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Prediction of the number of oocytes based on AMH and FSH levels in IVF candidates

IVF adaylarında AMH ve FSH seviyelerine göre oosit sayısının tahmini

Nur Dokuzeylül Güngör [1], Tuğba Gürbüz [2]

[1] Department of Gynecology and IVF Clinic, Bahcesehir University Göztepe Medical Park Hospital, Istanbul, Turkey
[2] Department of Gynecology and Obstetrics, Medistate Hospital, Istanbul, Turkey

ORCID ID of the author(s)
NDG: 0000-0002-7234-3876
TG: 0000-0003-3555-3767

Corresponding author/Sorumlu yazar:
Tuğba Gürbüz
Address/Adres: Rüzgarlıbahçe Mahallesi. Cumhuriyet Caddesi. No: 24, Medistate Hastanesi, Beykoz, İstanbul, Türkiye
E-mail: drtgurbuz@hotmail.com

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Abstract

Aim: The purpose of this study was to predict the number of oocytes using anti-Mullerian hormone (AMH) and Follicle Stimulating Hormone (FSH) levels in In Vitro Fertilization (IVF) candidates.

Methods: This retrospective cohort study included 121 (23 cases with poor response, 92 cases with normal response, and 6 cases with excessive response) infertile patients. We examined the relationship between AMH and FSH levels and the number of oocytes. The method used in this study was to consider the number of oocytes as the response variable and identify the effective factors. All analyses were performed with free R software.

Results: The results show that although the ovarian response is associated with serum AMH levels and the women's age (P=0.04 and P<0.001, respectively), the negative linear and binomial regression yield a significant collinearity and the natural logarithm of AMH alone can be a good estimate of the ovarian response (P<0.001). ROC curves in logistic regression were used to determine cutoff points. The results of this regression showed that the natural logarithm of AMH with a cutoff value of 1.36 ng/ml can determine the line between the poor and normal ovarian response (P<0.01). The natural logarithm of AMH cannot determine the cutoff value of normal and excessive ovarian response (P>0.05).

Conclusion: The use of serum AMH levels is important for the prediction of poor ovarian response.

Keywords: Anti-Mullerian hormone, Ovarian response, Oocytes

Öz

Amaç: Bu çalışmanın amacı IVF tedavisi planlanan olgularda serum AMH ve FSH düzeylerini kullanarak oosit sayısını tahmin etmektir.

Yöntemler: Bu retrospektif kohort çalışma 121 infertil hastayı (düşük over rezervi (n=23), normal over rezervi (n=92) ve yüksek over rezervi (n=6)) kapsamaktadır. Çalışmada AMH, FSH ve oosit sayıları arasındaki ilişki değerlendirilmiştir. Oosit sayısı değişken yanıt olarak kabul edilip etkileyen faktörler tanımlanmıştır. Tüm analizler ücretsiz R software ile yapılmıştır.

Bulgular: Sonuçlar, over cevabının serum AMH seviyeleri ve ayrıca kadınların yaşı (sırasıyla P=0,04 ve P<0,001) ile ilişkili olmasına rağmen, negatif doğrusal ve binom regresyonunun, anlamlı bir eşdoğrusallık ve tek başına AMH'nin doğal logaritmasının olduğunu gösterdiğini göstermektedir ve over cevabı için iyi bir tahmini olabilir (P<0,001). Kesim noktalarını belirlemek için lojistik regresyondaki ROC eğrileri kullanılmıştır. Bu regresyonun sonuçları, 1,36 ng/ml'lik bir kesme değerine sahip doğal AMH logaritmasının, zayıf ve normal yumurtalık tepkisi arasındaki sınırı belirleyebileceğini gösterdi (P<0,01). AMH'nin doğal logaritması, normal ve aşırı over cevabının kesim değerini belirleyemez (P>0,05).

Sonuç: AMH özellikle düşük over rezervinin tahmininde predikte edici önemli bir faktördür.

Anahtar kelimeler: Anti-Mülleryan hormon, Over cevabı, Oosit

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Introduction

Infertility, a problem related to the reproductive abilities of humans and a global public health challenge [1], can cause enormous treatment costs, personal distress, and sometimes ostracism and discrimination [2]. The prevalence of infertility has significantly increased recently [3]. Epidemiological investigations have demonstrated the prevalence of infertility experienced by couples was 8 to 12% worldwide [4]. However, infertility involved 13% of the female population [5]. Some procedures, particularly In Vitro Fertilization (IVF), have significantly contributed to the success of fertility aids. IVF, an assisted reproductive technology (ART), is used to cure infertility in females [6]. One of the crucial factors affecting IVF success is the number of oocytes produced by the ovary in response to hormonal stimulation [7]. In other words, IVF success is limited by poor ovarian response, which is observed in 10 to 15% of women undergoing IVF [8].

Achieving competent quality oocytes is one of the essential parameters of IVF [19]. Pregnancy rates in IVF are related to the number of oocytes [10]. The fertilization method also affects the number and the quality of embryos obtained from in vitro maturation of human oocytes [11]. Lack of fertility or low fertility in the IVF cycle is due to the hormone levels achieved through ovulation induction protocols [12]. In ART, success depends on obtaining large numbers of high-quality eggs [13]. Gonadotropin hormones such as Follicle-Stimulating Hormone (FSH) primarily regulate menstrual cycles in women. Infertile women with high FSH levels have a poor response to ovulation [14]. FSH stimulates the conversion of androgen to estrogen in granulosa cells by enzymes of the aromatase system [15].

Some studies have identified ovarian volume and antral follicles as a way of determining the number of oocytes. FSH, serum inhibin B levels, and Antral Follicle Count (AFC) levels show the number of oocytes [16]. Anti-Müllerian Hormone (AMH), which is produced by the ovarian granulosa cells and disappears during menopause, has been recently used to predict ovarian response [17]. These functions include inhibition of primary follicle utilization, FSH-dependent growth inhibition, and selection of perinatal follicles and small antral follicles [18].

AMH is used to evaluate fertility potential and ovarian response in IVF due to the relationship between serum levels of AMH and the number of primary antral follicles [17]. According to Chang HJ et al., ideally determining ovarian reserve involves measuring AMH levels, due to better sensitivity and specificity. In the early stages of ovarian reserve depletion, Inhibin-B, FSH and estradiol levels are low. Then the serum levels of these three hormones undergo changes late in the reproductive process when the ovarian reserve reaching a critical level reduces the chance of pregnancy [20]. However, serum levels of AMH are independent of the menstrual cycle and are not affected by GnRH agonists or oral contraceptives [21].

The follicular response to gonadotropin stimulation also decreases due to reduced ovarian reserve [22]. Results of the IVF are strongly dependent on ovarian stimulation with gonadotropins and ovarian response [23]. The purpose of this study is to predict the number of oocytes using AMH levels and FSH levels in IVF candidates.

Materials and methods

This retrospective study was conducted between November 2014 and September 2019 at the Gynecology and IVF Department. The procedures were conducted in accordance with the regulations established by the Clinical Research and Ethics Committee and the Helsinki Declaration of World Medical Association. The study was carried out with the permission of Research Ethics Committee of Beykoz University (Permission granted/CAAE number. 2020/8.1, Decision no: 1). Signed informed consents were obtained from all patients. The study included 121 infertile patients (23 cases with poor response, 92 cases with a normal response, and 6 cases with excessive response).

We considered the number of oocytes as the response variable to find the effective factors i.e. AMH and FSH; and our covariates included maternal age and body mass index (BMI). The number of oocytes was thoroughly and precisely analyzed once quantitatively, and then qualitatively.

We ran three different models, logistic regression, linear regression, and negative binomial regression, to see which one fits better with our dataset. In the logistic regression model, response variables were defined as poor response, normal response, and excessive ovarian response. Regarding multiple regression, these responses were considered continuous variables. The response variable included the number of oocytes released in the period to be studied based on the negative binomial model. In the case of logistic regression, we recorded our response at three distinct levels. Patients were divided into three groups in which normal ovarian response contained 6-15 oocytes, the poor ovarian response contained 5 or less oocytes, and excessive ovarian response contained >15 oocytes.

Statistical analysis

Statistical analysis was performed with free R software. $P<0.05$ was considered statistically significant. To calculate the relationship between the ovarian number and AMH and FSH, we applied the Pearson correlation test because our response variable was not normal. To compare the different results of the oocyte in terms of the distribution of variables, we applied ANOVA with the following multiple comparisons. We used the Pearson correlation test to find the relationship between the number of ovarian responses, AMH and FSH.

Results

Analysis of variance (ANOVA)

Results of the ANOVA model on the variables in the study show that there is a significant difference among three groups in terms of AMH and women's age at a probability level of 5% ($P=0.04$, and $P<0.001$, respectively). Based on these results, FSH values in the poor response group were not significantly higher than those in the normal and excessive response groups ($P=0.48$). However, the AMH value also showed a significant difference between two poor and excessive response groups but none of them showed a significant difference from the normal group. In this research, the ages of the women were equal between the poor and normal response groups and the age of the excessive response group was significantly lower than that of the two other groups (P=0.04) (Table 1).

Negative binomial and linear regression

To evaluate the relationship between the studied variables and Ovarian Response, two negative binomial and linear multivariate regression models were used. In both models, the independent variables were included in the model based on the AIC value and finally, the significant variables remained in the model. On this basis, variables of the mother's age, AMH, and the natural logarithm of AMH remained in the linear regression model. The highest coefficient rate of the R-square relates to the natural logarithm of AMH, which could elaborate 24% of the changes in the ovarian reserve in the model. AMH and age of the women have been considered responsible for 4% and 2.5% of the changes. Variance Inflation Factor (VIF) below 5 for the variables indicates the weak collinearity. In the negative binomial model, both variables of AMH and normal logarithm of AMH remained in the model, with the difference being that the P-value of AMH in the negative binomial model is significantly lower than that of the linear regression model (Table 2). The Penalized B-spline regression model in Figure 1 also shows predictability of the ovarian response to the natural logarithm of AMH which first shows an increasing and then a decreasing slope.

ROC and Cut-off for both models indicate that AUC in the poor ovarian response is 0.796 and the Cutoff is 1.36 ng/ml based on the crossover of sensitivity and specificity against probability. At this point, the sensitivity and specificity of the model are 78% and 76%, respectively (Figure 2).

Table 1: Comparing the mean of the research variables in poor, normal, and excessive response groups (different letters indicate significant difference at a probability level of 0.05)

Variable	Ovarian Response		
	Poor Response (n=23)	Normal Response (n=92)	Excessive Response (n=6)
FSH (mIU/ml)	9.62(2.67^{a})	7.04(2.47^{b})	5.64(0.75^{b})
AMH (ng/ml)	1.42(1.35^{b})	2.77(2.24^{ab})	3.33(0.82^{a})
Age (y)	32.52(3.9^{a})	30.88(4.04^{a})	26.3(3.67^{b})
BMI (kg/m^2)	23.83(1.85^{a})	25.32(5.27^{a})	25.5(4.46^{a})

FSH: Follicle Stimulating Hormone, AMH: Anti-Mullerian Hormone, BMI: Body Mass Index

Table 2: Results of the stepwise method in the linear regression modeling and negative binomial regression model

Model	Variables	Coefficients					
		Estimate	Std. Error	Partial R-square	VIF	P-value	Model R-square
Multiple Regression	Age	-0.132	0.064	0.025	1.08	0.04	0.31
	AMH	-0.62	0.25	0.04	4.49	0.015	
	Ln AMH	3.47	0.71	0.244	4.63	<0.001	
	FSH	0.166	0.233	-	5.9	0.48	-
Negative Binomial Regression	AMH	-0.131	0.038	-	4.49	<0.001	-
	Log AMH	0.69	0.118		4.63	<0.001	
	FSH	0.031	0.033	-	5.9	0.35	-

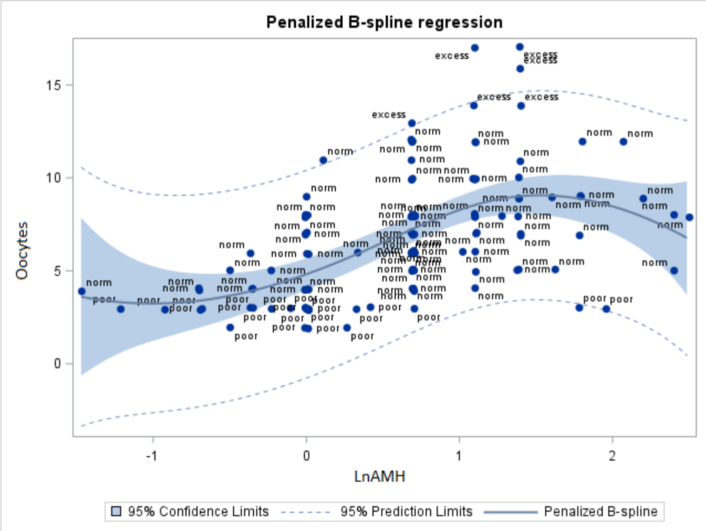

Figure 1: Penalized B-spline regression model in prediction of the ovarian response based on the natural logarithm of AMH

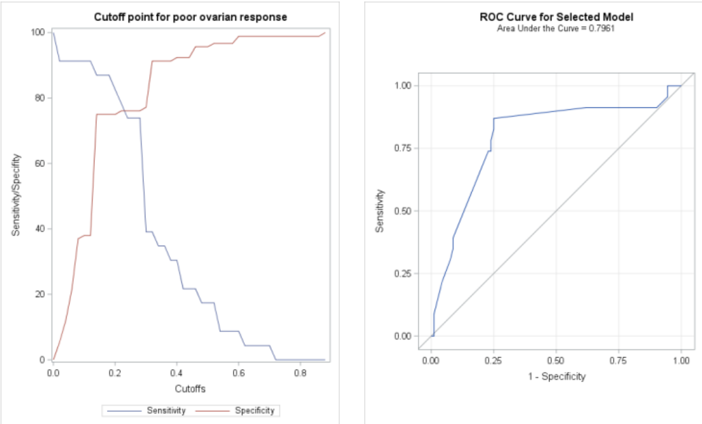

Figure 2: ROC for prediction of poor ovarian response using the AMH natural logarithm indicating a sensitivity and specificity of 78% and 76%, respectively with an optimal cut-off of 0.31 and AUC of 0.796

Discussion

The results of the current study demonstrated that every unit decrease in the log scale of AMH increased the value by 6.7% for the poor response group compared to the normal group. The optimal cut-off point of AMH for predicting poor response was 1.36 ng/ml, and the area under the ROC curve was 0.796. This finding revealed that AMH could be a useful marker to distinguish between poor ovarian response and the normal response levels with the cut-off point of 1.36 ng/ml of AMH blood levels with an area under the curve of 80%. The result of the negative binomial regression model demonstrated that one unit increase in the log of AMH blood levels increased the odds of releasing an oocyte by 50%.

Besides, based on the negative binomial regression model, FSH has a significant effect on the response so that every unit of increase in FSH increases the odds of a single response unit by 3%. Therefore, FSH levels had a significant effect on the number and quality of fertile oocytes. In most assisted reproductive procedures, basal FSH levels are often used as prognostic factors for ovarian function.

Different studies have investigated the correlation between AMH and FSH levels and the quality and number of oocytes in fertile and infertile women. Some of these studies will be reviewed to study the correlation between these two hormones and the quality of ovulation in women.

In women of reproductive age, the granulosa cells of the prenatal and small antral follicles are responsible for AMH secretion. As mentioned previously, AMH regulates ovarian response and follicular steroidogenesis. Hence, AMH can be used as a valuable predictor of ovarian response [24]. In a study conducted by Xiao-dong et al. [24] evaluating the levels of AMH in women of reproductive age, all participants were divided into three groups based on their age: Group A (24-30 years), group B (31-35 years) and group C (36-41 years). The study showed a negative correlation between age and AMH (r=-0.416, $P<0.05$), so that the AMH serum levels in women of older childbearing age are lower (2.10 (1.61)) than the women of middle childbearing age (3.52 (2.17)) and women of lower childbearing age (5.81 (3.98)). Another valuable result of this included that the number of infertile women with lower AMH serum levels (<1.1 ng/mL) was significantly higher than that of the fertile women ($P<0.05$) [24].

There were some methods for ovarian stimulation before IVF in infertile women [25]. GnRH agonist long protocol is one of these approaches used for evaluating the ovarian response and decreasing the potential bias in a study on the Iranian infertile women. In this study, Heidar et al. [26] evaluated ovarian stimulation using AMH serum levels. A positive pregnancy test was found in 30 % of patients. One unit increase in the AMH log scale has led to a 64% decrease in the odds ratio of having a poor response in comparison with the normal response (OR 0.36, 95% CI 0.19–0.68). Besides, every unit increase in the AMH log scale increased the odds of releasing an oocyte by 24% (OR 1.24, 95 % CI 1.14–1.35) [26].

A study by Aydın et al. [27] in patients undergoing ICSI showed that all women received the long protocol. The results yielded that 32 women had a good response (72.7%) and 12 women had poor responses (27.3%) and the serum level of AMH in poor and good responses group was significantly different ($P<0.01$). To evaluate the cut-off point of AMH, ROC analysis was used, demonstrating that the cut-off point of AMH was 1.90 ng/ml and women with good response had higher AMH serum levels (>1.90 ng/ml). Also, the age of patients with >1.90 ng/ml AMH serum levels was lower (31.47 (3.77)) than that of patients with <1.90 ng/ml AMH serum levels (33.67 (3.93)); but this difference was not significant ($P=0.09$) [27]. Our results show that AMH can be an applicable predictor of the number of oocytes.

The predictive role of FSH and LH serum levels on the third day of the menstrual cycle was demonstrated in Iranian women undergoing ART. According to the results, there is a significant correlation between FSH serum levels and the number of oocytes ($P=0.041$), the number of metaphase II oocytes ($P=0.049$), and pregnancy ($P=0.017$) but the optimum effect was found in patients with FSH: 10-15 miu/Ml. Besides, there was no significant correlation between LH serum levels and the mentioned variables, but optimum effects were observed in patients with LH $\geq$ 8 [28].

Applicability of FSH and AMH were independently demonstrated in different studies for the evaluation of IVF success, while some others evaluated the discordant and concordant combinations of AMH and FSH measurements to predict ovarian responses in women undergoing ART. Buyuk et al. [29] evaluated the correlation between AMH and ovarian responses in women with elevated early follicular FSH levels. Results of the study found a significant correlation between AMH serum levels and the number of retrieved oocytes (r=0.55). Patients with ≥0.6 ng/mL AMH serum levels had 11 (1.3) retrieved oocytes, while patients with ≤0.6 ng/mL AMH serum levels had 5.6 (0.6) retrieved oocytes. There is no significant correlation between AMH serum levels and the age of participants. Besides, clinical pregnancy in patients with ≥0.6 ng/mL AMH serum levels was insignificantly higher than that among women with ≥0.6 ng/mL serum levels (28% vs. 14%), ($P=0.1$) [29].

Ligon et al. [30] evaluated discordant and concordant values of AMH and FSH on live birth rate and IVF cycle cancellation rate. The live birth rate of patients with normal AMH and elevated FSH was higher than those of patients with low AMH and normal FSH (39% vs. 26%). The live birth rate in patients with normal AMH and normal FSH (concordant) was higher than in any other group (44%). Besides, the IVF cycle cancellation rate in patients with normal AMH and FSH was lower than that of other groups (4%) and this rate was higher in patients with elevated FSH and low AMH compared to other groups (30%) [30].

Limitations

The main limitation of our study is the low number of patients. Retrospective design analyses are among other limitations of this study. Further studies with larger patient cohort are needed to derive conclusions supported by statistical analysis.

Conclusion

Our study results and other reviewed studies show that there is a significant correlation between AMH serum levels and the quality of ovarian responses (particularly the number and quality of oocytes). The rate of FSH varies in distinct groups of ovarian response and there is a significant relationship between FSH and ovarian response rate but predicting ovarian response rate using FSH was not successful. Besides, AMH levels vary in different groups of ovarian responses, and there is a significant relationship between AMH and ovarian response. AMH can be used to predict poor ovarian response. The age of women, despite having significant differences in different groups of ovarian responses, has not been successful in predicting poor ovarian responses.

FSH level is not a good predictor of poor ovarian response. Also, it is not possible to correctly predict ovarian reserve. Therefore, further studies should be conducted on both FSH and AMH variables and age classification to evaluate the ovarian responses in infertile patients.

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