

Genetic and clinical predictors of ovarian response in assisted reproductive technology

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Abstract. Several factors are known to influence ovarian response to rFSH stimulation such as age, antral follicle count (AFC), and basal FSH level. Mutation of allele Ser680Asn in FSHR gene was responsible to ovarian resistance toward exogenous FSH. The aim of this study is to develop a prediction model of ovarian response to COS in IVF. This study was a prospective cohort study. One hundred and thirteen women undergoing their first cycle of IVF in Yasmin IVF Clinic Jakarta were recruited to this study. Clinical datas included were age, BMI, and AFC. Basal FSH and E2 as well as serum AMH was measured from peripheral blood taken at second day of cycle. Bsr-1 enzyme is used to identify the polymorphism in exon 10 position 680 with RFLP technique. Three genotype polymorphism, Asn/Asn (255 bp ribbon), Asn/Ser (97 bp and 158 bp), and Ser/Ser (97 bp, 158 bp, and 255 bp). AFC has the highest predictor for ovarian response with AUC 0.922 (CI 95% 0.833-1.000). AMH also showed high predicting value (AUC 0.843 CI 95% 0.663-1.000). The multivariate analysis revealed combination of AFC, AMH, age, and basal FSH is a good model for ovarian response prediction (AUC=0.97). No significant relation between Asn/Asn, Asn/Ser, or Ser/Ser genotype FSHR polymorphism with ovarian response ($p = 0.866$) and total dose of rFSH ($p = 0.08$). This study showed that model combination of AFC, AMH, patient's age and basal FSH are very good to predict number of mature oocytes.

1. Introduction

In vitro fertilization (IVF) is known as the last option for infertile couple. The process involved controlling hypothalamus-pituitary-ovary axis exogenously to achieve multiple follicle growth. The European Society of Human Reproduction and Embryology (ESHRE) report in 2012 showed that of 139978 IVF cycles, the clinical pregnancy rates per aspiration and per transfer were stable with 29.4 (29.1% in 2011) and 33.8% (33.2% in 2011), respectively [1]. Compared to other ART methods, IVF has the highest success rate thus making it the most chosen one. Ovarian response consists of follicle and endocrine response toward exogenous FSH stimulation. Adequate response is defined as minimal three mature oocytes obtained during ovum pick up (OPU). Another criteria could be at least three



follicles observed with 18-20 mm diameter and total serum estradiol level at least 600 pg/ml obtained before OPU. Experts proposed tailored ovarian stimulation to achieve good response. Poor responder groups are known to have high cancellation rate and low pregnancy rate. Also, prediction of ovarian response is useful to determine optimal dose of recombinant FSH (rFSH) for each patient. The incorrect dose could cause supraphysiological estrogen serum level that decrease endometrial receptivity due to the premature luteinization phenomenon. This event occurred in 44.5% in 55 cycles observed at our clinic.

Several factors are known to influence ovarian response to rFSH stimulation. Based on a study by Popovic-Todorovic *et al.*, age, ovarian volume measurement, antral follicle count (AFC), smoking status, and basal FSH level are significant variables to predict the initial rFSH dosage to achieve adequate ovarian response [2]. Bancsi *et al.* found a prediction model for poor responder which consist of AFC, FSH basal level, and inhibin level with 89% accuracy level (sensitivity 0.75, specificity 0.95, and ROC AUC 0.92) [3]. Another intriguing issue is whether polymorphism of FSH receptor. Mutation of allele Ser680Asn in FSHR gene was responsible to ovarian resistency toward exogenous FSH. There was higher poor response in homozygous Ser/Ser group compared to Asn/Asn and Asn/Ser group (36% to 14.4%, respectively) [4]. A comprehensive consideration of clinical, hormonal, and genetic parameters could support a better prediction for ovarian response. This could lead to a better stimulation program and educational material for doctor and client.

2. Materials and Methods

This study is a prospective cohort study to 113 cycles performed from July 2010-July 2011 in our fertility clinic. We included all IVF patients. Clinical datas included were age, duration of infertility, body mass index, and AFC. The ultrasound parameter was measured by Aloka SSD-500 type with transvaginal *probe* with minimal frequency of 7 MHz. Cut off point for AFC is 10 for both ovaries. Intra- and interobserver variability are controlled by limiting the US operator to three persons only. Basal FSH and estradiol level as well as serum AMH level was measured from peripheral blood taken at second day of cycle. The FSH and estradiol examination was run by AxSYM System (Abbott(R), 1997) machine. AMH examination was run by *kinetic microplate reader* VMAX type and Softmax software.

Analysis for FSHR polymorphism was done by isolating the DNA from peripheral blood sample in EDTA preservatives. Polymerase chain reaction is done with GeneAMP, PCR system 9700 (Perkin Elmer) machine to achieve 35 cycles of amplification with 255 bp DNA ribbon. Bsr-1 enzyme is used to identify the polymorphism in exon 10 position 680 with the restriction fragment length polymorphism technique (RFLP). This will result in three genotype polymorphisms, Asn/Asn (255 bp ribbon), Asn/Ser (97 bp and 158 bp), and Ser/Ser (97 bp, 158 bp, and 255 bp).

As a dependent variable, ovarian response is categorized into two different groups – good and poor responder. The cut-off value is three oocytes retrieved during ovum pick-up procedure. This study was analyzed using SPSS 20.0 (IBM(C)) software for statistics. All independent variables were considered as ordinal datas and analyzed with t-test or Mann-Whitney test. The ROC-AUC curve analysis were performed to rank the highest predictor for ovarian response. All significant variables were then included for multivariate analysis using logistic regression, from which the odds ratio (OR) for each variables could be ranked. Validation for the formula was made by computing the ROC, AUC, and Hosmer-Lemeshow test.

3. Results and Discussion

3.1 Results

The clinical characteristics of subjects are described in Table 1. One hundred and thirteen patients were involved in this study. There is a significant mean difference between age, antral follicle count, basal FSH, AMH, and initial rFSH dosage among poor responder and good responder. Good responders have a tendency to be younger, with shorter duration of infertility, leaner, have more amount of AFC, less basal FSH, higher AMH, lower total rFSH initial dose, and higher rFSH total

dose. Forty percent poor responder groups were gone through long protocol, while other 45% have short protocol, and 15% with normal cycle. Fifty six percent good responders were gone through long protocol, while 43.3% short protocol and no subjects with natural cycle.

Table 1. Clinical characteristics of the study subjects

Variables	Poor responders (n=19)	Good responders (n=94)
Age (year)	40 (30-48)	35 (22-44)
AFC (follicles)	3.1+1.9	9.3+5.4
BMI (kg/m ²)	26.2 (17.9-30.5)	22.5 (16.6-28.4)
Basal FSH (mIU/ml)	23.5+34.6	7.1+2.3
Basal estradiol (pg/ml)	45.8+33.4	45.4+29.5
AMH (ng/ml)	0.9 (0.05-6.2)	2.35 (0.2-9.8)
Initial rFSH dose	225+50	214.3+56.7
Total rFSH dose	1,725 (450-4,200)	2,250 (275-4,875)
Mature oocyte count	2 (1-3)	9(4-31)

Based on bivariate analysis (see Table 2), basal FSH and AFC have significant relation with ovarian response in rFSH administration ($p < 0.01$ for both variables). Age and AMH level were significantly related with ovarian response based on Mann-Whitney test ($p < 0.001$ for both variables). Based on ROC curve, AFC has the highest predictor for ovarian response with AUC 0.922 (CI 95% 0.833-1.000). The cut-off values for each significant variable in predicting ovarian response are mentioned in Table 3.

Table 2. Relation among independent variables and ovarian response

Variables	p-value
Age (year) ¹	0.001*
AFC ²	<0.01*
BMI (kg/m ²) ¹	0.489
Basal FSH (mIU/ml) ²	<0.001*
Basal estradiol (pg/ml) ²	0.922
AMH (ng/ml) ¹	0.001*
Initial rFSH dose ²	0.715
Total rFSH dose ¹	0.46

¹ Mann-Whitney test

² Unpaired t-test

Table 3. AUC and cut-off values for significant variables

Variables	AUC	Cut-off	CI 95%
AFC	0.922	5	0.833-1.000
AMH (ng/dl)	0.843	1.222	0.663-1.000
Age (year)	0.749	38.5	0.592-0.906
Basal FSH (mIU/ml)	0.100	7.45	0.000-0.222
Total rFSH dose (mIU/ml)	0.221	1,787.5	0.044-0.399

The multivariate analysis for significant variables was performed to calculate probability of good response. The obtained formula is: Formula 1: $Y = 11.785 + .531AMH + .520AFC - .334age - .226FSH$. Probability is calculated by entering Y value to the second formula: Formula 2: $p = 1/(1+\exp(-Y))$. Younger patient has better ovarian response with OR 0.72 (CI 95% 0.46-1.13). Lower basal FSH level (OR 0.79 CI95% 0.66-4.41), higher AFC (OR 1.68 CI 95% 0.69-4.13) and AMH (OR 1.70 CI 95% 0.66-4.41) are clinically significant for good response. This analysis is still worth

published although there is no statistical significance. The consideration was based on many previous studies regarding ovarian response. (4-6, 10, 14). The formula above has good discrimination and calibration value based on AUC of 0.970 (CI 95% 0.911-1.000) and Hosmer-Lemeshow test ($p = 0.402$).

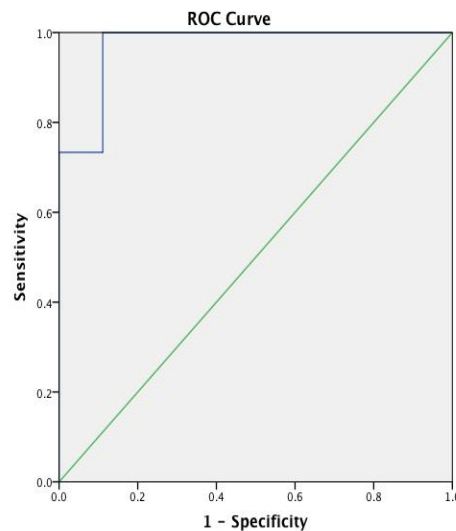


Figure 1. ROC curve of logistic regression for ovarian response

Based on linear regression test, the independent variables could predict the mature oocyte count. Formula 2: mature oocyte count = $14.811 + 1.032 \cdot \text{AMH} + 0.069 \cdot \text{AFC} - 0.262 \cdot \text{age} - 0.076 \cdot \text{FSH}$. This formula could explained 37.5% mature oocyte count. The highest correlation were held by basal FSH, AMH, age, and AFC. One-hundred-nine subjects were evaluated for the FSHR gene polymorphism analysis. There was no significant relation between FSHR gene polymorphism and ovarian response ($p = 0.866$). The characteristics for FSHR polymorphism is listed in Table 4. There was no significant relation between total rFSH dosage and FSHR gene polymorphism ($p = 0.082$). The relation of that is listed in Table 5.

Table 4. FSHR gene polymorphism analysis (n=109)

Table 1. F508del gene polymorphism analysis (n=103)							
	Polymorphism						p-value
	Asn/Asn		Asn/Ser		Ser/Ser		
	n	%	n	%	n	%	
Good responder	41	80.4	42	87.5	8	80	0.866
Poor responder	10	19.6	6	12.5	2	20	
Total	51	100	48	100	10	100	

Table 5. Relation of total rFSH dosage and FSHR gene polymorphism

	Asn/Asn	Asn/Ser	Ser/Ser	p
Total rFSH dosage	2,384+889.36	2,229+866.92	2,311+1,371.83	0.082

3.2 Discussion

Controlled ovarian stimulation is used widely for infertility patient. Recombinant FSH is used IVF patient to suppress the hypothalamus axis. To achieve adequate ovarian response, COS dosage should be measured individually. According to a study of 267 long protocol IVF patients in Denmark, prediction of COS could be made by calculating several factors such as AFC, ovarian Doppler vascularization score, and smoking habits [2]. The importance of calculating individual dose for COS lies in the risks of the stimulation. Underdosage could cause no follicle growth, while overdosage cause supraphysiologic estradiol level thus poor oocyte quality and premature luteinization [5]. Based

on this study, age, basal FSH level, AMH level, and AFC were significantly related to ovarian response in COS. There were no significant difference in ovarian response related to variation of basal estradiol level, BMI, and FSHR gene polymorphism. In 130 IVF cycles conducted in Netherlands, basal FSH level ($p < 0.001$), inhibin B ($p < 0.001$), basal estradiol ($p = 0.049$), and AFC ($p < 0.001$) are significant factors for ovarian response. Age is almost significant for prediction of ovarian response ($p = 0.07$) [6]. Bivariate analysis must be proceeded with multivariate analysis to achieve higher prediction for ovarian response.

AFC was proven to have the highest AUC. The similar result was also showed by AMH (AUC 0.843 CI 95% 0.663-1.000). AMH has an advantage to AFC regarding its independency of intra-cycle variability. Based on a meta-analysis of five AFC studies and nine AMH studies in year 2011, AMH and AFC shared the same sensitivity value of 82% while higher specificity was seen in AFC (80% vs 76%, respectively). No statistical differences were found between AUC in AMH or AFC [7]. An analysis of prediction model based on AFC, basal FSH level, and inhibin B level from 120 IVF cycles in Netherlands showed AUC 0.90 with $p = 0.36$ from Hosmer-Lemeshow test [6]. Development of prediction model is shown to be important for COS. A scoring system to count individualized dose to start COS was made from multivariate analysis from Denmark study [2]. The ability to predict ovarian response as well as the individualized initial rFSH is important to prevent stimulation side effects, stimulation failure, and reduce unnecessary cost for the patient.

Author	Year	Sample	Country	Ser/Ser(%)	Asn/Asn(%)
Perez-mayorga, <i>et al.</i> [8]	2000	161	Germany	26	29
Karalok [9]	2011	182	Turkey	30	20
Mohiyideen [10]	2012	421	UK	19	30

There was no significant relation between total rFSH dosage and FSHR polymorphism. The same result was observed from a study of 161 IVF patient in Germany [8]. This result may be due to the small sample size of this study. The range of age may affect the study result, because the possibility of decreasing FSH receptor becomes a bias in observing FSHR gene polymorphism. Another study conducted in UK has also concluded non-significant relation between FSHR gene polymorphism and other markers of ovarian reserve such as AMH and AFC [10]. This conclusion can be explained by the relation of AMH and AFC with amount of follicles in ovaries which is not established in FSHR gene polymorphism.

4. Conclusion

This study is a pioneer study done in Asian subjects which certainly has different characteristics than Caucasian patients. We should bear in mind the increasing need for IVF and other ART methods in Asian population. Even so, this study should proceed with another study using matching subjects. Some reference studies use certain age range, number of cycles, and excluded certain characteristics such as PCOS patient to achieve higher significancy.

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