CEOG Clinical and Experimental Obstetrics & Gynecology

The predictor markers of ovarian response in poor responders under 40 years of age

B. Vural[1], Y. Çakıroğlu[1], F. Vural[2]

[1] Kocaeli University Faculty of Medicine, Department of Obstetrics and Gynecology, Kocaeli
[2] Haydarpaşa Numune Teaching Hospital, Obstetrics and Gynecology Clinic, Istanbul (Turkey)

Summary

Objective: To explore the ovarian reserve markers in predicting ovarian response and pregnancy rates in poor responder patients undergoing in vitro fertilization (IVF). *Material and Methods:* A total of 140 women < 40 years with poor ovarian response (POR), who underwent IVF were included in the study. The clinical findings compared with normal responder controls (n= 250). Regression analysis was used to search the correlation between the number of the total oocyte count retrieved and independent variables as age, FSH, LH, AMH, AFC, and E2 on the hCG day. *Results:* AUC ROC curve were AMH 0.804, AFC 0.701, E2 on hCG day 0.786, FSH 0.705, LH 0.527, and E2 0.479, age 0.707, respectively. E2 levels on hCG day and AMH levels were independent markers of POR. None of the factors were predictor of pregnancy rate. *Conclusion:* The serum E2 levels on the hCG day and AMH levels predict ovarian response, but not pregnancy rates.

Key words: In vitro fertilization; Anti-Müllerian hormone; Poor ovarian response; Cycle cancellation; Ovarian reserve; Pregnancy rate.

Introduction

The accurate assessment of functional capacity of the ovary before controlled ovarian hyperstimulation (COH) is an essential issue in the success of infertility treatment. Ovarian reserve describes the number and quality of oocytes pooled in each ovary and it declines with an increasing age, resulting in a decrease in female reproductive function [1, 2]. However, chronological age does not always reflect biological age, and they may not always correlate with each other [3]. Various studies have assessed ovarian reserve with different ovarian reserve markers such as age, follicle stimulating hormone (FSH), estradiol (E2), antral follicle count (AFC), and anti-Müllerian hormone (AMH). However, the results are variable depending on the population and markers studied [4-19]. There are some disadvantages of day 3 FSH levels and AFC that are the most common markers used. Firstly, FSH level may not be an accurate marker for ovarian reserve due to cyclic fluctuation of hormone. Also, high FSH values occur late in the aging process. Secondly, the accuracy of measurements of AFC depend on ultrasonographer [6-18]. AMH measurement is the most famous test with promising results. However, the results of the previous studies varied due to heterogeneity of population and laboratories and kits [6-8]. Regarding FSH levels that increased in later phases of ovarian aging, AMH seems to be more valuable and earlier marker of ovarian aging in the studies [6-18].

Poor ovarian response is a sign of ovarian ageing, and it is an important limiting factor in vitro fertilization (IVF) success. Although the incidence is unknown, it is encountered in approximately 10-15% of women undergoing IVF [19]. The delayed childbearing age has increased the rate of poor ovarian response (POR) [20]. There is a paucity of studies until Bologna criteria for POR definition [21]. The Bologna standards define the poor response. The diagnosis performed by existence of two or more of the following features such as advanced maternal age or risk factors for POR, previous POR, and abnormal ovarian reserve test [22]. Introduction of these diagnostic criteria is a significant step toward reproducibility and homogeneity of the studies. Most of the studies among POR include cases with advanced age [7-21]. In this study different from the others, the authors examined women under 40 years of age. This current study aimed to investigate the predictive role of ovarian reserve markers in ovarian response of poor responder patients under 40 years of age.

Materials and Methods

This study was designed retrospectively among women undergoing COH in an Assisted Reproductive Techniques Unit of Kocaeli University. The local ethics committee approved the study. Clinical details of all treatment cycles prospectively entered into a computer, which were retrieved for analysis, retrospectively.

A total of 140 patients who fulfilled the inclusion criteria be-

Revised manuscript accepted for publication February 3, 2015

Table 1. — *The characteristic findings of participants.*

	Min-Max	Mean±SD
Patient age (years)	19-40	32.1±5.2
Gravida	0-8	0.49±0.98
Parity	0-3	0.02±0.17
Abortion	0-6	0.28±0.77
Ectopic pregnancy	0-3	0.09±0.37
BMI (kg/m^2)	16.2-32	22.2±3.2
Couple's age (years)	23-60	35.2±5.9
Duration of marriage (years)	1-30	7.2±4.9
Duration of infertility (years)	1-20	6.1±4.1

Table 2. — *The comparison of biochemical and hormonal values of poor and normal responders.*

	Poor responders	Normal responders	*p values*
BMI (kg/m^2)	24.8±4.6	24.2±4.1	0.311
HOMA-IR	2.1±1.4	2.0±1.4	0.872
FSH (mIU/ml)	8.7±4.1	7.2±2.5	0.000
LH (mIU/ml)	5.8±3.0	5.4±3.0	0.239
E_2 (pg/ml)	54.0±39.1	56.1±93.6	0.729
AMH (ng/ml)	1.0±1.44	1.9±1.9	0.000
AFC	8.8±5.7	12.8±6.7	0.000
Basal progesterone (ng/ml)	4.5±7.0	8.5±9.4	0.250
Testesterone (ng/ml)	27.7±13.8	35.1±17.2	0.02
DHEA-S (ng/ml)	162.1±67.3	213.9±89.5	0.04

tween July 2011 and November 2012 enrolled in the study. For the comparison of hormonal and clinical findings, normal-responder, age-matched patients selected with 1:2 ratio on the same week of oocyte retrieval (OR). After exclusion criteria, a total of 250 normal-responder young women were included. All patients underwent detailed infertility evaluation. Patients with previous ovarian-uterine-tubal surgery, polycystic ovary syndrome or OR >15, obesity (BMI > 35 kg/m^2), endocrine diseases, over 40 years of age were not included in the study.

The primary interventions of patients were measures of day 3 FSH, luteinizing hormone (LH), E2, and AMH on random days. Transvaginal ultrasonography was performed on all patients during the follicular phase to exclude any pelvic pathology and AFC (total number of follicles with two- to five-mm diameter). Gen II microELISA method was used in AMH measurements, with high sensitivity (0.017 ng/mL). This method has 5 % intra-assay variations and 8% inter-assay variability. The immunoassay method was used to measure FSH, LH, and E2 levels ("ECLIA" method).

Patients were monitored during COH protocol via serial measurements of serum E2, LH, progesterone level, and ultrasonographic examinations. All subjects underwent GnRH antagonist or agonist long protocol.The recombinant-human chorionic gonadotropin (rhCG) alpha administered if one or more follicles (> 17 mm size) develop during COH protocol. If no follicle developed or serum progesterone level was more than 1.5 ng/ml on the hCG day, the cycle was cancelled. OR was carried out under transvaginal ultrasound under sedation-analgesia. Patients with OR < three oocytes were accepted as poor responders. OR= 4-15 oocytes were accepted as normal responders.

The data analysis was done by using SPPS. 18 software and MedCalc software version 12.3.0. All the data examined within 95% confidence interval, and a p-value < 0.05 was considered statistically significant. The data in the text presented as mean, standard deviation, and percentage. The comparison of parametric/non-parametric variables done via independent samples t-test or chi-square test. Multivariate linear regression analysis was performed to explore the effects of AFC, AMH, FSH, LH, E2, and hCG day E2 on OR. Logistic regression analysis was used to explore the associates of FSH, AFC, AMH, and pregnancy rates (PR). The sensitivity and cut-off values of tests were evaluated by receiver operating characteristic (ROC) analysis.

Table 3. — *A comparison of normal and poor responders with respect to clinical findings, ovarian response, and stimulation doses.*

	Poor responders	Normal responders	*p values*
Gonadotropin initiation dose	357.7±95.3	292.4±83.8	0.00
Total gonadotropin dose	3145.0±1062.7	2717.6±1090	0.00
Duration of gonadotrophin day	9.1±1.9	9.2±1.6	ns
hCG day E2 (pg/ml)	937.4±768.9	1712.1±872.9	0.00
hCG day LH (mIU/ml)	3.2±3.1	2.3±2.3	0.02
hCG day progesterone (ng/ml)	1.5±7.1	1.0±0.6	ns
Total oocyte count	2.4±1.1	8.8±2.9	0.00
M_2 oocyte count	2.0±1.1	6.7±2.9	0.00
Pregnancy rate (%)	20.1	39.2	0.00

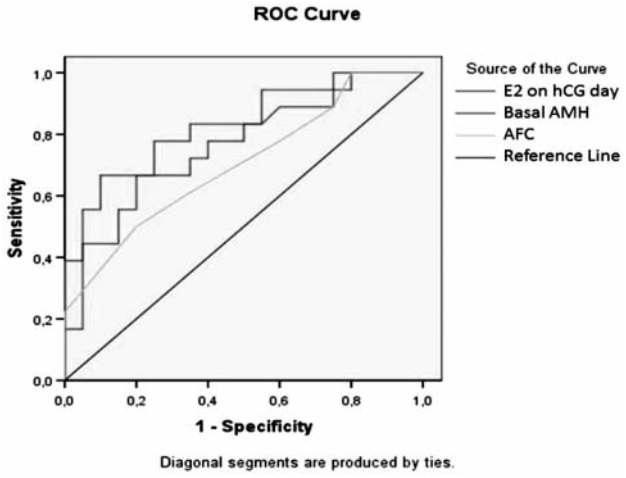
Results

The age of the 390 women ranged from 19 to 40 years with a mean duration of infertility was 6.1 ± 4.1 years. Table 1 presents the characteristic findings. Of the 390 patients enrolled, 140 (35.9 %) patients were poor responders, while 250 (64.1%) women were normal-responders.

Table 2 shows a comparison of biochemical and hormonal findings. BMI, blood glucose, homeostatic model assessment-insulin resistance (HOMA-IR), basal LH, E2, and progesterone were similar. However, FSH levels were significantly higher in poor responders, while AMH, AFC, total testosterone, and dehydroepiandrosterone sulfate (DHEA-S) levels significantly decreased. Gonadotropin initiation dose, total gonadotropin doses, and cycle cancellation rates were considerably higher in poor responders. The hCG day E2, LH, metaphase two (MII) oocyte count, and pregnancy rates were significantly lower. Table 3 presents the details of the comparison.

A poor ovarian response was defined as fewer than three oocytes; ovarian reserve markers performed well in the prediction of poor response. The area under the curve (AUC) ROC curve for ovarian reserve markers were AMH (0.804, p < 0.01), AFC (0.701), E2 on hCG day (0.786), FSH (0.705), LH (0.527), and E2 (0.479), age (0.707), respectively. Figures 1a and 1b show the results of the ROC curve analysis.

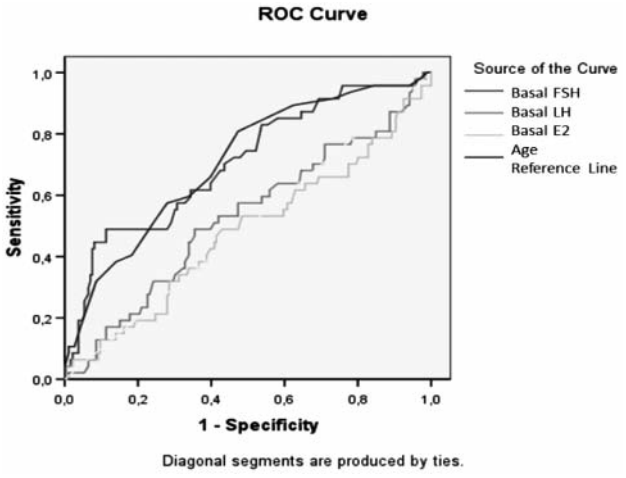
The present authors determined the cut-off values, sensitivity, and specificity of AMH levels for poor response to be 1.09 ng/mL, 80%, and 55.2 %, respectively. The values below the cut-off level were estimated as poor responder



Area under the curve (AUC):

Test result variable(s)	Area	Std. error (a)	Asymptotic sig.(b)	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
E_2 level on hCG day	0.786	0.073	0.003	0.643	0.930
AMH on day 3	0.804	0.074	0.001	0.659	0.949
AFC	0.701	0.085	0.034	0.535	0.868

Figure 1a. — Results of the ROC curve analysis of serum AMH, AFC, and E_2 level on hCG day in poor responders.



Area under the curve (AUC):

Test result variable(s)	Area	Std. error (a)	Asymptotic sig. (b)	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Basal FSH (mIU/ml)	0.705	0.044	0.000	0.619	0.791
Basal LH (mIU/ml)	0.527	0.049	0.572	0.431	0.622
Basal E_2 (mIU/ml)	0.479	0.050	0.652	0.381	0.576
Age (yr)	0.707	0.042	0.000	0.625	0.790

Figure 1b. — Results of the ROC curve analysis of serum FSH, LH, E_2, and age in poor responders.

Table 4. — *A linear regression model for predictors of the total oocyte number (independent variables: age, FSH, LH, AMH, AFC, hCG day E_2).*

	p value	Confidence interval
Age (years)	0.328	-0.288-0.099
FSH (mIU/ml)	0.071	-0.553-0.024
LH (mIU/ml)	0.780	-0.484-0.367
AMH (ng/ml)	0.016	0.259-2.20
E_2 on hCG day(pg/ml)	0.015	0.000-0.003
AFC	0.763	-0.607-0.449

populations. The serum AMH cut-off level in patients with cycle cancellation was 0.72 ng/ml with 75% sensitivity and 56% specificity. If AMH was 0.08, 99.8% of cycles were cancelled.

Total number of oocytes retrieved was related to a variety of factors. There was a negative relation to chronological age ($p = 0.00$; r = - 0.393), day 3 FSH level ($p = 0.00$; r= - 0.302). There was a positive relation to AFC ($p = 0.00$; r = 0.518), E2 level on hCG day ($p = 0.00$; r = 0.571), AMH level ($p = 0.00$; r = 0.529). However, the correlation between the total number of oocytes and BMI, insulin level, HOMA-IR, day 3 LH, day 3 E2, day 3 progesterone, and endometrial thickness were insignificant.

Linear regression analysis was used to search the correlation between the number of total oocytes (dependent variable) and independent variables as age, FSH, LH, AMH, AFC, and E2 on an hCG day. According to this model, AMH and an hCG day E2 levels were independent predictors of the OR (Table 4). If FSH level > 10 mIU/ml, AFC < 6, AMH < 0.72 ng/ml were accepted as associates, none of the factors had an independent effect on pregnancy rates.

Discussion

In this study, ovarian reserve markers predicting poor ovarian response in women under 40 years of age were researched. Ovarian reserve determination maintains the optimization of follow-ups of patients undergoing IVF. Optimizing the treatment protocol according to ovarian reserve parameters will lead to optimal gonadotropin doses, adequate protocols, and sufficient information before the start of induction [17]. Although all ovarian reserve parameters related to the oocyte number, only AMH and the hCG day E2 levels were independent predictors of ovarian response, but not PR. The present study has a limitation of retrospective design. However, this study differs from the others that evaluated AMH cutoff values in women under 40 years of age to predict POR.

Several studies have assessed of ovarian reserve with

different ovarian reserve markers [9-25]. Recently, AMH measurements in predicting ovarian response gains priority [2, 7-16]. FSH, LH, AMH, AFC, and E2 on the hCG day were considered as an independent variables, and a regression analysis model was used for the oocyte count and pregnancy rates. These results were similar to results of Sahmay *et al.* [10]. The results of AUC ROC curve analysis for poor ovarian response showed that AUC ROC was 0.804 for AMH, 0.701 for AFC, and 0.786 for E2 on hCG day, respectively. The present authors determined the cut-off level of AMH as 1.09 ng/ml. When the AMH level was higher than 1.09 ng/ml, the number of retrieved oocytes were significantly higher. In addition, when AMH level was below 0.72 ng/ml, cycle cancellation would probably be seen and also in cases with AMH values lower than 0.08 ng/ml, almost all the cycles cancelled. The present results suggested that AMH levels may aid in proper decision-making before stimulation program in women under 40 years of age. Although various studies indicated the predictive value of AMH measurements before COH protocols, there are no standard cut-off values for AMH measurements. Previous studies reported several cutoff values for a POR that ranged from 0.1 to 2 ng/ml: approximately a 20-fold variation exist between the results [13-15]. Since different commercial kits can cause different results from different laboratories, it is difficult to reach a consensus on cut-off values [13-16].

In conclusion, AMH and hCG day E2 levels were independent predictors of ovarian response. In young, poor responders, measurement of AMH before COH protocol was the most sensitive marker to predict ovarian response, but had no effect on pregnancy rates. Despite the measurements of AMH in ART are promising, prospective studies in different age groups are needed.

References

[1] Te Velde E.R., Pearson P.L.: "The variability of female reproductive ageing". *Hum Reprod Update*, 2002, *8*, 141.

[2] Kalaiselvi V.S., Saikumar P., Prabhu K., Presthath Kristhna G.: "Antimullerian Hormone-A Novel Marker for Assesing the Ovarian Reserve in Women with Regular Menstrual Cycles". *J. Clin. Diagn. Res.,* 2012, *6*, 1636.

[3] Gougeon A., Echochard R., Thalabard J.C.: "Age-related changes of the population of human ovarian follicles: Increase in the disappearance rate of non-growing and early-growing follicles in aging women". *Biol. Reprod.*, 1994, *50*, 653.

[4] Panchal S., Nagori C.: "Comparison of anti-mullerian hormone and antral follicle count for assessment of ovarian reserve". *J. Hum. Reprod. Sci.*, 2012, *5*, 274.

[5] Broer S.L., Mol B.W., Hendriks D., Broekmans F.J.: "The role of antimullerian hormone in prediction of outcome after IVF:comparison with the antral follicle count". *Fertil. Steril*., 2009, *91*, 705.

[6] La Marca A., Broekmans F.J., Volpe A., Fauser B.C., Macklon N.S.: "Anti-Mullerian hormone (AMH): what do we still need to know?". *Hum. Reprod*., 2009, *24*, 2264.

[7] La Marca A., Sighinolfi G., Radi D., Argento C., Baraldi E., Artenisio A.C., *et al.*: "Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART)". *Hum. Reprod., Update*, 2010, *16*, 113.

[8] Avi B.H., Jacob F., Yasmin Z., Onit S., Israel M., Benjamin F.: "Correlations between antral follicle count and ultrasonographic ovarian parameters and clinical variables and outcomes in IVF cycles". *Gynecol. Endocrinol*., 2012, *28*, 432.

[9] Visser J.A., Themmen A.P.: " Anti-mullerian hormone and folliculogenesis". *Mol. Cell. Endocrinol*., 2005, *234*, 81.

[10] Sahmay S., Cetin M., Ocal P., Kaleli S., Senol H., Birol F., *et al*.: "Serum anti-Müllerian hormone level as a predictor of poor ovarian response in in vitro fertilization patients". *Reprod. Med. Biol*., 2011, *10*, 9.

[11] Chimote N., Chimote M., Murkherjee A., Mehta B.: "Day zero serum antimullerian hormone (AMH):A beter predictor of ovarian response than basal AMH in down regulated ART cycles". *Fertil. Steril*., 2008, *90*, 217.

[12] Nath N., Chimote M., Murkherjee A., Chimote N.: "Correlationship of anti-mullerian hormone (AMH) in follicular fluid with oocyte, embryo quality and pregnacy outcome in ART cycles". *Fertil. Steril.,* 2008, *90*, 218.

[13] Satwik R., Kochar M., Gupta S.M., Majumdar A.: "Antimullerian hormone cut-off values for predicting poor ovarian response to exogenous ovarian stimualtion in in vitro fertilization". *J. Hum. Reprod. Sci*., 2012, *5*, 206.

[14] Gnoth C., Schuring A.N., Friol K., Tigges J., Mallmann P., Godehardt E.: " Relevance of anti-Mullerian hormone measurement in a routine IVF program" *Hum. Reprod*., 2008, *23*, 1359.

[15] Alviggi C., Humaidan P., Ezcurra D.: " Hormonal, functional and genetic biomarkers in controlled ovarian stimulation: tools for matching patients and protocols". *Reprod. Biol. Endocrinol.*, 2012, *10*, 1.

[16] Fréour T., Mirallié S., Bach-Ngohou K., Denis M., Barrière P., Masson D.: "Measurement of serum anti-Müllerian hormone by Beckman Coulter ELISA and DSL ELISA: comparison and relevance in assisted reproduction technology (ART)". *Clin. Chim. Acta*, 2007, *375*, 162.

[17] Vural B., Çakıroğlu Y., Vural F., Filiz S.: "Hormonal and functional biomarkers in ovarian response". *Arch. Gynecol. Obstet*., 2014, *289*, 1355.

[18] Coughlan C., Yuan X., Nafee T., Yan J., Mariee N., Li T.: " The clinical characteristics of women with recurrent implantation failure". *J. Obstet. Gynaecol*., 2013, *33*, 494.

[19] Pellicer A., Lightman A., Diamond M.P., Russell J.B., DeCherney A.H. "Outcome of *in vitro* fertilization in women with low response to ovarian stimulation". *Fertil. Steril.,* 1987, *47*, 812.

[20] Te Velde E.R.: "Ovarian ageing and postponement of childbearing". *Maturitas*, 1998, *30*, 103.

[21] Kyrou D., Kolibianakis E.M., Venetis C.A., Papanikolaou E.G., Bontis J., Tarlatzis B.C.: "How to improve the probability of pregnancy in poor responders undergoing *in vitro* fertilization: A systematic review and meta-analysis". *Fertil. Steril.,* 2009, *91*, 749.

[22] Ferraretti A.P., La Marca A., Fauser B.C., Tarlatzis B., Nargund G., Gianaroli L., *et al*.: " ESHRE consensus on the definition of 'poor response' to ovarian stimulation for *in vitro* fertilization: The Bologna criteria". *Hum. Reprod.,* 2011, *26*, 1616.

[23] Jirge P.R., Chougule S.M., Gavali V.G., Bhomkar D.A.: " Impact of dehydroepiandrosterone on clinical outcome in poor responders: A pilot study in women undergoing in vitro fertilization, using bologna criteria". *J. Hum. Reprod. Sci*., 2014, *7*, 175.

[24] Celik E., Bastu E., Dural O., Yasa C., Buyru F.: " Relevance of anti-Müllerian hormone on in vitro fertilization outcome". Clin. Exp. Obstet. Gynecol.,, 2013, *40*, 66.

[25] Matalliotakis I., Cakmak H., Arici A., Goumenou A., Fragouli Y., Sakkas D.: "Epidemiological factors influencing IVF outcome: Evidence from the Yale IVF program". *J. Obstet. Gynaecol*., 2008, *28*, 204.

Address reprint requests to:
F. VURAL, M.D.
Orhan mah. Bağtepe sok.
Erenkaya park evleri. A2-2 Bağçeşme / İzmit
41100 Kocaeli (Turkey)
e-mail: fisunvural@yahoo.com.tr