF), and found that AMH had higher specificity, sensitivity and accuracy than follicle-stimulating hormone, which also indicated that AMH could not only diagnose female POF, but also predict the outcome of IVF/ICSI-ET in older patients.

Later, we analyzed the independent factors underlying the failure of clinical pregnancy to occur in patients with IVF/ICSI-ET, and found that older age was the independent risk factor, while higher AMH, bAFC and number of high-quality embryos were protective factors. Park et al. [22] conducted statistics and research on 188 women aged 40-44 who received IVF/ICSI-ET, and through multivariate logistic regression analysis, AMH was identified to be the only factor significantly affecting the clinical pregnancy rate. This led us to suspect that AMH levels might also be predictive in older women in our study.

However, there are still some shortcomings in our study. First, the research participants included in this study were all patients receiving IVF/ICSI-ET, without the enrollment of healthy controls, so we are not clear about the comparison of test indicators and outcomes. Secondly, the outcome of our study was based on the acquisition of pregnancy. As for whether AMH and bAFC can also predict the live birth rate, we need to carry out follow-up studies to improve our study. Last but not the least, the female infertility factors were mainly considered in our study, but in reality, many infertility pathologies are related to male reproductive function, that's why male detections, such as sperm and chromatin structure determination, are applied in clinical practice [23, 24]. We hope to improve the IVF/ICSI-ET pregnancy outcome prediction scheme by combining these male related detection indicators in the follow-up study, so as to better manage infertile couples.

In summary, AMH and bAFC can well predict the outcome of IVF/ICSI-ET in older patients. Older age is an independent risk factor for the failure of clinical pregnancy in patients, while higher AMH, bAFC and number of high-quality embryos are protective factors.

Disclosure of conflict of interest

None.

Address correspondence to: Hui Su, Department of Reproductive Medicine, Hengshui People's Hospital, No.180 Renmin Road, Hengshui 053000, Hebei Province, China. Tel: +86-13256857482; E-mail: suhui1982@163.com

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