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ORIGINAL ARTICLE

Relationship between follicular fluid and serum anti-Mullerian hormone levels and pregnancy rate in ART cycles

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KEYWORDS
Anti-Mullerian hormone;
Oocyte quality;
ART success rates

Abstract
Objective: This study is aimed at determining the serum and follicular fluid level of anti-Mullerian hormone, its quality, and relationship between anti-Mullerian hormone level with assisted reproductive technology (ART) success rates.
Method: In 50 infertile patients who underwent the ART cycle, follicular fluid and serum levels of anti-Mullerian hormone were measured on oocyte retrieval day. Patients were then divided into two groups of fertile and non-fertile.
Results: Pregnancy test result was positive in 13 and negative in 37 patients. Serum and follicular fluid level of anti-Mullerian hormone was higher among the first group than the second group ($P < 0.05$). Mean follicular fluid level of anti-Mullerian hormone was 5.12 and 4.23 among the first and second groups, respectively. Mean serum anti-Mullerian hormone level was 1.76 in the first group and 1.49 in the second group.
Conclusions: Higher level of anti-Mullerian hormone in serum and follicular fluid has a significantly positive correlation with the success rate in ART cycles and oocyte quality; therefore, anti-Mullerian hormone can be a positive predictor of ART success cycles.
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Abbreviations: WHO, World Health Organization; IVF, in vitro fertilization; AMH, anti-Mullerian hormone; ART, assisted reproductive technology; BMI, body mass index.

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PALABRAS CLAVE
Hormona antimülleriana;
Calidad de los ovocitos;
Tasas de éxito de las técnicas de reproducción asistida

Relación entre los niveles de hormona antimülleriana en suero y líquido folicular y la tasa de embarazo en ciclos de técnicas de reproducción asistida

Resumen
Objetivo: Este estudio tiene como objetivo establecer los niveles de la hormona antimülleriana en suero y líquido folicular, su calidad y la relación entre el nivel de hormona antimülleriana y las tasas de éxito de las técnicas de reproducción asistida (TRA).
Método: Se midieron los niveles de hormona antimülleriana en suero y líquido folicular el día de recuperación de los ovocitos de 50 pacientes infértiles en que se realizó el ciclo de TRA. Se dividió a las pacientes en 2 grupos: fértiles e infértiles.
Resultados: El resultado de la prueba de embarazo fue positivo en 13 pacientes y negativo en 37. El nivel de hormona antimülleriana en suero y líquido folicular fue mayor en el primer grupo que en el segundo ($p < 0.05$). El nivel medio de la hormona antimülleriana en el líquido folicular fue de 5.12 y 4.23 en el primero y segundo grupo, respectivamente. El nivel medio de la hormona antimülleriana en suero fue de 1.76 en el primer grupo y de 1.49 en el segundo grupo.
Conclusiones: Un nivel más elevado de la hormona antimülleriana en suero y líquido folicular tiene una correlación muy positiva con la tasa de éxito en los ciclos de TRA y la calidad de los ovocitos; por tanto, la hormona antimülleriana puede ser un factor pronóstico positivo de los ciclos de éxito de las TRA.
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Summary of relevance

General criticisms:

Few studies dealt with association between follicular fluid level of AMH and oocyte quality, ART success rates, as well as correlation between serum and follicular fluid level of AMH.

What is already known:

Higher level of AMH in serum has a significantly positive correlation with the success rate in ART cycles.

What this paper adds:

Serum AMH level is an appropriate reflection of its follicular fluid level. Higher level of AMH in the follicular fluid revealed a better quality of the obtained oocytes. Follicular fluid AMH level can be a positive predictor of ART success cycles.

Introduction

According to the World Health Organization (WHO), the clinical definition of infertility is the inability to get pregnant after one year of natural and persistent sexual intercourse without prevention. According to the studies, about 50–80 million people around the world experience some form of infertility during their life.[1] Today, modern assisted reproductive technologies have revolutionized the evaluation and treatment of infertility.

First and still the commonest, modern assisted reproductive technique is called in vitro fertilization (IVF) assisted a large number of infertile couples to experience a successful pregnancy.

IVF consists of a completely synchronized sequence of actions which begin with severe and controlled stimulation of ovary by exogenous gonadotropins. Oocyte retrieval will then occur from ovaries under trans-vaginal ultrasound guidance. After IVF, the embryos will be transferred into the uterus as trans-cervical. Chance of IVF success depends on several factors, most of which cannot unfortunately be identified before the advanced stages of the treatment cycle or even near the end of the treatment. Success of these methods can be predicted by various indicators; the major ones include: Mother's age, the ovarian reserve status, and the previous reproductive capability. Ovarian reserve is described as the size and the quality of follicular reserve of remaining ovarian. There are different sonographic and biochemical methods to measure it. An appropriate method for measuring the ovarian reserve is the measurement of the anti-Mullerian hormone (AMH) level.[2]

AMH is secreted from antral and small pre-antral follicles of ovary into the follicular fluid. In female infant, the serum AMH level is immeasurable but its level increases until puberty and pubarche time. Its level in the serum remains constant in the entire reproductive period of a woman. Also, its level does not depend on gonadotropin and its changes are negligible between two menstrual cycles and during the menstrual cycle.[3–6]

Anti-Mullerian hormone in follicular fluid has a direct autocrine–paracrine effect on granulosa cells, the quality of oocytes and embryo.[7,8]

In previous studies, its lesser extent is accompanied with the poor response to the ovarian stimulation and also with reducing the quality of the oocytes, embryo quality and the pregnancy rate.[9] Such relationship has not been seen about any of the hormones as an indicator of ovarian reserve.[10] Several studies have examined AMH level in serum and its relationship with successful pregnancy rates in assisted reproductive technology (ART) cycles. However, few studies dealing with its rate in the follicular fluid as an environment in which the oocytes grow and evolve, oocyte quality, and ART success rate.[10]

Purpose of this study is to evaluate anti-Mullerian hormone level in the follicular fluid and the serum of the infertile person and its relationship with the success of assisted reproductive techniques. Considering an almost 40% success rate for the assisted reproductive procedure (IVF) and the enormous cost that infertile couples pay, it seems essential to provide a series of methods for predicting this procedure's success rate before starting the treatment.

Materials and methods

In this cross-sectional study, 50 patients who were candidates for IVF or ICSI treatments were selected. They had already referred to the infertility center of Ahwaz Imam Khomeini Hospital.

Inclusion criteria were an age of less than 35, a body mass index (BMI) below 25, the presence of both ovaries, menstrual cycles of 25–35 days, normal hormonal status, and a lack of other hormonal disorders such as thyroid disease and hyper prolactinemia. Exclusion criteria were a history of failed treatment in the ART cycles, endometriosis and the history of pelvic surgery, history of chemotherapy and serum FSH level higher than 10 IU/L. After approving the study by the research ethics committees, written informed consents were obtained from all the patients.

All the patients were treated under an agonist (short or long) or an antagonist. The follow-up of patients was performed by trans-vaginal sonography and by one individual. HCG was prescribed for all patients when at least 3 follicles reached a size of more than 18 mm in diameter and transvaginal oocyte retrieval was done 36 h later. On the day of oocyte retrieval, the follicular fluid samples obtained from the first follicle were collected without being contaminated with blood. Samples of 5 cc serum were also collected.

In all patients, progesterone was prescribed in the form of tablets from the day of oocyte retrieval, suppositories or injections for luteal phase support.

Oocyte maturity

Oocyte maturity was examined under condition of the corona radiata and the cumulus granulosa cells, as described previously.[11] Mature oocytes includes oocyte-corona-cumulus complexes with a large and loose cumulus and distinct corona radiata, but complexes with a small and dense cumulus and opaque corona radiata were defined as immature.

Oocyte quality

Oocyte quality was estimated by assessing granulosa cells under a fluorescence microscope following fixation and staining with Hoechst 33258 fluorescent dye (0.5 mg/mL in distilled water; Japan). Oocyte quality was scored based on the incidence of apoptotic granulosa cells with condensed/fragmented nuclei, as described previously.[12]

Detection of oocyte's prognosis

Oocytes were observed under a dissecting microscope 20 h after IVF to determine the number of pronuclei. Oocytes with two pronuclei were distinguished as fertilized oocytes and cultured for an additional 24 h. After culture period, the embryos were assessed morphologically, as described previously.[13] The embryos with equal blastomeres and no fragmentation have a good quality.

Embryo transfer and pregnancy detection

Embryos were transferred in the second to the fifth day after oocyte retrieval in different numbers and grades for each patient. In this study, the criterion of being pregnant was positive BHCG serum 14 days after embryo transfer.

Blood and follicular fluid samples were centrifuged on the day of oocyte retrieval at 3000 rpm for 10 min and were then stored at −21 °C until all samples were collected. After the complete collection of samples, follicular fluid and serum AMH levels were measured by Ansh Labs kit, the product of Germany, and by the ELISA method. Quantitative variables were reported as mean and standard deviation and the qualitative variables were reported as frequency and relative percentages using the SPSS software (version 23).

Statistical analysis

T-test was used to compare the quantitative variables and Chi-square test was used to compare qualitative variables. In cases where the distribution was not normal, non-parametric tests were used. In this study, the level of significance was considered less than 0.05.

Role of the funding source

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Results

From among the patients referring to the infertility center of Ahwaz Imam Khomeini Hospital in 2016, fifty people who had the inclusion criteria were selected. The age range of patients was 17–35 and the mean age was 29 (Table 1). Among them, 42 patients (84%) had primary infertility and 8 patients (16%) had secondary infertility (Table 2).

Patients were divided into two groups based on the pregnancy test results; the first group included 13 patients (26%)

Table 1 Average age distribution of fertile and infertile women under study.

Standard deviation	Mean	Number	Pregnancy test results
2.93	30.46	13	Positive
4.29	28.64	37	Negative

Table 2 Frequency distribution of infertility variable in women under study.

Percentage frequency	Frequency	Infertility variable
84	42	Primary
16	8	Secondary
100	50	Total

with a positive pregnancy test and the second group comprised 37 patients (74%) with a negative pregnancy test.

In all the patients (both groups with positive and negative pregnancy tests), anti-Mullerian hormone level in follicular fluid and serum were positively correlated with each other: in the patients with higher levels of anti-Mullerian hormone in serum, AMH level in follicular fluid was also higher. This correlation was stronger in the first group which was statistically significant [*P* value <0.05] (Table 3). In the first group, the average of anti-Mullerian hormone level in the serum and follicular fluid had no significant difference with the second group (5.12 versus 4.23 for follicular fluid and 1.77 versus 1.50 for serum) (Table 4).

AMH level in follicular fluid had a direct relationship with the quality of oocytes in both groups: in the patients with the higher hormone level in follicular fluid, the quality of oocytes was also better. This relationship was statistically significant (*P* value <0.05) (Table 5). AMH level in serum had a direct relationship with the quality of oocytes in both groups. Put differently, in the patients with the higher hormone level in serum, the quality of oocytes was also better. This relationship was statistically significant (*P* value <0.05) (Table 5).

In both groups, there was not any relationship between AMH level in follicular fluid and serum and the number of obtained oocytes (Table 6).

Discussion

Briefly, The AMH levels in follicular fluid and serum were positively correlated with each other in all patients. In this study, a positive relationship was observed between the quality of obtained oocytes and the AMH level in follicular fluid and serum. No positive relationship was observed among the AMH level of serum and follicular fluid, the number of oocytes, and the chance of pregnancy.

Over the past decade, several studies have been conducted in this field. Relevant studies in England,[14] United States,[15] France,[16] Japan,[17] Turkey,[18] Kuwait,[19] and Iran[20] regarding the relationship between AMH and the quality of oocytes have all been in agreement with our study.

There was a positive relationship between serum AMH level and the quality of oocytes in a meta-analysis performed in China[21] but the results were ambiguous about follicular fluid. However, the results about serum AMH level were consistent with our results. Unlike our study, no such relationship was observed regarding the quality of oocytes in the studies in the Netherlands[22] and Egypt.[23] There was no positive relationship between the number of obtained oocytes and the AMH level in our study where the results were similar to the study in the Netherlands.[24] Unlike the findings of our study, a positive relationship has been obtained in a number of studies[15,19,25–27] on this subject.

In our study, there was no relationship between the AMH level in serum and follicular fluid from one hand and the pregnancy rate after ART on the other. In this regard, our results were consistent with those of the studies in Denmark,[28] the Netherlands,[22] and Italy.[27] In a study carried out in Egypt,[23] no relationship was observed between serum AMH level and the pregnancy rate. However, a positive relationship was observed between the follicular fluid AMH level and the pregnancy rate, which is different from the results of our study.

Inconsistent with what the researchers obtained in this study, the studies in London,[14] United States,[27] Greece,[12] France,[15] Kuwait,[19] and Iran[29] reveal that there is a positive relationship between the pregnancy rate and the AMH level.

On the other hand, a study in India showed that the lower level of AMH will be with the higher chance of pregnancy. This, of course, is not consistent with the findings of our study and the other studies.

Conclusion

This study showed that serum AMH level is an appropriate reflection of follicular fluid AMH level. In other words, serum assessment can be a replacement for measuring AMH in follicular fluid for diagnostic purposes. Our study, like most other studies, highlights that there is a positive relationship between the AMH levels of serum and follicular fluid and the quality of oocytes observed in all the patients.

Since infertility is a multifactorial problem and the quality of oocytes is one of the vital factors in the results of ART cycles, other causes of infertility have also contributed to no achievement of pregnancy despite the acceptable quality of oocytes in the present study. Small size of samples has also affected the results. Finally, although we did not achieve a positive relationship between fertility rate and AMH level, due to the fact that there is an obvious relationship between AMH level and the quality of oocytes and the quality of collected embryos which have a high potential for implantation, we can be hopeful that the AMH level can be used as a strong predictor of the quality of the obtained oocytes and, consequently, a potential for reproduction in ART cycles.

It is recommended that further studies be done in a larger sample size to achieve stronger results. Of course, due to the small sample size, it was not possible to determine the threshold for anti-Mullerian hormone. More extended studies can hopefully help determine a threshold for AMH to be used in predicting the success of ART cycles.

Table 3 Correlation between the variables of the average AMH level in follicular fluid and the average AMH in serum in two groups of women under study with positive and negative pregnancy tests.

P-value	Pearson correlation coefficient	Number	Variable	Group
<0.001	0.78	37	Correlation of anti-Mullerian hormone in the follicular fluid with the serum	Women with the negative pregnancy test
<0.001	0.86	13	Correlation of anti-Mullerian hormone in the follicular fluid with the serum	Women with the positive pregnancy test

Table 4 Correlation between the variables of the average AMH level in follicular fluid and serum and the pregnancy test results in women under study.

P-value	Standard deviation	Average	Number	Pregnancy test results	Variable
0.56	6.48	5.12	13	Positive	Anti-Mullerian hormone in follicular fluid
	3.85	4.32	37	Negative	
0.71	3.14	1.77	13	Positive	Anti-Mullerian hormone in serum
	1.82	1.50	37	Negative	

Table 5 Correlation between the variable of the average AMH level in follicular fluid and blood serum and the quality of oocytes in women under study.

Pearson correlation coefficient		Number	Variable
0.035	0.30	50	Correlation of anti-Mullerian hormone in follicular fluid with the quality of oocyte
0.002	0.44	50	Correlation of anti-Mullerian hormone in blood serum with the quality of oocyte

Table 6 Relationship between the variables of the average AMH level in follicular fluid and blood serum and the number of oocytes in women under study.

Pearson correlation coefficient		Number	Variable
0.67	0.06	50	Correlation of anti-Mullerian hormone in follicular fluid with the oocyte number
0.96	0.007	50	Correlation of anti-Mullerian hormone in blood serum with the oocyte number

Conflict of interest

This work has received research grant support from Ahvaz Jundishapour University of Medical Sciences. All other authors report no conflicts of interest relevant to this article.

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