CORRELATION OF SERUM ANTI-MULLERIAN HORMONE LEVELS WITH MATURE OOCYTE COUNT AND BLASTOCYST FORMATION IN *IN VITRO* FERTILIZATION CYCLES

Do Ngoc Lan[1], Trinh The Son[1*]
Doan Thi Hang[1], Nguyen Minh Phương[1]

Abstract

Objectives: To evaluate the correlation between serum anti-Mullerian hormone (AMH) levels and the total number of oocytes, the proportion of mature oocytes, and the proportion of good-quality blastocysts. ***Methods:*** A retrospective observational study was conducted on 435 women (18 - 45 years of age) undergoing *in vitro* fertilization (IVF) at the Military Institute of Clinical Embryology and Histology from June 2023 to December 2024. Patients were divided into four groups: Women < 35 years old with low AMH (< 1.2 ng/mL, n = 60) and normal AMH (≥ 1.2 ng/mL, n = 119), and women ≥ 35 years old with low AMH (n = 96) and normal AMH (n = 160). ***Results:*** The antral follicles count (AFC) was significantly higher in the normal AMH group compared to the low AMH group (16.33 ± 6.24 vs. 8.83 ± 4.26, p < 0.001) among women < 35 years, and (12.92 ± 6.29 vs. 7.26 ± 3.46, p < 0.001) among women ≥ 35 years. The median number of mature oocytes (MII) was higher in the normal AMH group (8 vs. 4 oocytes) in women < 35 years, and (6 vs. 3 oocytes) in women ≥ 35 years. The median number of usable blastocysts differed significantly (p < 0.001) between low and normal AMH groups: 2 vs. 4 blastocysts in women < 35 years, and 1 vs. 3 blastocysts in women ≥ 35 years. However, the proportions of mature oocytes and blastocyst formation showed no significant differences across the groups. ***Conclusion:*** Serum AMH levels were positively correlated with the number of mature oocytes and usable blastocysts, but were not significantly associated with oocyte or blastocyst quality in IVF cycles.

Keywords: Anti-Mullerian hormone; *In vitro* fertilization; Intracytoplasmic sperm injection; Good-quality blastocyst; Mature oocytes.

[1]Military Institute of Clinical Embryology and Histology, Vietnam Military Medical University
[*]Corresponding author: Trinh The Son (trinhtheson@vmmu.edu.vn)

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INTRODUCTION

Ovarian reserve is a biological indicator that reflects the reproductive potential of women, expressed through the number and quality of remaining oocytes in the ovaries. Among the currently available biomarkers, AMH is considered to be a highly valuable marker for assessing ovarian reserve and predicting ovarian response in IVF cycles. AMH is one of the factors of the transforming growth factor-β superfamily, which is a glycoprotein secreted by granulosa cells of primordial and antral follicles [1]. Serum AMH levels show minimal variation during menstrual cycles but tend to decrease with age and have a well-established clinical value [2]. In addition, factors influencing the IVF outcome include maternal age, AFC, AMH, male factors, ovarian stimulation protocols, and embryo quality. Among these, evaluating ovarian reserve and predicting ovarian response are of critical importance. Several studies worldwide have demonstrated that serum AMH levels may reflect the "biological age" of the ovary, and serum AMH levels within the range of 1.66 - 4.52 ng/mL have been associated with better oocyte quality and higher blastocyst formation potential [3]. However, studies evaluating the association between AMH and blastocyst quality outcomes remain limited. Therefore, we conducted this study to: *Evaluate the correlation between serum AMH levels and the total number of oocytes, the proportion of mature oocytes, and the proportion of good-quality blastocysts.*

MATERIALS AND METHODS

1. Subjects

A total of 435 IVF cycles were included.

** Inclusion criteria:* Women aged 18 to 45 years. IVF treatment was conducted (antagonist protocol) at the Military Institute of Clinical Embryology and Histology; AMH testing was conducted within 12 months prior to the IVF cycle; the presence of good-quality blastocysts on day 5 or day 6.

** Exclusion criteria:* Patients with history of oophorectomy or amenorrhea; patients with thyroid disorders; oocyte donation cycles; infertility due to male factor, including surgically retrieved sperm, donor sperm, or severe oligozoospermia (< 5 million/mL).

- Patients with polycystic ovary syndrome (PCOS).

** Study location and time:* The study was carried out at Military Institute of Clinical Embryology and Histology between June 2023 and December 2024.

2. Methods

** Study design:* A retrospective observational study.

** Study variables:*

Grouping: Age groups: < 35 years old and ≥ 35 years old [4]; AMH groups: Low AMH (< 1.2 ng/mL); Normal AMH (≥ 1.2 ng/mL) [5].

Study parameters: AMH concentration.

Baseline characteristics: Age, BMI, type of infertility, duration of infertility, AFC, sperm concentration.

IVF outcomes: Number of follicles, number of retrieved oocytes, number of

mature oocytes (MII), maturation rate (MII/total oocytes), number of 2PN (two-pronuclear) embryos, fertilization rate (2PN/MII), number of usable blastocysts, blastocyst formation rate (usable blastocysts/2PN), number of good-quality blastocysts (classified according to Munné et al, 2019) [6].

* *Data analysis:* Data were collected from medical records and analyzed using Stata version 17. Normally distributed variables were expressed as $\overline{X}$ ± SD, while non-normally distributed variables were expressed as median [IQR]. Descriptive statistics were used for qualitative and quantitative variables. Mean values were compared using Student's T-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. Proportions were compared using the Chi-squared test. Scatter plots with Pearson's correlation coefficient (R) were applied to evaluate correlations.

3. Ethics

The study was approved by the Ethics Council of Military Institute of Clinical Embryology and Histology, Vietnam Military Medical University. The data used in this study were collected and analyzed with the approval of Military Institute of Clinical Embryology and Histology. The authors declare that there are no conflicts of interest related to this study.

RESULTS

1. Baseline characteristics of patients

Table 1. Baseline characteristics of AMH groups.

Characteristics	Age < 35 years		p	Age ≥ 35 years		p
	Low AMH (n = 60)	Normal AMH (n = 119)		Low AMH (n = 96)	Normal AMH (n = 160)	
AMH median [IQR]	0.81 [0.51 - 1.03]	3.81 [2.59 - 4.99]	< 0.001	0.76 [0.41 - 1.00]	2.42 [1.68 - 3.58]	< 0.001
Age	31.07 ± 2.97	30.47 ± 2.74	0.184	39.90 ± 3.03	37.54 ± 2.87	< 0.001
BMI	21.51± 2.01	21.12 ± 2.30	0.263	21.93 ± 2.25	21.83 ± 2.44	0.743
Infertility type n, (%)			0.029			0.251
Primary	27 (45)	34 (28.57)		17 (17.71)	20 (12.5)	
Secondary	33 (55)	85 (71.43)		79(82.29)	140 (87.5)	
Duration of infertility (years)	3.13 ± 2.28	3.25 ± 2.08	0.728	4.93 ± 4.03	4.38 ± 3.22	0.228
AFC	8.83 ± 4.26	16.33 ± 6.24	< 0.001	7.26 ± 3.46	12.92 ± 6.29	< 0.001
Sperm concentration ($x10^6$/mL)	52.17 [25.61 - 78.26]	50 [31.22 -74.27]	0.708	59.61 ± 35.95	53.86 ± 30.39	0.173

The mean age and AFC were significantly higher in the normal AMH group ($p < 0.001$), whereas the mean age differed significantly only in women aged ≥ 35 years.

2. Ovarian stimulation response: Number and quality of oocytes and blastocysts

Table 2. Ovarian stimulation outcomes.

Characteristics	< 35 years		p	≥ 35 years		p
	Low AMH (n = 60)	Normal AMH (n = 119)		Low AMH (n = 96)	Normal AMH (n = 160)	
Total follicles, median [IQR]	5.5 [4 - 8]	13 [10 - 18]	< 0.001	4 [2 - 6]	9.5 [7 - 13]	< 0.001
Total retrieved oocytes, median [IQR]	5 [3 - 8]	10 [7 - 14]	< 0.001	3 [2 - 5]	8 [5 - 12]	< 0.001
MII oocytes, median [IQR]	4 [2.5 - 6]	8 [5 - 11]	< 0.001	3 [2 - 4]	6 [4 - 10]	< 0.001
MII rate (%)	271/347 (78.10%)	1022/1308 (78.13%)	0.988	303/363 (83.47%)	1181/1455 (81.17%)	0.311

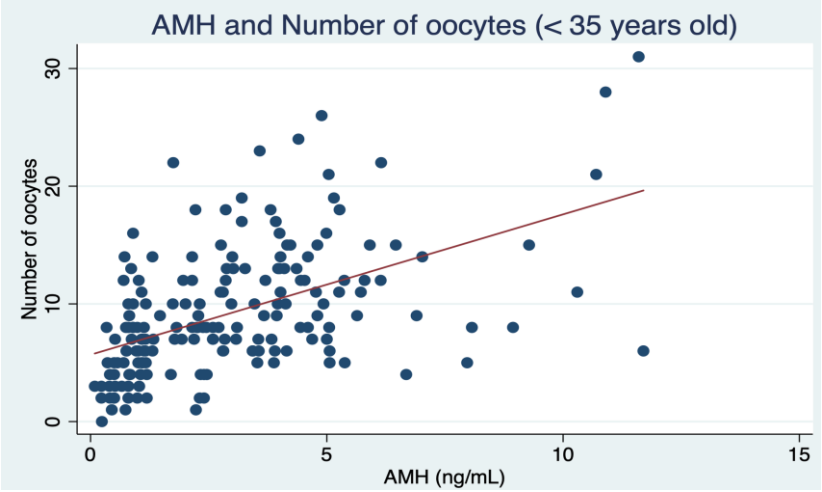
The total number of follicles, retrieved oocytes, and mature MII oocytes was significantly higher in the normal AMH group compared with the low AMH group ($p < 0.001$). However, the MII maturation rate showed no significant difference across the groups.

Table 3. IVF outcomes.

Characteristics	< 35 years		p	≥ 35 years		p
	Low AMH (n = 60)	Normal AMH (n = 119)		Low AMH (n = 96)	Normal AMH (n = 160)	
2PN embryos median [IQR]	3 [2 - 4.5]	5 [4 - 9]	< 0.001	2 [1 - 3]	5 [3 - 7.5]	< 0.001
Fertilization, n (%)	200/271 (73.80)	755/1022 (73.87)	0.980	220/303 (72.61)	874/1181 (74.00)	0.622
Usable blastocysts, median [IQR]	2 [1 - 3]	4 [2 - 6]	< 0.001	1 [0 - 2]	3 [1 - 5]	< 0.001
Blastocyst formation n (%)	133/200 (66.50)	479/755 (63.44)	0.423	119/220 (54.09)	505/874 (57.78)	0.323
Good-quality blastocysts, median [IQR]	1 [0 - 2]	1 [0 - 3]	0.001	0 [0 - 1]	1 [0 - 2]	< 0.001
Good-quality blastocysts n (%)	64/133 (48.12)	224/479 (46.76)	0.782	48/119 (40.34)	226/505 (44.75)	0.382

The normal AMH group had a significantly higher number of blastocysts and good-quality blastocysts compared with the low AMH group ($p < 0.001$). However, there was no significant difference in the proportion of good-quality blastocysts across the groups.

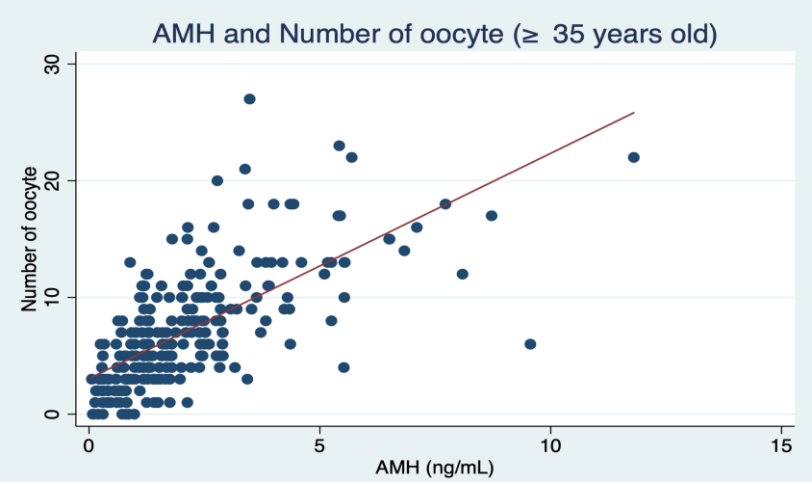
3. Correlation between AMH and IVF outcomes



y = 1.1931x + 5.6775, R = 0.5124, p < 0.001

Figure 1. Correlation between AMH and the number of oocytes (< 35 years).

The figure clearly demonstrates a significant positive correlation between AMH levels and the number of retrieved oocytes (R = 0.5124; p < 0.001). As serum AMH concentration increases, the number of oocytes obtained also increases proportionally.



y = 1.9301x + 3.0496, R = 0.6838, p < 0.001

Figure 2. Correlation between AMH and the number of oocytes (≥ 35 years).

The figure shows a significant positive correlation between the number of mature oocytes and serum AMH levels.

DISCUSSION

Our study was conducted on 435 IVF cycles, stratified by age (≥ 35 and < 35 years) and serum AMH levels (< 1.2 ng/mL and ≥ 1.2 ng/mL) [5]. The mean number of retrieved oocytes was significantly higher in the normal AMH group in both age categories. Similarly, the number of mature oocytes and good-quality blastocysts also showed significant differences across the groups, favoring those with normal AMH levels. Linear regression analysis revealed a moderate positive correlation between AMH and the number of oocytes ($R = 0.5124$; $p < 0.001$), with regression equation $y = 1.1931x + 5.6775$. Our findings are consistent with the report by Heidary et al. (2024), in which a strong correlation between serum AMH levels and the number of MII oocytes (rho = 0.624, $p < 0.001$) was observed [3]. However, our results indicated that AMH was not a reliable predictor of oocyte or blastocyst quality. This contrasts with Heidary's findings which demonstrated significant correlations between AMH and top-quality blastocysts (types A and AB; rho = 0.419 and 0.446, $p < 0.001$) [3]. This divergence may be attributed to differences in sample size, patient selection criteria (our study excluded PCOS patients), and embryo grading, Munné (2019) criteria. Conversely, Dai et al. (2020) in a study on 492 IVF/ICSI cycles in women ≥ 37 years of age, concluded that AMH level was not predictive of oocyte quality or blastocyst developmental potential, despite its correlation with the number of retrieved oocytes and MII oocytes [7]. This finding is consistent with our results in women ≥ 35 years, suggesting that biological age may exert a stronger influence on oocyte and embryo quality than AMH levels in older patients. This also explains the differences observed between age groups (< 35 and ≥ 35 years) in our dataset. Although many studies support AMH as a reliable predictor of oocyte yield, its role in predicting embryo quality remains inconclusive. A randomized clinical trial conducted by Munné et al (2019) demonstrated that embryo selection by preimplantation genetic testing for aneuploidy (PGT-A) did not improve cumulative pregnancy rates compared with morphological selection, except for women aged 35 - 40 years. This highlights the importance of embryonic genetic status (euploidy/aneuploidy) in determining pregnancy outcomes in addition to AMH. Overall, our study provides further evidence that AMH reflects ovarian reserve and oocyte yield. However, its predictive value for oocyte competence and good-quality blastocyst formation remains limited. Discrepancies among studies may be attributable to variations in patient age, population characteristics, exclusion criteria, and embryo assessment methods. However, our study has certain limitations. Its retrospective design inherently

restricts the ability to establish causal relationships. In addition, only univariate analyses were performed; applying multivariate regression models could more accurately delineate the independent effect of serum AMH on the studied reproductive outcomes.

CONCLUSION

Serum AMH levels show a positive correlation with the number of mature oocytes and usable blastocysts, but not with oocyte or blastocyst quality in IVF cycles.

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