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Original Article

Clinical significance of combined detection of anti-Mullerian hormone and follicular output rate in women of late reproductive age

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Abstract: Objective: This study was designed to explore the clinical significance of anti-Mullerian hormone (AMH) combined with follicular output rate (FORT) in women of late reproductive age. Methods: A total of 258 women (age range: 35-45 years old) who underwent pre-pregnancy examination in our hospital were collected as the research group (RG), among whom 184 were treated with *in vitro* fertilization-embryo transfer (IVF-ET). Concurrently, 126 women aged 24-30 years who came to our hospital for pre-pregnancy examination were enrolled as the control group (CG). AMH and FORT were detected and compared between the two groups to analyze the clinical significance of the two in women of late reproductive age. Results: Compared with the CG, AMH was decreased statistically in the RG (P<0.05). AMH was statistically higher in the regular menstrual group than in the menstrual disorder group (P<0.05), and FORT was statistically higher in the pregnancy group in comparison with the non-pregnancy group (P<0.05). AMH decreased with age (P<0.05), while FORT did not correlate with any notable difference among the three subgroups (P>0.05). High, medium and low AMH groups showed no significant difference in the number of retrieved oocytes and transplantable embryos, as well as FORT (P<0.05). A lower AMH level, was correlated with fewer number of retrieved oocytes and transplantable embryos, and higher the FORT level. Significant differences were present among the high, middle and low FORT groups regarding the number of retrieved oocytes and transplantable embryos, the clinical pregnancy rate and AMH level (P<0.05). The lower the level of FORT was, the less the number of retrieved oocytes and transplantable embryos was, the lower clinical pregnancy rate was, and the higher the AMH level was. Conclusions: AMH decreases gradually in women with an increase of age, and FORT can effectively predict pregnancy outcome. AMH detection combined FORT is of great significance in predicting the ovarian reserve function in women of late reproductive age.

Keywords: AMH, FORT, women of late reproductive age, ovarian reserve function

Introduction

In the past 5 decades, many high-income men and women have delayed starting a family [1], and as China's family planning policy changes [2], many older couples also have the desire to have a child, which has led to an increasing number of women of late reproductive age being seen in clinical practice for reproductive health care. Studies have shown that as women grow older, the fertility rate shows a trend of continuous decline [3]. Age-related fertility decline mainly stems from spontaneous follicular failure and degeneration [4], the underlying cause of which is ovarian reserve dysfunction [5]; that is, the function of fertilizable oocytes in the ovarian cortex becomes reduced which is determined by the number and quality of follicles in the ovary [6]. The fertility boom for women occurs in their early 20s, begins to decline in the third decade of life, and declines sharply after the age of 35 [7]. Therefore, compared with young women, the clinical detection of fertility in elderly women is helpful.

Accurate measurement of ovarian reserve function is crucial for clinical evaluation of fertility in elderly women. Studies have shown that anti-

Mullerian hormone (AMH) is the best ovarian reserve measure that can effectively predict female reproductive life span and ovarian dysfunction under various clinical conditions [8]. AMH, a marker of ovarian reserve, is considered to be completely periodic-independent, with no difference among observers except for a small decrease in the late follicular stage [9]. However, AMH alone has limitations in evaluating ovarian reserve function. In some studies, follicular output rate (FORT), a novel evaluation index, has been proposed [10]. FORT is considered to be a favorable index to evaluate the hormone response of follicles to exogenous recombinant follicles, which makes it a promising qualitative index of ovarian function [11].

Therefore, this paper evaluated the ovarian reserve function of women of late reproductive age by detecting AMH and FORT in two groups of patients, and analyzed their clinical significance.

Materials and methods

General data

Aged 35-45 years old with an average age of (40.32±4.31) years, 258 women who underwent pre-pregnancy examination in Hengshui People's Hospital were collected as the research group (RG), among whom 184 patients who received *in vitro* fertilization-embryo transfer (IVF-ET) treatment were included in the IVF-ET group. Additionally, 126 women (24-30 years old, mean age: 27.43±2.52 years) from the same period were set as the control group (CG). Inclusion criteria: All the pregnant women were accompanied by their family members at admission, and had not taken any sex hormone drugs within the last 3 months, with complete clinicopathological data. Exclusion criteria: Those with family history or previous history of mental illness, history of autoimmune deficiency, history of severe organ disease, history of drug dependence, history of ovarian and uterine surgery, or inability to cooperate with the examination due to aphasia, restlessness, unconsciousness or communication impairment were excluded. The Ethics Committee of Hengshui People's Hospital approved the study protocol. All the enrolled participants were informed about the details of this study, and provided written informed consent to participate.

Methods

On the 3rd day of menstruation, fasting venous blood (5 ml) was collected from all the participants and placed into anticoagulant tubes to coagulate for 60 min (20-25°C). Then the samples were centrifuged at 1369.55xp and 4°C for 15 min with a centrifuge (Sichuan Shuke Instrument Co., Ltd., Chengdu, China, TG 112), and then put into a cryogenic freezer at -70°C for storage. Determination of serum AMH expression (Human AMH ELISA kit, Wuhan Fine Biotech Co., Ltd., Wuhan, China, EH0528) was performed by Enzyme-linked immunosorbent assay (ELISA) following the manufacturer's protocol. The subjects who received IVF-ET were treated with luteal phase long regimen of short-acting gonadotropin-releasing hormone-agonist (GnRH-a) + recombinant follicle-stimulating hormone (rFSH, Guenafin, Serono, Switzerland)/human menopausal gonadotropin (HMG, Lizhus) + human chorionic gonadotropin (hCG, Serono, Switzerland). While those receiving FSH underwent daily vaginal B-ultrasound examination to record the AFC (number of follicles 2-8 mm in diameter), and those treated with HCG received daily vaginal B-ultrasound examination to record the PFC (number of follicles 16-22 mm in diameter), so as to calculate the FORT value (PFC/AFC × 100%).

Outcome measures

The general clinical data and AMH were compared between the RG and CG. Subjects in the IVF-ET group were further subdivided into the regular menstrual group (108 cases) and the menstrual disorder group (76 cases), as well as the pregnancy group (72 cases) and the non-pregnancy group (112 cases), and were additionally categorized into the high age (>42 years old), middle age (42-38 years old), and low age (<38 years old) groups according to menstruation, pregnancy and age, so as to compare AMH and FORT between or among subgroups. According to the quartile method (>75%, 75-25%, <25%), subjects in the IVF-ET group were divided into the corresponding high, medium, and low groups based on AMH and FORT values to compare the treatment outcomes among the three subgroups.

Statistical methods

The experimental results were analyzed by SPSS 20.0 (IBM Corp, Armonk, NY, USA), and

Table 1. Comparison of general information (x±sd)/[n (%)]

	Research group (n=258)	Control group (n=126)	t/X^2	P
Average age (years old)	40.32±4.31	27.43±2.52	31.06	0.00
BMI (kg/m^2)	22.64±3.57	22.59±3.61	0.13	0.90
Drinking history			0.08	0.77
Yes	52 (20.16)	27 (21.43)	-	-
No	206 (79.84)	99 (78.57)	-	-
Smoking history			0.85	0.36
Yes	49 (18.99)	29 (23.02)	-	-
No	209 (81.01)	97 (76.98)	-	-
Residence			0.42	0.52
Urban	124 (48.06)	65 (51.59)	-	-
Rural	134 (51.94)	61 (48.41)	-	-
Ethnicity			2.37	0.12
Han	202 (78.29)	107 (84.92)	-	-
Ethnic minorities	56 (21.71)	19 (15.08)	-	-

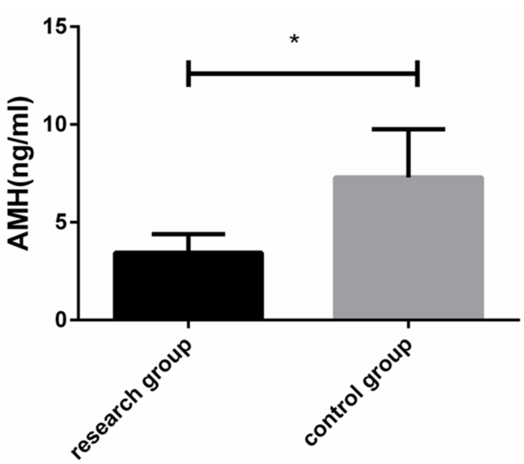

Figure 1. Comparison of AMH between the two groups. The AMH level in the research group was statistically lower than that in the control group. Note: * indicates P<0.05 compared between the two groups.

visualized using GraphPad Prism 7 (GraphPad Software Inc., San Diego, USA). The counting data were represented by [n (%)], and compared by Chi-square test between or among groups. The measurement data, recorded as (x±sd), were compared by t test between groups. A *p*-value <0.05 was considered significant.

Results

Comparison of general data

No noteworthy differences were observed in age, body mass index (BMI), smoking history or drinking history (P>0.05) between the RG and the CG, while a significant difference was present concerning average age (P<0.05). **Table 1**.

Comparison of AMH between the RG and CG

Figure 1 shows the comparison of AMH between the RG and CG. Compared with the CG, the AMH in the RG was decreased statistically (P<0.05).

Comparison of AMH and FORT between the regular menstrual and disorder group

The comparison of AMH and FORT between the regular menstrual group and the menstrual disorder group is shown in **Figure 2**. AMH was statistically higher in the regular menstrual group than in the menstrual disorder group (P<0.05), while FORT did not differ statistically between the two subgroups (P>0.05).

Comparison of AMH and FORT between the pregnancy and non-pregnancy groups

The comparison of AMH and FORT between the pregnancy group and non-pregnancy group is shown in **Figure 3**. No marked difference was observed in AMH between the two subgroups (P>0.05), but the FORT was statistically higher in the pregnancy group as compared to non-pregnancy group (P<0.05).

Comparison of AMH and FORT among the high, middle and low age groups

AMH and FORT were compared among the high, middle and low age groups, as shown in **Figure**

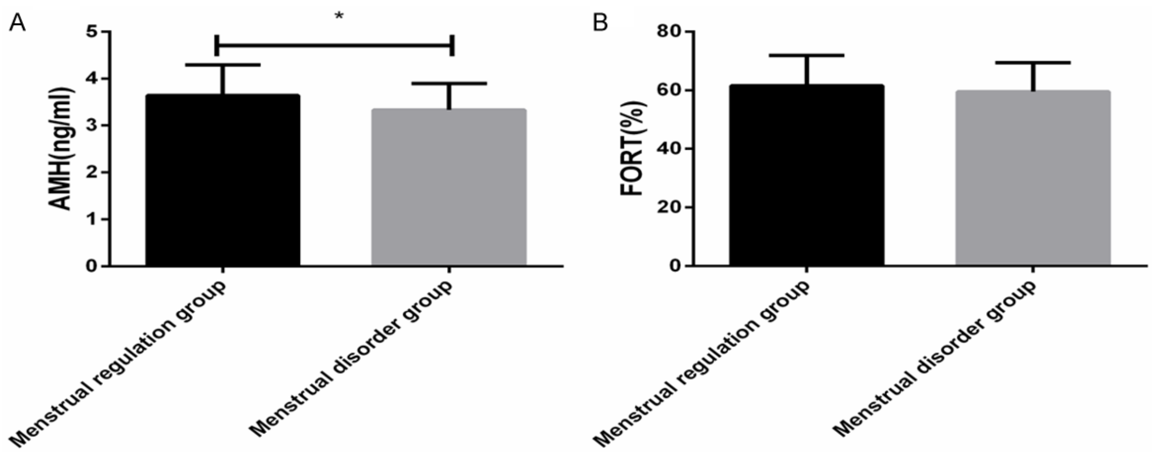

Figure 2. Comparison of AMH and FORT between the regular menstrual and disorder group. A: The AMH level in the regular menstrual group was statistically higher than that in the menstrual disorder group. B: The FORT level showed no significant difference between the regular menstrual group and menstrual disorder group. Note: * indicates P<0.05 compared between the two groups.

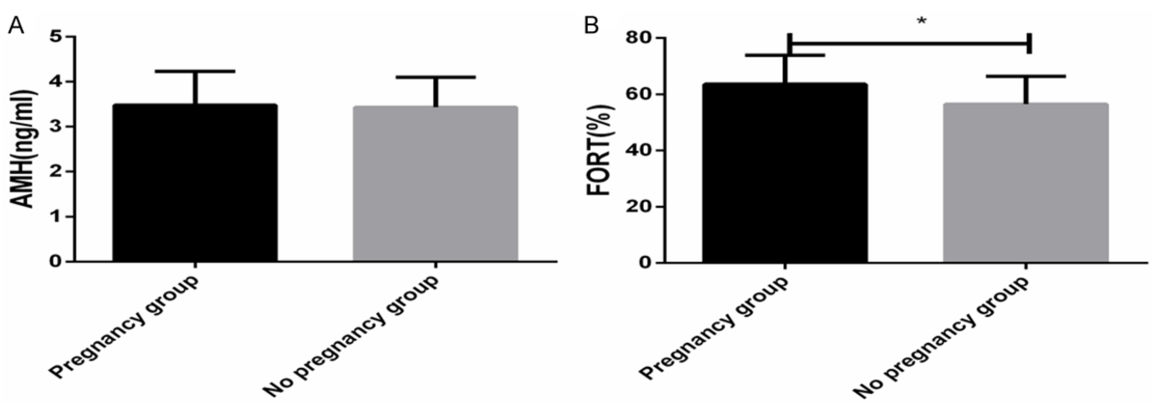

Figure 3. Comparison of AMH and FORT between the pregnancy and non-pregnancy groups. A: There was no significant difference in AMH between the pregnancy group and non-pregnancy group. B: The FORT was statistically higher in the pregnancy group than in non-pregnancy group. Note: * indicates P<0.05 compared between the two groups.

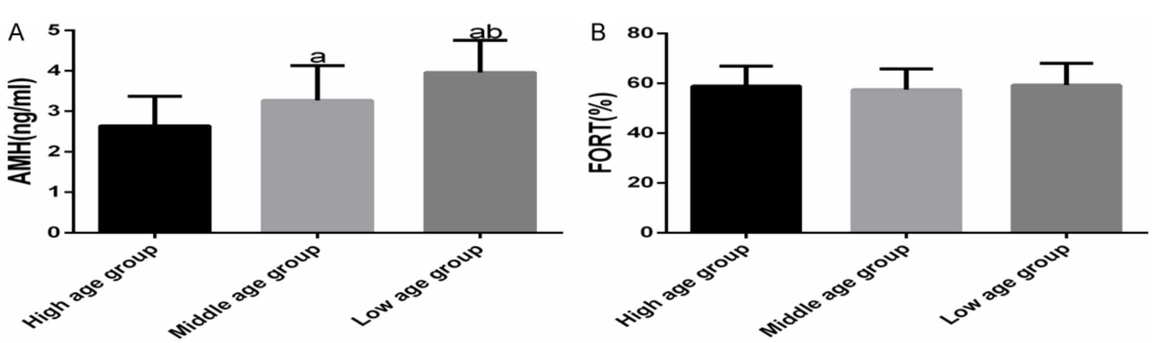

Figure 4. Comparison of AMH and FORT among the high, middle and low age groups. A: AMH increased with the decrease of age. B: There was no significant change in FORT with the decrease of age. Note: a indicates P<0.05 compared with the high age group, and b indicates P<0.05 compared with the middle age group.

4. AMH decreased with the increase in age (P<0.05), but there was no notable difference in FORT among the three subgroups (P>0.05).

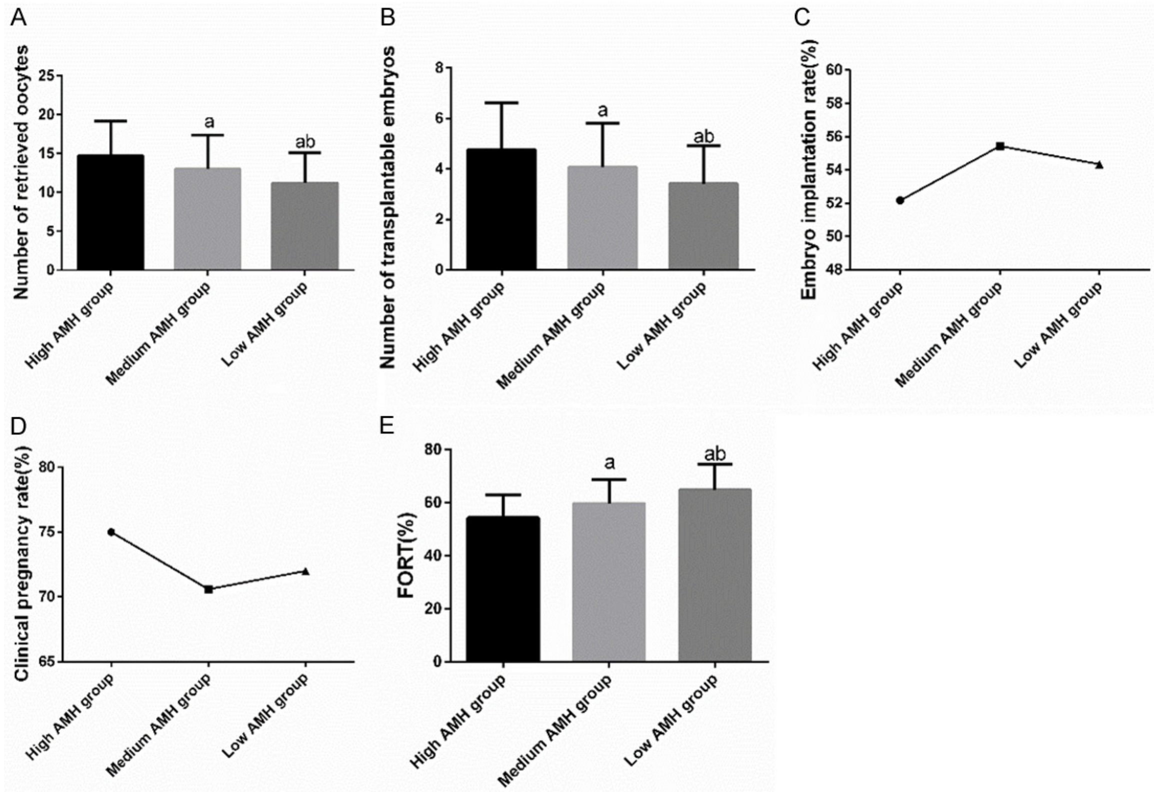

Figure 5. Comparison of FORT and treatment outcomes among the high, medium and low AMH groups. A: The number of retrieved oocytes decreased with the decrease of AMH. B: The number of transplantable embryos decreased with the decrease of AMH. C: There was no significant difference in embryo implantation rate among the three groups. D: There was no significant difference in clinical pregnancy rate among the three groups. E: FORT increased with the decrease of AMH. Note: a indicates P<0.05 compared with the high AMH group, and b indicates P<0.05 compared with the medium AMH group.

Comparison of FORT and treatment outcomes among the high, medium and low AMH groups

The comparison of FORT and treatment outcomes among the high, medium and low AMH groups is shown in **Figure 5**. Significant differences were not found concerning embryo implantation rate and clinical pregnancy rate among the three subgroups (P>0.05), while significance was present regarding the number of retrieved oocytes and transplantable embryos, and FORT (P<0.05). A lower AMH indicated a lower number of retrieved oocytes and transplantable embryos, and a higher FORT level.

Comparison of AMH and treatment outcomes among the high, middle and low FORT groups

Figure 6 shows the comparison of AMH and treatment outcomes among the high, middle and low FORT groups. The embryo implantation rate revealed no significant difference among the high, middle and low FORT groups (P>0.05). However, marked differences were observed in the number of retrieved oocytes and transplantable embryos, clinical pregnancy rate and AMH (P<0.05). A lower FORT indicated a lower number of retrieved oocytes and transplantable embryos, a decreased clinical pregnancy rate, and a higher AMH level.

Discussion

Assisted pregnancy and fertility for women of late reproductive age has become a global occurrence [12]. The ovarian reserve function of women will continue to decline with age, and the rates of pregnancy and live births from such women are statistically decreased compared with those of women of younger childbearing age [13]. At present, an objective and accurate index is needed to evaluate the ovarian reserve function of women of late reproductive age, which is conducive to formulating personalized

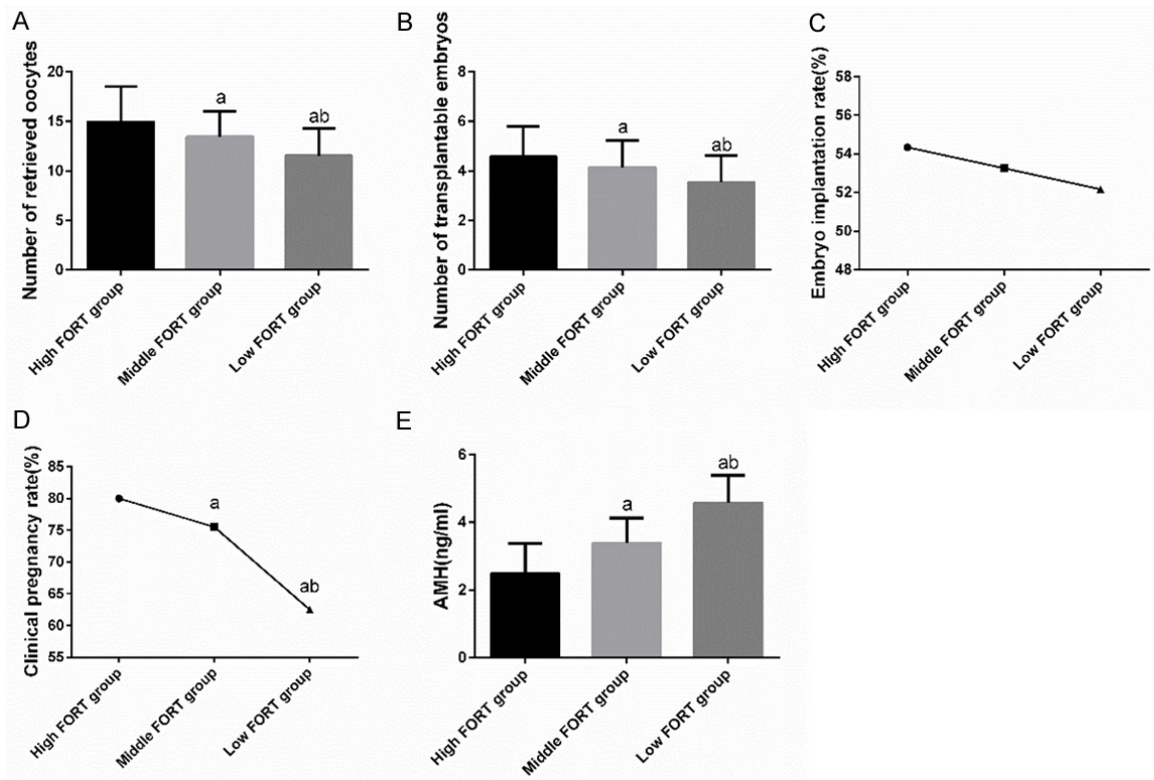

Figure 6. Comparison of AMH and treatment outcomes among the high, middle and low FORT groups. A: The number of retrieved oocytes decreased with the decrease of FORT. B: The number of transplantable embryos decreased with the decrease of FORT. C: There was no significant difference in embryo implantation rate among the three groups. D: The clinical pregnancy rate decreased with the decrease of FORT. E: AMH increased with the decrease of FORT. Note: a indicates $P<0.05$ compared with the high FORT group, and b indicates $P<0.05$ compared with the middle FORT group.

treatment and ovulation induction program to obtain better pregnancy outcomes [14].

AMH, a unique member of the TGF-β family and responsible for the development and differentiation of the reproductive system; it signals through its own dedicated type II receptor, the anti-Muller's hormone receptor II (AMHR2), thus providing the only ligand-receptor pair in the wider TGF-β family [15]. AMH is negatively correlated with age in women with normal ovarian function [16]. Studies have shown that AMH is not only an important ovarian reserve reproductive marker, which is vital in the recruitment of primitive follicles and follicle growth dependent on FSH, but also a predictor of various assisted reproductive technologies such as IVF [17]. While FORT can accurately predict oocyte yield and is an independent variable affecting clinical pregnancy rate in IVF/ICSI cycles; the higher the FORT value, the better the oocyte yield and clinical pregnancy rate of unexplained infertile women treated with IVF/ICSI with normal ovarian response [19]. Therefore, in this study, the combined detection of AMH and FORT was used for ovarian reserve function evaluation of women of late reproductive age.

According to reports, AMH is the best endocrine marker for evaluating the ovary of healthy women and can be an indicator of fertility and pregnancy cycle and outcome of assisted reproductive technology in women of late reproductive age [20]. Its concentration will slowly decrease with age [21], which is basically consistent with the results in our study that the AMH in the RG was statistically lower than that in the CG. Also, it is shown that serum AMH concentration decreases by about 6% a year with age, until the depletion of primordial follicles around 5 years before menopause [22]. This agrees with the results that AMH decreased with age in the high, middle and low age groups of participants, further indicating that AMH is related to age and is an important index to predict reserve function of women. We also found that the regular menstrual group showed statistically higher AMH levels and lower ovarian reserve function decline than the menstrual disorder group, indicating that even if the menstrual cycle of women aged over 35 was normal, the AMH level still decreased significantly. However, no noteworthy difference was observed in FORT level between the two groups. Referring to the relevant literature, it was found that FORT, which can effectively evaluate the ovarian reserve function of women, will not be affected by factors such as age, BMI or basic FSH level [23], indicating that FORT alone has some limitations in assessing women's ovarian reserve function. Hence, AMH and FORT should be tested together. In the present study, no significant difference was observed in AMH levels between the pregnancy and non-pregnancy groups, but the FORT level of the former was statistically higher than that of the latter, indicating that the pregnancy success rate in older women increased with the increase of FORT level. Therefore, patients in the IVF-ET group were further allocated into three subgroups according to high, medium and low AMH or FORT levels to compare treatment outcomes. This revealed no significant differences in embryo implantation rate and clinical pregnancy rate among the high, medium and low AMH groups, but significant differences in the number of retrieved oocytes and transplantable embryos, with FORT level; moreover, a lower AMH indicated a lower number of transplantable embryos, and a higher FORT level. Previously, AMH and FORT were identified to be negatively associated, which was similar to the result obtained in this study [24]. As mentioned earlier, the clinical pregnancy rate and fertilization rate gradually increased with the improvement of FORT level, as an independent variable affecting the clinical pregnancy rate in IVF/ICSI cycle, the higher FORT value is, the better the oocyte yield and clinical pregnancy rate of infertile women with unknown causes but normal ovarian response who are treated with IVF/ICSI [19]. The results obtained in this study are basically consistent with the preceding results; that is, there were notable differences in the number of retrieved oocytes and transplantable embryos, the clinical pregnancy rate and AMH among the high, middle and low FORT groups; moreover, the lower FORT was, the lower the number of retrieved oocytes and transplantable embryos and the clinical pregnancy rate was, while the higher the AMH level. This is also in line with the study "Exploring the relationship between FORT and clinical prognosis in patients with polycystic ovary syndrome", which concluded that the pre-ovulation follicular count in the high FORT group was statistically higher than that of the corresponding medium and low FORT groups, and the number of oocytes, high quality embryo rate and clinical pregnancy rate was reduced from the high to low FORT groups [10]. It also suggests that elevated FORT and AMH lead to increased number of transplantable embryos, and improved cumulative pregnancy rate.

In this study, AMH and FORT were tested in two groups of participants to evaluate the ovarian reserve function of women of late reproductive age, and to analyze the clinical significance of these two indexes. However, this paper still has certain limitations. The specific mechanism of action of AMH and FORT in women of late reproductive age remains to be clarified. In addition, the exploration of the specific mechanism of the negative correlation between AMH and FORT is also warranted. Therefore, we will constantly improve the research design to provide more scientific reference for clinical practice, with a view to better improve the fertility of women of late reproductive age.

Collectively, AMH decreases gradually in women with the increase of age, and FORT can effectively predict pregnancy outcomes. AMH combined FORT has an important value in predicting the ovarian reserve function in women of late reproductive age.

Disclosure of conflict of interest

None.

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References

[1] Sylvest R, Koert E, Vittrup I, Birch Petersen K, Nyboe Andersen A, Pinborg A and Schmidt L. Status one year after fertility assessment and counselling in women of reproductive age-a

qualitative study. Ups J Med Sci 2018; 123: 264-270.
[2] Jiang Q, Yang S, Li S and Feldman MW. The decline in China's fertility level: a decomposition analysis. J Biosoc Sci 2019; 51: 785-798.
[3] Garcia D, Brazal S, Rodriguez A, Prat A and Vassena R. Knowledge of age-related fertility decline in women: a systematic review. Eur J Obstet Gynecol Reprod Biol 2018; 230: 109-118.
[4] Isono W, Wada-Hiraike O, Kawamura Y, Fujii T, Osuga Y and Kurihara H. Administration of oral contraceptives could alleviate age-related fertility decline possibly by preventing ovarian damage in a mouse model. Reprod Sci 2018; 25: 1413-1423.
[5] Morin SJ, Patounakis G, Juneau CR, Neal SA, Scott RT and Seli E. Diminished ovarian reserve and poor response to stimulation in patients <38 years old: a quantitative but not qualitative reduction in performance. Hum Reprod 2018; 33: 1489-1498.
[6] Lensen SF, Wilkinson J, Leijdekkers JA, La Marca A, Mol BWJ, Marjoribanks J, Torrance H and Broekmans FJ. Individualised gonadotropin dose selection using markers of ovarian reserve for women undergoing in vitro fertilisation plus intracytoplasmic sperm injection (IVF/ICSI). Cochrane Database Syst Rev 2018; 2: CD012693.
[7] Zhang W, Tian Y, Xie D, Miao Y, Liu J and Wang X. The impact of peak estradiol during controlled ovarian stimulation on the cumulative live birth rate of IVF/ICSI in non-PCOS patients. J Assist Reprod Genet 2019; 36: 2333-2344.
[8] Broer SL, Broekmans FJ, Laven JS and Fauser BC. Anti-Mullerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update 2014; 20: 688-701.
[9] Pilsgaard F, Grynnerup AG, Lossl K, Bungum L and Pinborg A. The use of anti-Mullerian hormone for controlled ovarian stimulation in assisted reproductive technology, fertility assessment and -counseling. Acta Obstet Gynecol Scand 2018; 97: 1105-1113.
[10] Tan X, Wen Y, Chen H, Zhang L, Wang B, Wen H, Zhang F, Wu Q and Du J. Follicular output rate tends to improve clinical pregnancy outcomes in patients with polycystic ovary syndrome undergoing in vitro fertilization-embryo transfer treatment. J Int Med Res 2019; 47: 5146-5154.
[11] Rehman R, Mustafa R, Baig M, Arif S and Hashmi MF. Use of follicular output rate to predict intracytoplasmic sperm injection outcome. Int J Fertil Steril 2016; 10: 169-174.
[12] Abbara A, Eng PC, Phylactou M, Clarke SA, Hunjan T, Roberts R, Vimalesvaran S, Christopoulos G, Islam R, Purugganan K, Comninos AN, Trew GH, Salim R, Hramyka A, Owens L, Kelsey T and Dhillo WS. Anti-Mullerian hormone (AMH) in the diagnosis of menstrual disturbance due to polycystic ovarian syndrome. Front Endocrinol (Lausanne) 2019; 10: 656.
[13] Ligon S, Lustik M, Levy G and Pier B. Low anti-mullerian hormone (AMH) is associated with decreased live birth after in vitro fertilization when follicle-stimulating hormone and AMH are discordant. Fertil Steril 2019; 112: 73-81, e71.
[14] Jaillard S, Sreenivasan R, Beaumont M, Robevska G, Dubourg C, Knarston IM, Akloul L, van den Bergen J, Odent S, Croft B, Jouve G, Grover SR, Duros S, Pimentel C, Belaud-Rotureau MA, Ayers KL, Ravel C, Tucker EJ and Sinclair AH. Analysis of NR5A1 in 142 patients with premature ovarian insufficiency, diminished ovarian reserve, or unexplained infertility. Maturitas 2020; 131: 78-86.
[15] Hart KN, Pepin D, Czepnik M, Donahoe PK and Thompson TB. Mutational analysis of the putative Anti-Mullerian Hormone (AMH) binding interface on its type II receptor, AMHR2. Endocrinology 2020; 161: bqaa066.
[16] Evliyaoglu O, Imohl M, Weiskirchen R and van Helden J. Age-specific reference values improve the diagnostic performance of AMH in polycystic ovary syndrome. Clin Chem Lab Med 2020; 58: 1291-1301.
[17] Umer S, Sammad A, Zou H, Khan A, Weldegebriall Sahlu B, Hao H, Zhao X, Wang Y, Zhao S and Zhu H. Regulation of AMH, AMHR-II, and BMPs (2,6) genes of bovine granulosa cells treated with exogenous FSH and their association with protein hormones. Genes (Basel) 2019; 10: 1038.
[18] Griesinger G, Teal V, McCrary Sisk C and Ruman J. Comparison of the follicular output rate after controlled ovarian stimulation with daily recombinant follicle-stimulating hormone versus corifollitropin alfa. Eur J Obstet Gynecol Reprod Biol 2019; 232: 101-105.
[19] Hassan A, Kotb M, AwadAllah A, Wahba A and Shehata N. Follicular output rate can predict clinical pregnancy in women with unexplained infertility undergoing IVF/ICSI: a prospective cohort study. Reprod Biomed Online 2017; 34: 598-604.
[20] Meczekalski B, Czyzyk A, Kunicki M, Podfigurna-Stopa A, Plociennik L, Jakiel G, Maciejewska-Jeske M and Lukaszuk K. Fertility in women of late reproductive age: the role of serum anti-Mullerian hormone (AMH) levels in its assessment. J Endocrinol Invest 2016; 39: 1259-1265.
[21] Hsieh HC, Su JY, Wang S and Huang YT. Age effect on in vitro fertilization pregnancy mediated by anti-Mullerian hormone (AMH) and

modified by follicle stimulating hormone (FSH). BMC Pregnancy Childbirth 2020; 20: 209.

[22] Khan HL, Bhatti S, Suhail S, Gul R, Awais A, Hamayun H, Enver F, Abbas S, Hassan Z, Nisar R, Sardar S and Asif W. Antral follicle count (AFC) and serum anti-Mullerian hormone (AMH) are the predictors of natural fecundability have similar trends irrespective of fertility status and menstrual characteristics among fertile and infertile women below the age of 40 years. Reprod Biol Endocrinol 2019; 17: 20.

[23] Rehman R, Irfan T, Jawed S, Hussain M and Ali R. Embryo quality in intracytoplasmic sperm injection: a quasi experimental design in Pakistan. J Pak Med Assoc 2018; 68: 1451-1455.

[24] Fatima S, Naqvi Q, Tauseef A, Qamar M, Khan QU, Akram T and Saeed M. Correlation of serum anti-mullerian hormone with ovarian follicle output rate in infertile females: a clomiphene citrate challenge test. Cureus 2020; 12: e8032.