Analysis of discordance between AMH and AFC in IVF patients: a retrospective cohort study

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Research

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Abstract

Background

Both anti-Mullerian hormone (AMH) and antral follicle count (AFC) predict ovarian reserve independently and have been shown to correlate well. However, it is quite common to encounter discordance between the two clinical indices. This objective of this study is to speculate which condition is favorable to patients and to decide what best to be done in cases of such discordance.

Methods

This retrospective cohort study analyzed the medical records of 714 women undergoing the first IVF cycle at the Center for Reproductive Medicine, the Affiliated Hospital of Nantong University, Nantong, Jiangsu between January 2017 and December 2020. All patients had their essential characteristics, baseline FSH, Estradiol, PRL, LH, Testosterone, CA125, AMH, and AFC recorded. Patients were classified into three groups according to whether their AMH and AFC values were in the lower quartile, upper quartile, or in between, namely Group A (Concordance, concordant in AMH and AFC), Group B (HTP, AMH concentration higher than predicted according to AFC) and Group C (LTP, AMH concentration lower than predicted according to AFC). SPSS 25.0 software package (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis.

Results

The entire study population has a good concordance between AMH and AFC. 240 women (33.61%) had discordant AMH and AFC values, and the proportion in Group B(HTP) and Group C(LTP) seemed similar (16.67% VS 16.94%). The three cohorts had similar age, infertile duration, FSH, Estradiol, PRL, LH, Testosterone, and CA125 levels. BMI progressively increased from Group B to Group A and to Group C. The ratio of patients, who had completely mature oocytes (COM), rapidly decreased from Group C to Group A and to Group B. Although no significant difference in CMO rate was observed among Group A, B, and C when stratified according to BMI, CMO rate was the highest (72.31%) when BMI < 18.5 kg/m^2 in all BMI categories.

Conclusions

Our analysis demonstrated a high frequency of clinical discordance between AMH and AFC in women undergoing IVF therapeutic regime. Patients' BMI and completely mature oocytes ratio are significantly different among distinct grouping based on their AMH and AFC partition.

Background

The ovarian reserve is usually estimated by serum anti-Mullerian hormone (AMH) concentration(1) and antral follicle count (AFC)(2), which are also often employed in in vitro fertilization (IVF) therapeutic regime(3–5).

AMH, also known as Mullerian-inhibiting substance, is a member of the transforming growth factor β family, which is secreted by granulosa from small, growing follicles(6–9). AMH exerts its biological effects on the ovaries generally by inhibiting the initial follicle recruitment and the FSH-dependent growth of the follicles and selection of pre-antral and small antral follicles through a transmembrane serine/threonine kinase type II receptor (AMHR II)(10–12). The absence of AMH has been shown to enhance FSH-induced follicle growth in female mice(10, 13). Currently, AMH has been widely used as a golden marker for evaluating ovarian reserve in females, particularly in the field of assisted reproduction(1, 14–16).

Antral follicle count (AFC), which consists of all ultrasonographically identified and counted antral follicles measuring 2–10 mm in diameter in both ovaries, is another reliable indicator of ovarian reserve owing to its close relationship to age-related declines in follicle counts(17–21). Although AFC may be more machine and operator-dependent and could have higher inter-operator variability compared to AMH, it correlates more directly with the ovarian reserve of a single ovary than AMH(1, 3, 5, 19, 22).

Systematic reviews showed that AMH and AFC had a very good correlation with each other. In 2003, Fanchin et al. demonstrated that AFC was closely related to serum AMH level on cycle day 3 in infertile women, more strongly than other hormonal markers, such as inhibin B, E2, and FSH(23). A strong positive correlation between AMH and AFC and paralleled fluctuations of AMH and AFC have also been demonstrated throughout the regular menstrual cycle(24–26). Yet, it is not unusual for IVF specialists to encounter a serum AMH level that is higher or lower than expected according to AFC, even when the measurement of AMH and counting of antral follicles are performed during the early follicular phase of the same menstrual cycle in a single clinical center(18, 27).

In this article, we conducted a retrospective analysis to evaluate the discordance between AMH and AFC in women undergoing their first IVF treatment, the reasons hiding in this phenomenon, and maybe the clinical implications.

Methods

Patient sampling

The medical records of 714 women at the Center for Reproductive Medicine, Affiliated Hospital of Nantong University between January 2017 and December 2020 were evaluated in the analysis. Only the first cycle was included for each patient to eliminate previous failure bias.

The exclusion criteria were as follows: patients with a history of ovarian or pelvic surgery, endometriosis, chemotherapy or radiation exposure, hormonal therapy in the past 6 months, fragile X premutation, and

abnormal karyotype or other endocrine diseases, including diabetes, thyroid dysfunction, and hyperprolactinemia.

During the study period, the type of protocol was determined by the doctor according to specific characteristics, such as ovarian reserve and follicle size.

All patients who had their essential characteristics, baseline FSH, Estradiol, PRL, LH, Testosterone, CA125, AMH, and AFC recorded during their evaluation were included in the study. The Ethics Committee of Affiliated Hospital of Nantong University has authorized us to use existing patient data anonymously, waiving the requirement for written consent from individual patients. The main clinical and pathologic variables of the study patients are shown in Table 2.

Classification of patients

In the precycle analysis, the 25th percentile and 75th percentile values of serum AMH concentration were 2.05 ng/mL and 5.58 ng/mL, whereas the values of AFC were 12 and 31, respectively. Patients were classified into three groups according to whether their AMH and AFC values were in the lower quartile, upper quartile, or in between (Table 1), namely Group A (Concordant, concordance in AMH and AFC), Group B (HTP, AMH concentration higher than predicted according to AFC) and Group C (LTP, AMH concentration lower than predicted according to AFC).

Clinical investigation

Ultrasonographic examinations were performed by reproductive medicine specialists, who had at least 5 years of experience in gynecological ultrasound and have undergone uniform training. During the early follicular phase of the menstrual cycle, follicles measuring 2–10 mm in diameter were counted. The number of follicles in each ovary was assessed by using the same ultrasound equipment with a 2D transvaginal probe 6.5 MHz (Toshiba, Nemio, Japan). The total number of follicles in both ovaries was used as the AFC.

Laboratory analyses

Serum FSH, Estradiol, PRL, LH, and Testosterone were measured on day 2 or 3 of the menstrual cycle with the use of Beckman Coulter Dxl 800 (Beckman Coulter, Inc., Brea, USA). Random serum CA125 and AMH levels, unrelated to cycle day, were measured by using enzyme-linked immunosorbent assay (ELISA) on ARCHITECH I2000SI (Abbott, Chicago, IL, USA) and iFlash 3000-H (YHLO Biotechnology Co., Ltd., Shenzhen, CHINA), respectively.

The functional sensitivity, the coefficients of variation for intra-assay precision (repeatability) and the inter-assay precision (intermediate precision) of the FSH, Estradiol, PRL, LH, Testosterone, CA125 and AMH assays were as follows: 0.20 mIU/mL, 3.10%-4.30%, 4.30%-5.60%; 15 pg/mL, ≤ 10%, ≤ 15%; 0.25 ng/mL, 1.42%-1.61%, 3.32%-6.92%; 0.20 mIU/mL, 3.80%-5.40%, 4.30%-6.40%; 0.10 ng/mL, 1.67%-3.93%, 4.22%-7.08%; 1.00 U/mL, 1.50%-3.20%, 1.90%-4.70%; and 0.10 ng/mL, ≤ 10%, ≤ 15%.

Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$.

Statistical analysis

SPSS 25.0 software package (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Patient characteristics were compared between the groups by using Mann–Whitney test. Continuous variables were compared using Student's t-test when data were normally distributed and by Mann–Whitney U when the data were not normally distributed. Chi-squared tests were used to compare proportions. All normal distribution measurement data are expressed as the mean ± standard deviation (SD). The level of significance was set at $P < 0.05$.

Results

In this study, 714 women, who underwent the first IVF treatment cycle, fulfilled the inclusion criteria, were included. The clinical and demographic parameters of the included subjects were listed in Table 2.

The correlation between AMH and AFC was visualized in Figure 1. A significant positive association of AMH and AFC was depicted in the entire study population (regression equation: AMH = 0.4167 × AFC; R^2= 0.462, $P < 0.001$).

To better elucidate the relationship between AMH and AFC, the two ovarian reserve markers were stratified into the following categories. The interquartile range (IQR) value of serum AMH concentration was 2.05-5.58 ng/mL, whereas which of AFC was 12-31. Patients were grouped according to whether their AMH and AFC values were in the lower quartile, upper quartile, or in between (Table 1).

Table 1 Grouping of the entire study subjects based on the interquartile range (IQR) values of serum AMH concentration(ng/mL) and AFC.

AMH	AFC			
≤ng/mL≤	≤12	12-31	≤31	
≤2.05	≤	≤	≤	
	124	49	5	178
2.05-5.58	≤	≤	≤	
	43	249	67	359
≤5.58	≤	≤	≤	
	0	76	101	177
	167	374	173	714

As shown in Table 1, subjects in □, □, and □ (474 out of 714, 66.39%) , had a concordance in AMH and AFC. In the rest 240 subjects (240 out of 714, 33.61%), those in □, □, and □ had AMH concentration higher than predicted(HTP) according to AFC (119 out of 714, 16.67%), while those in □, □, and □ had it lower than predicted(LTP) according to AFC (121 out of 714, 16.94%). Patients were then divided into three groups based on the concordance between AMH and AFC levels provided by Table 1:

Group A (Concordant): □ + □ + □ (AMH<2.05 ng/mL and AFC<12, AMH 2.05-5.58 ng/mL and AFC 12-31, AMH□5.58 ng/mL and AFC□12);

Group B (HTP): □ + □ + □ (AMH 2.05-5.58 ng/mL and AFC<12, AMH□5.58ng/mL and AFC≤31);

Group C (LTP): □ + □ + □ (AMH<2.05 ng/mL and AFC≥12, AMH 2.05-5.58 ng/mL and AFC□31).

The clinical and biochemical characteristics of subjects after grouping were presented in Table 2. As seen in Table 2, the three cohorts had similar age, infertile duration, FSH, Estradiol, PRL, LH, Testosterone, and CA125 levels.

Table 2 Comparison of clinical and biochemical characteristics of patients in different groups. P < 0.05 was considered statistically significant. Data were presented as n (%) or mean ± standard deviation (SD).

Variables	Group A (Concordant) (N=474)	Group B (HTP) (N=119)	Group C (LTP) (N=121)	Total (N=714)	P
Age (years)	30.07±4.59	29.54±4.08	29.29±4.43	29.85±4.49	0.059
BMI (kg/m^2)	22.65±3.43	22.56±3.46	23.81±3.87	22.83±3.54	0.015*
Infertile duration (years)	3.41±2.63	3.32±2.36	3.31±2.53	3.38±2.57	0.914
FSH (mIU/mL)	7.85±3.24	7.18±1.99	7.09±2.04	7.61±2.90	0.082
Estradiol (pg/mL)	51.89±32.24	52.29±20.15	50.57±22.82	51.73±29.06	0.367
PRL (pg/mL)	277.46±132.39	299.48±148.77	279.09±129.20	281.41±134.78	0.589
LH (pg/mL)	5.11±3.16	5.31±3.45	5.07±3.33	5.13±3.23	0.711
Testosterone (ng/mL)	0.54±2.52	0.68±2.72	0.43±0.15	0.54±2.33	0.512
CA125 (U/mL)	17.46±10.12	16.24±7.84	16.78±8.79	17.14±9.56	0.579
AFC	22.21±15.19	18.22±8.70	30.55±12.37	22.96±14.33	0.000*
AMH (ng/mL)	4.20±3.44	5.82±2.66	3.01±1.56	4.27±3.18	0.000*
Fully mature retrieved oocytes(n)	333 (70.25%)	74 (62.18%)	93 (76.86%)	500	0.045*

Interestingly, BMI (P=0.015) progressively increased from Group B(HTP) to Group A(Concordant) and to Group C(LTP) (from 22.56±3.46 kg/m^2 to 22.65±3.43 kg/m^2 and to 23.81±3.87 kg/m^2, P=0.015). The ratio of patients, who had completely mature oocytes (COM), rapidly decreased from Group C(LTP) to Group A(Concordant) and to Group B(HTP) (from 76.86% to 70.25% and to 62.18%, P=0.045).

According to BMI, general population is classified into five categories: underweight (BMI<18.5 kg/m^2), normal weight (BMI 18.5-24.9 kg/m^2), class I obesity-overweight (BMI 25.0-29.9 kg/m^2), class II obesity - obesity (BMI 30.0-39.9 kg/m^2), and class III obesity -extreme obesity (BMI≥40 kg/m^2). The range of BMI in this study sample was 14.90-36.10 kg/m^2. To get a better understanding of BMI-related AMH and AFC discordance, women were subdivided into the following groups on account of the above BMI classification criteria (Table 3, P=0.009). The upper numbers were the observed values, and the numbers in the brackets were the expected ones. As shown in Table 3, the observed value in Group A (concordant) was notably higher than the expected one when BMI≤24.9 kg/m^2 (BMI<18.5 kg/m^2 and BMI 18.5-24.9 kg/m^2), which means in this BMI region, concordant in AMH and AFC was more common. Concurrently,

the corresponding situation in Group B (HTP) generally occurred when BMI<18.5 kg/m^2 and BMI 25.0-29.9 kg/m^2, while in Group C (LTP), widely appeared when BMI≥25.0 kg/m^2 (BMI 25.0-29.9 kg/m^2 and BMI 30.0-39.9 kg/m^2).

Table 3 The number of patients in different groups sub-stratified according to BMI categories. P < 0.05 was considered statistically significant. Data were presented as n (%).

BMI classification (kg/m^2)	Group A (Concordant) (N=474)	Group B (HTP) (N=119)	Group C (LTP) (N=121)	Total (N=714)	P
Underweight <18.5	47 (43.2)	14 (10.8)	4 (11.0)	65	0.009
Normal weight 18.5-24.9	321 (311.4)	72 (78.2)	76 (79.5)	469	
Class I obesity overweight 25-29.9	92 (101.6)	30 (25.5)	31 (25.9)	153	
Class II obesity obesity 30-39.9	14 (17.9)	3 (4.5)	10 (4.6)	27	

Our previous studies showed that Group C (LTP) patients had the highest BMI, and meanwhile the supreme completely mature oocytes (CMO) rate. On the contrary, Group B(HTP) patients had the lowest BMI, and simultaneously the worst CMO rate. We proposed whether a lower BMI was accompanied by a worse CMO rate in different grouped specimens, and vice versa. Although no significant difference in CMO rate was observed among Group A, B, and C when stratified according to BMI (P=0.719, Table 4), CMO rate was the highest (72.31%) when BMI< 18.5 kg/m^2 in all BMI categories. The highest rate of CMO in Group A(Concordant) was seen when BMI< 18.5 kg/m^2 (72.34%), in Group B(HTP) when BMI≥ 25.0 kg/m^2 (100%), and in Group C(LTP) when BMI 18.5-24.9 kg/m^2 (81.58%).

Table 4 The ratio of completely mature oocytes in different groups sub-stratified according to BMI categories. P < 0.05 was considered statistically significant. Data were presented as n (%).

BMI classification (kg/m^2)	Group A (Concordant) (N=474)	Group B (HTP) (N=119)	Group C (LTP) (N=121)	Total (N=714)	P
Underweight □18.5	34/47 (72.34%)	11/14 (78.57%)	2/4 (50.00%)	47/65 (72.31%)	0.719
Normal weight 18.5-24.9	230/321 (71.65%)	41/72 (56.94%)	62/76 (81.58%)	332/469 (71.00%)	
Class I obesity□overweight□ 25-29.9	62/92 (67.39%)	19/30 (63.33%)	21/31 (67.74)	102/153 (66.67%)	
Class II obesity□obesity□ 30-39.9	7/14 (50.00%)	3/3 (100.00%)	8/10 (80.00%)	18/27 (66.67%)	

Discussion

It is important for patients undergoing assisted reproduction treatment to evaluate ovarian reserve precisely. Both AMH and AFC predict ovarian reserve independently and have been shown to correlate well, although AFC may have a higher inter-operator variability compared to AMH. However, it is quite common for IVF specialists to encounter serum AMH level that is higher or lower than expected according to AFC. It impels us to speculate which condition is favorable to patients and to decide what best to be done in cases of such discordance.

Our initial results showed a strong correlation between AMH and AFC, and then, we classified the two measurements into three intervals based on their respective lower and upper quartiles. The cut-offs for AFC were 12 and 31, and for AMH were 2.05 and 5.58 ng/mL, respectively. There was a high level of discordance (33.61% of cases) between AMH and AFC, and the incidence of HTP and LTP seemed similar (16.67% VS 16.94%). The further division demonstrated that BMI progressively increased from Group B(HTP) to Group A(Concordant) and to Group C(LTP). The ratio of patients, who had completely mature oocytes (COM), rapidly decreased from Group C(LTP) to Group A(Concordant) and to Group B(HTP). BMI classification ulteriorly authenticated the significance of BMI in the discordance between AMH and AFC. This urged us to speculate if Group B(HTP) had the worst ratio of completely mature oocytes (COM) was due simply to its lowest BMI. We found a negative correlation, although statistically nonsignificant, between BMI and completely mature oocytes rate altogether. The highest rate of completely mature oocytes in Group A(Concordant) could be seen when BMI < 18.5 kg/m^2(72.34%), in Group B(HTP) when BMI ≥ 25.0 kg/m^2(100%), and in Group C(LTP) when BMI 18.5–24.9 kg/m^2(81.58%).

There have been widely differing versions of the reasons for the discordance between AMH and AFC. The AFC refers to the number of follicles with a diameter of 2–10 mm in both ovaries and is a direct marker of

the recruitable follicular cohort(28). AMH is a dimeric glycoprotein produced by granulosa cells of pre-antral and small antral follicles, and it indirectly reflects the population of early growing follicles. These pre-antral and small antral follicles are not able to completely develop into maturity leading to oocyte being yielded(3). Thus, the AMH level is not exactly reflecting the population of antral follicles counted in our AFC measurement. Moreover, the intra- and inter-menstrual cycle AMH variations may contribute to discordance in some cases(29).

A negative correlation between AMH and BMI has been previously reported in late reproductive-age women(30), young women using oral contraceptive pills(31), and infertile women with (diminished ovarian reserve) DOR but not in women with normal ovarian reserve (NOR)(32). In our analysis, we have found a significant role of BMI in the discordance between AMH and AFC. Group A(Concordant) had a moderate BMI and a medium ratio of completely mature oocytes (COM). Group B(HTP) had the lowest BMI and meanwhile, the worst ratio of completely mature oocytes (COM) and Group C(LTP) was its opposite. But we could not jump to a conclusion that a patient with AMH concentration higher or lower than predicted according to AFC will have a worse or better ratio of completely mature oocytes owing to individual BMI.

Conclusions

In conclusion, approximately 33.61% of patients undergoing the first IVF therapeutic regime have discordance between AMH and AFC. Patients having a concordance in AMH and AFC have an intermediate ratio of completely mature oocytes and a moderate BMI. Those whose AMH concentration lower than predicted according to AFC have an obviously higher ratio, and their BMI are also above the average. The BMI is not an independent element that would influence the complete maturity of oocytes. This study is limited by its retrospective nature and the small number of patients, making it difficult to generalize results. Such theoretical consideration, however, is needed to be elucidated by future clinical and basic studies.

Declarations

Ethics approval and consent to participate

This study has been approved by the Ethics Committee of Affiliated Hospital of Nantong University.

Consent for publication

Not applicable.

Availability of data and materials

Datasets during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Yannan Chen, Xia Wang and Di Wang designed the study, conducted literature searches, selected articles for inclusion, performed all statistical analyses, and drafted the first draft of the manuscript. Xiaoling Gu, Xi Guan and Minyan Yu reviewed the study design, extracted data, planned the statistical analyses and drafted the first version of the manuscript. Panpan Yang and Shuping Zhong extracted data and assessed study quality. The manuscript has been revised and approved by all authors.

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Figures

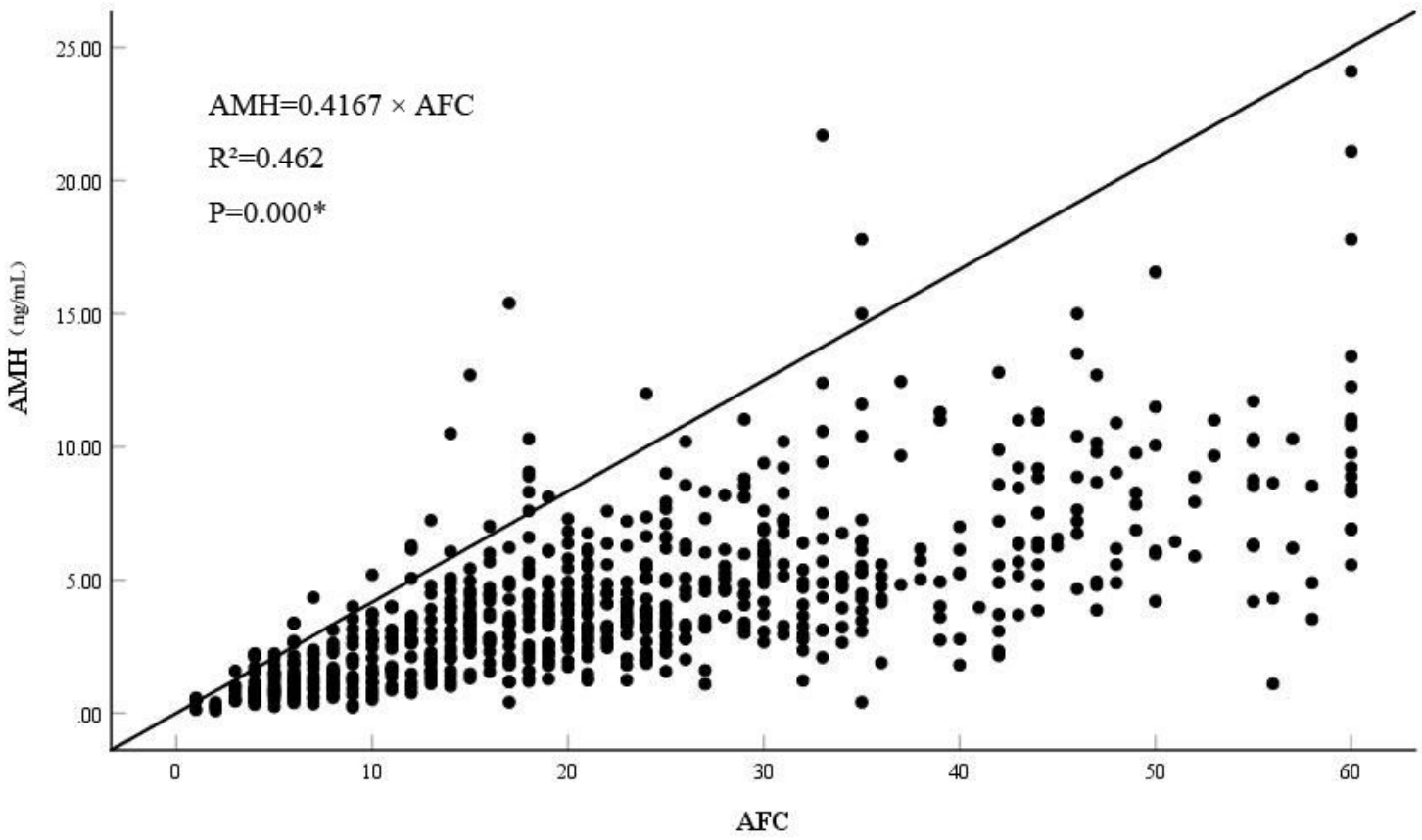

Figure 1

The relationship between serum anti-Müllerian hormone (AMH) concentration and antral follicle count (AFC) in the entire study population.