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Original Article

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The Association of AMH Level with the Number and Quality of Oocytes in Women Undergoing IVF/ICSI: A Single-Center Study

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Abstract

Background: The recognized role of Anti-Müllerian hormone (AMH) as a marker for women's biological age and ovarian reserve prompts debate on its efficacy in predicting oocyte quality during IVF/ICSI. Recent findings challenging this view compelled us to conduct this study to examine the correlation between AMH levels and quantity/quality of oocytes in IVF/ICSI procedures.

Methods: The data were collected retrospectively from the medical records of 320 women between 25-42 years old. The included patients were divided into two groups: the high AMH group (>1.1 *ng/ml*) and the low AMH (=<1.1 *ng/ml*) group. The high AMH group comprised 213 patients, while the low AMH group consisted of 107 patients. Spearman's correlation coefficient and Multinomial logistic regression were computed to assess the relationships between different variables.

Results: Significant positive correlations were detected between AMH level and the number of aspirated follicles (rho=0.741, p<0.001), retrieved oocytes (rho=0.659, p< 0.001), M2 oocytes (rho=0.624, p<0.001), grade A embryos (rho=0.419, p<0.001), and grade AB embryos (rho=0.446, p<0.001. In contrast, AMH levels had negative associations with the number and duration of cycles (p<0.05). AMH emerged as a statistically significant independent predictor of the number of M2 oocytes.

Conclusions: Serum AMH level could represent the quantity and quality of oocytes following IVF/ICSI treatments. Future studies should aim to delve deeper into the correlations between AMH levels and both the quality and quantity of embryos. Additionally, it would be beneficial to consider the influence of sperm factors, as well as assess pregnancy rates.

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Introduction

Approximately 10% of couples at reproductive age require in vitro fertilization (IVF) as an assisted reproductive technology (ART) option; however, only 5% of oocytes in an ART cycle can result in successful pregnancy (1). IVF and intracytoplasmic sperm injection (ICSI) are the most common ART treatments, with a 40-60% chance of embryo implantation (2). Several independent factors have been introduced to predict IVF/ICSI outcome, including the total number of good-quality embryos, total number of embryos, age, antral follicle count (AFC), and the number of oocytes and their quality (3-5). Additionally, the number of 10–14 *mm* follicles could affect the

cumulative outcome of IVF/ICSI as a marker of oocyte quality. Morphological assessment of oocytes influences pregnancy success rate (6). Furthermore, the impact of embryo quality on the cumulative outcome of IVF/ICSI positively correlates with age, especially in women over the age of 40 (3).

So far, the need for finding a suitable and accurate marker to reflect ovarian reserve and subsequently predict pregnancy has been growing. Anti-Müllerian hormone (AMH) is a peptide growth factor produced by the granulosa cells of ovarian follicles (7). Serum AMH level can reflect the biological age of women as the driving factor for ovarian follicles to produce oocytes; it has been observed that AMH levels can provide insights into ovarian function earlier than both follicle-stimulating hormone (FSH) levels and antral follicle count (AFC) (8). Although AMH levels do not change significantly throughout the menstrual cycle (9), they tend to decrease steadily with advancing age. The AMH level of 1.66-4.52 *ng/ml* is associated with good-quality oocytes, which can result in the development of higher-quality embryos (10). However, the cut-off values of AMH for the prediction of poor response are yet to be determined. Previous studies revealed a positive correlation between the AMH level and the oocyte quality and quantity in IVF/ICSI cycles, regardless of age (11, 12). Higher levels of AMH in animal models also indicated better oocyte collection and a higher number of embryos following fertilization (13). However, some clinical studies reported that in an IVF cycle, AMH level could not predict the oocyte quality in women below the age of 40 years (14). Therefore, the role of AMH as a predictor of oocyte quality in ART remains controversial (15).

Furthermore, elevated AMH level was associated with an increased rate of clinical pregnancy in women over 40 who underwent IVF/ICSI treatment (16). The purpose of the current study was to evaluate AMH level as a marker of oocyte quality during ART cycles.

Methods

Study design: The serum level of AMH and its association with the quality and quantity of oocytes and embryos were evaluated retrospectively in 320 women between 25 and 42 years old who underwent IVF/ICSI at Vali-E-Asr Infertility Clinic affiliated to Tehran University of Medical Sciences (Tehran, Iran). The data was collected from patients' medical records. The patients were scheduled for 523 IVF/ICSI cycles from October 2019 to November 2021. Baseline serum AMH concentrations were measured by ultrasensitive ELISA (Beckman-Coulter Ireland Inc., Ireland) with a functional sensitivity of 0.2 *ng/ml* and intra and inter-assay coefficient of variability of 8 and 12%, respectively.

The study included women who had no prior history of ovarian surgery and no exposure to cytotoxic drugs or pelvic radiation therapy. Patients with a history of endometriosis, unilateral oophorectomy, pelvic radiotherapy, or chemotherapy, as well as those with allergies or contraindications to oral estradiol or progesterone treatment, were excluded from the study.

The first cycle characteristics in each patient were recorded, including the total duration of the cycle, the total number of aspirated follicles and retrieved oocytes, the assessment of oocyte quality, the total number of embryos, and embryo quality. The total number of cycles for each person was also determined.

Oocyte quality was morphologically assessed based on the maturation of the oocyte nucleus, distinguishing between metaphase 2 (M2) as mature oocytes, metaphase 1 (M1) as intermediate mature oocytes, and germinal vesicle (GV) as immature oocytes. The embryos were categorized as A and AB (good), B (fair), and C (poor) based on criteria including the number of cells, degree of fragmentation, and similarity indices of the embryo.

Based on the serum level of AMH, the included patients were divided into two groups: the high (H) AMH group (AMH >1.1 *ng/ml*) and the low (L) AMH (AMH=<1.1 *ng/ml*) group. The optimal AMH cut-off of 1.1 *ng/ml* (sensitivity 89% and specificity 35%) was selected based on the findings of a previous study conducted by Harris et al. (17). According to the study conducted by Zhang et al. (18), the number of mature oocytes (M2) was divided into three groups: low (0-2 M2 oocytes), intermediate (3-6 M2 oocytes), and high (≥7).

This study was approved by the Medical Ethics Committee of Tehran University of Medical Sciences (No. IR.TUMS.IKHC.REC.1400.108) following the Declaration of Helsinki. The Medical Ethics Committee of Tehran University of Medical Sciences waived the requirement for informed consent due to the retrospective nature of the study.

Stimulation protocols

Antagonist protocol: After daily administration of gonadotropins at a dose of 150-225 *IU* from cycle-day 2/3 for five days, the GnRH antagonist (flexible) protocol was initiated, while closely monitoring the follicle size of the patients. Subcutaneous administration of the GnRH antagonist (0.25 *mg* cetrorelix) (Cetronax; Ronak Pharmaceutical Co., Iran) was initiated when follicular size reached 13-14 *mm*. HCG was administered after at least one follicle reached a mean size of 17-19 *mm*.

Progestin-primed ovarian stimulation (PPOS) protocol: PPOS protocol was performed by administering gonadotropin and Duphaston (20 *mg/day* dydrogesterone; Abbott Laboratories, USA) from cycle day two until the dominant follicle diameter reached 17-19 *mm*. Then, HCG was injected to trigger ovulation.

Long protocol: The long GnRH agonist protocol commenced with the administration of an oral contraceptive on menstruation day five, alongside 50 international units *(IU)/day* of GnRH agonist starting from cycle-day 21 (1 *mg/1ml* of buserelin, Cinafact; Cinnagen, Iran). Following the onset of menstruation on cycle day 2, daily administration of human menopausal gonadotropin (hMG, Merional; IBSA, Switzerland) was initiated, with the dose adjusted based on the patient's age and medical history. Concurrently, the GnRH agonist dose was reduced to 20 *IU/day*. Subsequently, after a transvaginal ultrasound, when follicles reached 17-19 *mm* in size, 10000 *IU* of human chorionic gonadotropin (hCG; Pooyesh Darou, Iran) injection was administered intramuscularly, and GnRH agonist was discontinued.

Minimal stimulation protocol: In minimal stimulation, patients received 100 *mg/day* clomiphene citrate (Iran Hormone Pharmaceutical Co., Iran) on days 3-7 of the cycle for five days, followed by 150 *IU/day* gonadotropin on days 7 and 8. When at least one follicle reached a mean diameter of 14 *mm*, a 0.25 *mg/day* GnRH antagonist was initiated. When the mean diameter of the follicle reached 17-19 *mm*, HCG was administered for the maturation of follicles. Thirty-six to forty *hr* after each stimulation, oocyte retrieval was performed.

Egg retrieval: The oocytes were denuded using hyaluronidase immediately after puncture. If the interval between hCG injection and retrieval was less than 36 *hr*, the cumulus-oocyte complexes were incubated for a maximum of two *hr* before injection to facilitate cytoplasmic maturation. Subsequently, the quality of the oocytes was assessed.

Embryo development: The commercial IVF Culture Media were utilized for embryo culture, and the embryos were maintained at a temperature of 37°*C* in an atmosphere containing 5% CO_2 and 20% O_2. The embryos were transferred to the recipient 3-5 days after preparation. The evaluation of the embryos followed the standards described by Tesarik et al. (19).

Statistical analysis: Statistical analysis was performed using SPSS version 26 (SPSS Inc., USA) and GraphPad Prism version 8.0 (GraphPad Software, USA). Continuous data were first examined by the normality and log normality test, and variables with abnormal distribution were reported as the median and interquartile range (IQR). Qualitative variables were expressed as numbers (percentages). To compare the groups, statistical analysis was performed using the Mann-Whitney U test and Chi-Square test. Spearman's rank correlation coefficient and multinomial logistic regression were computed between different variables. The $p<0.05$ were considered statistically significant.

Results

Population characteristics: In this study, 320 patients were evaluated with a median (IQR) age of 35.0 (30.0-38.7) years. The total basal AMH level was 2.3 (0.7-4.5) *ng/ml* in the patients. The AMH level was negatively correlated with the age of participants [rho=-0.444, 95% CI (-0.5308 to -0.3492); $p<0.001$]. More than half of the patients were diagnosed with female factor infertility (55.0%), followed by male factor infertility (20.4%), and a combination of both (15.1%). Moreover, 70.8% (223 of 315 participants) had primary infertility. History of abortion and live birth was reported in 47 (14.7%) and 51 (16.3%) patients, respectively. To obtain a comprehensive view, the patients were divided into low AMH (L) and high AMH (H) groups and their results were compared. One hundred seven patients were categorized as L group and 213 as H group. The participants in the H group were remarkably younger than the L group [34.0 (29.0-37.0) *vs.* 38.0 (34.0-40.0) years, ($p<0.001$)]. Body mass index (BMI) was not significantly different between the two groups ($p=0.747$). The duration of infertility was longer in the H group, with 4.0 (2.0-7.0) years,

compared to the L group with 2.0 (1.0-5.0) years (p=0.001). The history of abortion was reported in 17 (15.9%) and 30 (14.1%) patients in the L and H groups, respectively (p=0.667). History of live birth was present in 18 (17.3%) and 33 (15.8%) patients of the L and H groups, respectively (Table 1).

Cycle characteristics: A total of 523 IVF/ICSI cycles were evaluated in 320 patients. The maximum number of IVF/ICSI cycles in a single patient was six in our study. A hundred and ninety-three women (60.3%) performed one cycle of oocyte retrieval, 75 (23.4%) did two cycles of oocyte retrieval, 35 (10.9%) did three cycles of oocyte retrieval, 12 (3.8%) did four cycles of oocyte retrieval, 2 (0.6%) did five cycles of oocyte retrieval, and 3 (0.9%) did six cycles of oocyte retrieval. Among 474 IVF/ICSI cycles for which data on the stimulation protocol was available, 256 (54.0%) cycles utilized antagonist protocol. The next most common protocol was PPOS with dydrogesterone, which was used in 158 (33.3%) cycles. The minimal protocol was employed in 21 (4.4%) cycles. These findings are summarized in table 1.

Correlation between AMH level and oocyte quality and embryo developmental parameters in the first cycle: Significant positive correlations were observed between AMH level and the first cycle characteristics, namely the number of aspirated follicles (rho=0.741, p<0.001), retrieved oocytes (rho=0.659, p<0.001), M2 oocytes (rho=0.624, p< 0.001), M1 oocytes (rho=0.409, p<0.001), GV oocytes (rho=0.337, p<0.001), total embryos (rho=0.577, p<0.001), grade A embryos (rho= 0.419, p<0.001), grade AB embryos (rho=0.446, p<0.001), and grade B embryos (rho=0.356, p< 0.001). On the other hand, there was a negative correlation between AMH level and the total number of performed cycles (rho=-0.255, p<0.001). After adjusting for age, significant correlations remained, although with lower rho levels (Table 2).

Multinomial logistic regression was used to analyze the relationship between AMH level, age, and M2 oocytes. The model exhibited a good overall fit (likelihood ratio test p<0.001). AMH (β=0.509, SE=0.080, Wald=40.933, p<.001) and age (β=-0.099, SE=0.028, Wald=12.481, p<0.001) were significant predictors of number of M2 oocytes between low and high M2 groups. An increase of one unit in AMH level was associated with a 1.663-fold increase in the number of M2 oocytes (95% CI: 1.423–1.944). In addition, when comparing low and intermediate M2 groups, AMH was also a significant predictor (β=0.297, SE=0.074, Wald=16.043, p<0.001) with a lower Exp(B) value of 1.346 (95% CI:1.164–1.556). These results suggest that AMH is an important predictor of M2 oocyte level regardless of age.

Comparison of low AMH and high AMH group: The median number of IVF/ICSI cycles in the H

Table 1. Baseline characteristics of the women in high and low AMH groups

Characteristics	Total (n=320)	H group (n=213)	L group (n=107)	p-value
Age (y), median (IQR)	35.0 (30.0-38.7)	34.0 (29.0-37.0)	38.0 (34.0-40.0)	**<0.001**
BMI, median (IQR)	25.9 (23.5-28.8)	26.0 (23.5-28.6)	25.7 (23.4-29.3)	0.747
Type of infertility (n=296)				
Primary, n (%)	223 (70.8)	151 (71.6)	72 (69.2)	0.668
Secondary, n (%)	92 (29.2)	60 (28.4)	32 (30.8)	
Duration of infertility, median (IQR)	3.0 (1.5-7.0)	4.0 (2.0-7.0)	2.0 (1.0-5.0)	**0.001**
History of abortion, n (%)	47 (14.7)	30 (14.1)	17 (15.9)	0.667
Prior live birth, n (%)	51 (16.3)	33 (15.8)	18 (17.3)	0.732
Stimulation protocols (n=319)				
Antagonist, n (%)	191 (59.9)	129 (60.8)	62 (57.9)	0.617
PPOS, n (%)	129 (40.4)	85 (40.1)	44 (41.1)	0.860
Long, n (%)	26 (8.2)	18 (8.5)	8 (7.5)	0.755
Minimal, n (%)	18 (5.6)	2 (0.9)	16 (15.0)	**<0.001**
Others, n (%)	9 (2.8)	2 (0.9)	7 (6.5)	**0.008**
AMH level, median (IQR)	2.3 (0.7-4.5)	3.5 (2.3-5.8)	0.4 (0.2-0.8)	**<0.001**

BMI: Body mass index; n: Number of patients; PPOS: Progestin-primed ovarian stimulation; AMH: Anti-Müllerian hormone

Table 2. Correlations between AMH level with oocyte and embryo number and quality before and after adjustment for age

Variables	Before adjustment		After adjustment	
	rho	p-value	rho	p-value
Number of follicles	0.741	<0.001	0.583	<0.001
Number of oocytes	0.659	<0.001	0.555	<0.001
Number of M1 oocytes	0.409	<0.001	0.301	<0.001
Number of M2 oocytes	0.624	<0.001	0.503	<0.001
Total number of embryos	0.577	<0.001	0.423	<0.001
Number of grade A embryos	0.419	<0.001	0.306	<0.001
Number of grade AB embryos	0.446	<0.001	0.283	<0.001
Number of grade B embryos	0.356	<0.001	0.365	<0.001

M1: Metaphase 1; M2: Metaphase 2; n: Number of patients

Table 3. Cycle characteristics and developmental indices of women in high and low AMH group

Variables	Total (n=320)	H group (n=213)	L group (n=107)	p-value
Number of follicles, median (IQR)	6.0 (3.0-9.0)	8.0 (5.0-13.0)	3.0 (2.0-5.0)	<0.001
Number of oocytes, median (IQR)	4.0 (2.0-8.0)	6.0 (4.0-11.0)	2.0 (1.0-4.0)	<0.001
Number of M1 oocytes, median (IQR)	1.0 (0-1.0)	1.0 (0-1.0)	0.0 (0-1.0)	<0.001
Number of M2 oocytes, median (IQR)	3.0 (1.0-7.0)	5.0 (3.0-8.0)	1.0 (1.0-3.0)	<0.001
Total number of embryos, median (IQR)	3.0 (1.0-6.7)	5.0 (3.0-8.0)	2.0 (1.0-3.0)	<0.001
Number of grade A embryos, median (IQR)	1.0 (0-2.0)	1.0 (0-2.0)	0 (0-1.0)	<0.001
Number of grade AB embryos, median (IQR)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	1.0 (0.5-2.0)	<0.001
Number of grade B embryos, median (IQR)	1.0 (0-2.0)	1.0 (0-3.0)	0.0 (0-1.0)	<0.001

M1: Metaphase 1; M 2: Metaphase 2; n: Number of patients. The median is shown along with 25th and 75th percentiles

group was notably lower than the L group [1.0 (1.0-2.0) *vs*. 2.0 (1.0-3.0), (p<0.001)]. Significant differences were observed in all of the analyzed IVF parameters between patients with low and high AMH levels (p<0.001, table 3). The number of aspirated follicles, total retrieved oocytes, M1, M2, and GV oocytes, and the total number of embryos and grade A, B, and AB embryos were significantly higher in the H group than in the L group (p<0.001) (Table 3).

Discussion

Recently, AMH has received significant attention as a promising indicator for assessing ovarian reserve and predicting pregnancy outcomes. In this study, AMH level and its association with the number and quality of oocytes were investigated in 320 women aged 25-42 who underwent ART. As anticipated (20, 21), a moderate negative correlation between AMH level and the age of participants was observed. In addition, AMH expression had significant positive relationships with the number of total retrieved oocytes, oocytes in different developmental states, total embryos, and good to fair-quality embryos. It was also found that AMH level is an independent significant predictor of M2 oocytes. This correlation of AMH level with the quality and quantity of oocytes and embryos has been consistently demonstrated in previous studies (11, 22, 23). However, none of the previous articles surveyed all developmental levels of oocytes to embryos. On the contrary, few studies exhibited that AMH cannot predict oocyte quality (14).

After categorizing patients based on their AMH level, no difference was detected in the BMI, type of infertility, history of abortion, and live birth. On the other hand, age and the duration of infertility were comparable between the groups. Unlike most studies (23, 24), Ghomian et al. reported the duration of infertility was longer in patients with higher AMH levels (25). However, this duration remained less than five years in both groups, with the highest success rate observed in ICSI group (26). Patients in the H group underwent fewer cycles and patients in the L group underwent a

minimal stimulation protocol, which is considered a mild and a patient-friendly approach. Additionally, the correlation of AMH level with the quality and quantity of oocytes and embryos was further confirmed in the pairwise comparison of our two groups. Importantly, although the significant impact of different stimulation protocols on oocytes number and quality is still disputable (27, 28), it is crucial to account for and control this effect in future studies. Strong correlations were observed between different parameters of oocyte and embryo, including the total number of follicles, total number of oocytes, M2 oocytes, embryos, and grade AB embryos. However, these correlations are not independent of AMH expression level.

In contrast to some studies (20, 29), there was no remarkable difference in the number of transferred embryos between the H and L groups. It is worth noting that the prevailing concept of increasing the probability of pregnancy by transferring a greater number of embryos has been challenged by recent studies (30, 31). Sun et al. claimed that despite AMH's role in developing good-quality embryos, it does not necessarily reflect the likelihood of clinical pregnancy (32). But according to a study conducted by Sahmay et al. (33), clinical pregnancy rates increase as AMH increases. This limitation is acknowledged in our study, since chemical and clinical pregnancy rates were not assessed.

Another crucial point to note is the influence of male factors in the development of high-quality embryos. While our primary focus was on investigating the impact of female factors on oocyte quality, a relationship between high AMH levels and the presence of good-quality embryos was observed. It is important to note that this observation was made without accounting for potential male factors that could influence embryo quality. This omission limits our ability to establish the exact underlying relationship and determine the specific contribution of male factors to the observed association between high AMH levels and good-quality embryos.

One limitation of our study is the absence of data on pregnancy rates in the patients. Therefore, future studies should thoroughly investigate not only the pregnancy rate but also the quality and quantity of oocytes and embryos to elucidate the role of AMH in IVF/ICSI outcomes. Furthermore, sperm and other male factors were not evaluated in the analysis of embryo quality assessment. It is crucial to emphasize that the primary focus of our study was to assess oocyte quality rather than embryo quality.

Conclusion

AMH level seems to be a reasonable marker in predicting the quantity and quality of oocytes following IVF/ICSI treatment. AMH level was inversely correlated with the age of participants, and those with higher AMH levels tended to undergo fewer IVF/ICSI cycles. Strong correlations were also present between different parameters of oocytes and embryos, including the total number of follicles, oocytes, MII oocytes, embryos, and good-quality embryos. According to our study, both AMH and age can independently predict the quantity of high-quality oocytes. Future studies should aim to delve deeper into the correlations between AMH levels and both the quality and quantity of embryos. Additionally, it would be beneficial to consider the influence of sperm factors as well as assess chemical and clinical pregnancy rates.

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Conflict of Interest

The authors declare no competing interests.

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