

Research Report

Predictive Factors for Pregnancy in IVF: An Analysis of 348 Cycles*Faktor penduga terjadinya kehamilan pada fertilisasi in vitro (FIV): Analisis terhadap 348 siklus***Budi Wiweko, Andon Hestiantoro, Kanadi Sumapraja, R. Muharam, Huthia Andriyana,
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Jakarta***Abstract****Objective:** To determine predictive factors for pregnancy after IVF.**Method:** The subject of this study were three hundred and forty eight IVF cycles in 266 couples who underwent controlled ovarian hyperstimulation in IVF cycles between January 2005 and March 2010. Categorical variables were compared using Chi Square test and continuous variables were analyzed using Independent t-test, $p < 0.05$ was considered statistically significant. Multivariate logistic regression analysis was done to test correlations between clinical variables and the occurrence of pregnancy.**Results:** The women's age significantly influenced pregnancy rate since women under 35 years old has the best chance for pregnancy (56.4%). Endometrial thickness on the day of hCG administration also significantly influenced pregnancy in IVF ($p < 0.001$) because 64.1% of pregnancy occurred if endometrial thickness ≥ 10.95 mm. Serum FSH on 3rd day of period that can predict ovarian reserve also has significance on pregnancy. On the other hand, 61.5% pregnancy occurred if more than 6 mature oocytes were retrieved ($p < 0.001$). Among 92 patients of 348 cycles we found strong correlation between AMH level with number of mature oocytes retrieved ($p < 0.001$; $r 0.659$). Logistic regression done revealed the couple with best chance of pregnancy can be described as follows: women with endometrial thickness ≥ 10.95 mm, number of mature oocytes > 6 and age under 35 years old.**Conclusion:** This study enabled the characterization of many prognostic factors for pregnancy.

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Keywords: in vitro fertilization, clinical pregnancy, age, mature oocytes, endometrial thickness**Abstrak****Tujuan:** Untuk menentukan faktor penduga terjadinya kehamilan pada FIV.**Metode:** Penelitian dilakukan terhadap 348 siklus FIV dari 266 pasangan yang menjalani hiperstimulasi ovarium terkendali antara Januari 2005 - Maret 2010. Variabel kategorik dibandingkan dengan uji *kai kuadrat* sedangkan variabel kontinu dianalisis dengan uji *t* tidak berpasangan. Nilai $p < 0,05$ dianggap bermakna. Untuk menilai hubungan antara variabel digunakan analisis multivariat dengan regresi logistik.**Hasil:** Usia perempuan memiliki hubungan bermakna terhadap kehamilan di mana 56,4% kehamilan terjadi pada perempuan berusia < 35 tahun. Ketebalan endometrium pada hari penyuntikan hCG juga berpengaruh terhadap tingkat kehamilan ($p < 0,001$) karena 64,1% kehamilan terjadi bila ketebalan endometrium $\geq 10,95$ mm. Kadar FSH basal yang digunakan untuk meramalkan cadangan ovarium, juga mempunyai hubungan yang bermakna dengan tingkat kehamilan. Sedangkan 61,5% kehamilan terjadi bila > 6 oosit matur berhasil dipetik ($p < 0,001$). Terdapat korelasi kuat antara kadar AMH dengan jumlah oosit matur yang berhasil dipetik pada 92 dari 348 siklus ($p < 0,001$; $r 0,659$). Setelah dilakukan regresi logistik diketahui bahwa peluang terbesar untuk hamil pada FIV bila ketebalan endometrium $\geq 10,95$ mm, jumlah oosit matur > 6 dan usia pasien kurang dari 35 tahun.**Kesimpulan:** Penelitian ini menunjukkan karakteristik beberapa faktor prognostik kehamilan pada FIV.

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Kata kunci: fertilisasi in vitro, kehamilan, usia, oosit matur, ketebalan endometrium**Correspondence:** Budi Wiweko, Division of Reproductive Immunoendocrinology Department of Obstetrics and Gynecology Medical Faculty of Indonesia University, Jakarta. Telephone: 021-3928720, Fax.: 021-3928719, Email: wiwekobudi@yahoo.co.id**INTRODUCTION**

As an option in the management of infertility with the highest rate of success, in vitro fertilization (IVF) has been commonly selected to obtain pregnancy. IVF is performed when infertility is associated with the uncorrected occlusion of bilateral tube, ovulation disorder, endometriosis of mild-to-severe degree, sperm factors which cannot be treated or corrected with surgery, or idiopathic infertility.

The mean of pregnancy rates in IVF ranged between 30 and 40%, with the factors of embryo quality and endometrial receptivity being suspected to play a major role in the failure of implantation.¹ The quality of embryo is largely dependent on the age of patient, causes of infertility, causes of oocytes, and the quality

of sperm. On the other hand, the quality of oocytes of a woman is largely dependent on ages, ovarian reserves which can be evaluated on the basis of FSH basal level. The receptivity of endometrium is evaluated on the basis of morphology and perfusion of the endometrium within the IVF cycles which can be observed in the endometrial thickness, estradiol level, and progesterone level on the day of hCG injection.

Various studies reported the factors affecting pregnancy in IVF, such as patient's age, ovarian reserve, number of oocytes retrieved, estradiol level, and endometrial thickness. However, these studies have not yet indentified the relationship between those factors and set them up as a model. Thus, this study aimed to evaluate the relationship between patient's age, length and causes of infertility, indications of

IVF, ovarian reserve, endometrial thickness, number of follicles, and mature oocytes, and estradiol and progesterone on the day of hCG injection in relation to the possibility of pregnancy in IVF, and to set them up as a predictive model.

It was expected that this study could be used to predict the possible occurrences of pregnancy in patients who are currently undergoing or have undergone IVF program, or who may use it in the future, to the extent that optimal services and support can be rendered to patients.

METHOD

The subjects of the study were 266 patients undergoing 348 IVF cycles at Yasmin Clinic of Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, from January 2005 to March 2010. The exclusion criteria were patients who did not follow the protocol of IVF to the completion.

The protocol of controlled ovarian hyperstimulation used was long-term protocol, short-term protocol, or natural cycle. The long-term protocol used a combination of *gonadotrophin-releasing hormone* (GnRH) and *recombinant-FSH* (r-FSH) agonists. Meanwhile, the short-term protocol employed combined GnRH antagonist and r-FSH. In several patients with diminished ovarian reserve, clomiphene citrate, femara, or *recombinant-LH* (r-LH) could be administered. All patients undergoing IVF cycles were administered *human chorionic gonadotrophin* (hCG) one or two days prior to the retrieval of oocytes. The administration of GnRH agonist, r-FSH, GnRH antagonists, clomiphene citrate, femara, hCG, embryo culture, and embryo transfer was performed on the basis of the protocol used at Yasmin Clinic, Dr. Cipto Mangunkusumo General Hospital, Jakarta.

The examination of transvaginal USG with Aloka SSD 3500 USG was carried out to evaluate the number of follicles and the thickness of the endometrium. Endometrial thickness was defined as the maximum distance between the ecogenic surfaces of the myometrium and endometrium in longitudinal axis. If there were three follicles with a diameter of > 17 mm, it was decided to resort to hCG injection and the oocytes were picked-up within 34 - 36 hours. After the pick-up had been completed, the quality and maturation of the oocytes were identified. The oocytes which were at metaphase II and possessed the polar bodies were considered to be mature oocytes. The pregnancy rates in IVF were assessed on the basis of the presence of gestational sac and the uterus within three to four weeks following the oocyte pick-up.

Data of medical records collected were recorded in the study forms which have been made specifically to facilitate data entry and analysis. The data were processed using SPSS 16.00 program, and data analysis were carried out with the following steps:

Bivariate Analysis

At this stage of analysis, we studied the relationship between two variables comprising the comparison between clinical variables, such as patient's age, type of infertility, causes of infertility in women and men,

insemination method, protocol of ovarian stimulation, FSH level and AMH basal level, progesterone and estradiol levels on the day of hCG injection, endometrial thickness on the day of hCG injection, the number of mature follicles, the number of mature oocytes, with dependent variables, i.e. pregnancy. Statistical tests used were chi square test for categorical variables and unpaired t test for continuous variables. P value < 0.05 was considered statistically significant.

Multivariate Analysis

This analysis was employed to identify the relationship between statistically significant clinical variables in bivariate analysis and dependent variables, i.e. pregnancy, and to determine the degree of strength between them by counting Odds Ratio (OR). Statistical test used was logistical regression analysis.

RESULTS

Table 1. Demographic data between pregnant IVF cycles and not-pregnant IVF cycles (total of 348 cycles).

Variable	Pregnant (n = 78)	Not-pregnant (n = 270)	P
Age (year)	33.32	35.64	0.000
Type of infertility			
Primary infertility	68 (21.6%)	247 (78.6%)	0.212
Secondary infertility	10 (33.2%)	22 (68.8%)	
Length of infertility (year)	6.71	7.46	0.135
Causes of infertility due to female factor			
Tubal factor	15 (4.3%)	35 (10.1%)	
Endometriosis	6 (1.7%)	51 (14.7%)	
Ovulation dysfunction	9 (2.6%)	20 (5.7%)	0.071
Other factor	8 (2.3%)	39 (11.2%)	
Idiopathic	40 (11.5%)	125 (35.9%)	
Causes of infertility due to male factor			
Azoospermia	7 (2%)	28 (8%)	
Severe oligospermia	23 (6.6%)	75 (21.6%)	0.965
Other factors	5 (1.4%)	15 (4.3%)	
Idiopathic	43 (12.4%)	152 (43.7%)	
Insemination method			
ICSI	69 (19.8%)	228 (66.5%)	0.845
Conventional	2 (0.6%)	6 (1.7%)	
Combined (ICSI+conventional)	7 (2%)	34 (9.8%)	
Protocol of stimulation			
Long-term protocol	60 (17.4%)	187 (54.2%)	0.102
Short-term protocol	18 (5.2%)	66 (19.1%)	
Natural cycles	0 (0%)	14 (4.1%)	
FSH basal level (mIU/ml)	6.48	7.94	0.009
AMH basal level (µg/ml)	3.11	3.10	0.991
Progesterone level on the day of hCG injection (ng/ml)	1.41	1.61	0.755
Estradiol level on the day of hCG (pg/ml) injection	2.47	2.14	0.137
Endometrial thickness on the day of hCG injection	11.46	10.26	0.000
Number of mature follicles (≥ 17 mm) oocyte pick-up day	8.37	6.36	0.000
Number of mature oocytes (metaphase II oocytes) oocyte pick-up day	7.94	5.77	0.000

Three hundred and forty eight cycles of IVF were performed in 266 couples undergoing IVF program from January 2005 to March 2010. The distribution

of patients' ages during period of IVF program was between 22 and 48 years of age. In the current study, there were 348 cycles which resulted in 78 pregnancies (22.4% per cycle), with the following details: single pregnancy 41 cases (11.8%), twin pregnancies 19 cases (5.5%), triple pregnancies 2 cases (0.6%), abortion during the first trimester 2 cases (0.6%), blighted ovum 8 cases (2.3%), disorder ectopic pregnancies 2 cases (0.6%).

Infertility caused by female-factors comprised 37.4%, by male-factors 28.7%, combined female and male factors 15.2%, and idiopathic factor 18.7%. Infertility due to female factor alone consisted of bilateral tubal occlusion (14.4%), endometriosis (16.4%), ovulation dysfunction (8.3%), other causes (13.5%), and idiopathic factor (47.4%). Statistically, the relationship between infertility factors in women and pregnancy was not significant ($p > 0.05$). By contrast, infertility associated with male factors was divided into three groups: azoospermia (10.1%), severe oligozoospermia (28.2%), other causes (5.7%), and idiopathic factor (56%). The protocol of ovarian stimulation employed was the long-term protocol (247 cycles, 71%), short-term protocol (84 cases, 24.1%), and natural cycles (14 cycles, 4%).

Table 2. Pregnancy rates based on age, basal FSH level, endometrial thickness, the number of mature follicles, the number of mature oocytes, type of infertility, length of infertility, causes of infertility due to female factor, protocol of stimulation and estradiol level on the day of hCG injection.

Predictive Factor	Pregnant	Not-pregnant
Age		
< 35 years	44 cycle 56.4%	101 cycle 37.4%
≥ 35 years	34 cycle 43.6%	169 cycle 62.6%
Basal FSH		
<10 mIU/ml	72 cycle 92.3%	224 cycle 83%
≥ 10 mIU/ml	6 cycle 7.7%	46 cycle 17%
Endometrial thickness		
< 10.95 mm	28 cycle 35.9%	106 cycle 39.3%
≥ 10.95 mm	50 cycle 64.1%	164 cycle 60.7%
Mature follicles		
< 8 mature follicles	35 cycle 44.9%	173 cycle 64.1%
≥ 8 mature follicles	43 cycle 55.1%	97 cycle 35.9%
Mature oocytes		
< 6 mature oocytes	30 cycle 38.5%	164 cycle 60.7%
≥ 6 mature oocytes	48 cycle 61.5%	106 cycle 39.3%
Type of infertility		
Primary	68 cycle 21.6%	247 cycle 78.6%
Secondary	10 cycle 33.2%	22 cycle 68.8%
Length of infertility		
≤ 7.5 years	45 cycle 57.7%	146 cycle 54.1%
> 7.5 years	33 cycle 42.3%	124 cycle 45.9%
Causes of infertility due to female factor		
Tubal factor	15 cycle 4.3%	35 cycle 10.1%
Endometriosis	6 cycle 1.7%	51 cycle 14.7%
Ovulation dysfunction	9 cycle 2.6%	20 cycle 5.7%
Other factors	8 cycle 2.3%	39 cycle 11.2%
Idiopathic	40 cycle 11.5%	125 cycle 35.9%
Stimulation protocol		
Long-term protocol	60 cycle 17.4%	187 cycle 54.2%
Short-term protocol	18 cycle 5.2%	66 cycle 19.1%
Natural cycle	0 cycle 0%	14 cycle 4.1%
Estradiol level on the day of hCG injection		
≥ 2000 pg/ml	36 cycle 46.2%	151 cycle 35.2%
< 2000 pg/ml	42 cycle 53.8%	119 cycle 64.8%

Variables which had significant relationship with pregnancy rates were patients' age, FSH basal level, endometrial thickness on the day of hCG injection, the number of mature follicles on the day of hCG injection, and the number of mature oocytes on the day of oocyte pick-up day. Furthermore, all these variables were divided into two groups in accordance with the threshold values obtained, along with their sensitivity and specificity values.

DISCUSSION

Patients with ages < 35 years when undergoing in vitro fertilization were more likely to obtain pregnancy by 56.4%, compared with patients with ages above 35 years who had 43.6% of such probability. This finding was consistent with other studies which suggested that ages were the primary predictive factors of pregnancy in women undergoing IVF program, and the number of oocytes successfully fertilized was greater such that the successful rates of implantation and clinical pregnancy were higher.²⁻⁵

Basal FSH levels were divided into two groups, i.e. normal basal FSH level (< 10 mIU/ml) and above-normal basal FSH level (≥ 10 mIU/ml). Pregnancies occurred in 92% of the patients with FSH < 10 mIU/ml, compared with 7.7% in patients with FSH ≥ 10 IU/l ($p = 0.041$). Although routine examinations were not recommended for predicting the outcome of IVF, these examinations were sufficient to predict the response of the ovaries.⁶⁻⁹ On the other hand, basal FSH level could be used as the predictor of IVF outcome.¹⁰ In 92 patients examined for their AMH levels, there was a significant relationship between AMH and the number of mature oocytes ($p < 0.001$; AUC 0.846; $r = 0.659$).

The threshold value of endometrial thickness that may result in pregnancy in patients undergoing in vitro fertilization was 10.95 mm. Based on the data in Table 2, the group with endometrial thickness of > 10.95 mm was likely to obtain pregnancy by 64.1%. This finding was consistent with other studies conducted which suggested endometrial thickness as the predictive factor of pregnancy in IVF.¹¹⁻¹⁴

The more mature follicles were found the likelier a woman to have pregnancy in IVF program. This finding was supported by the study conducted which demonstrated that the small number of mature follicles - as a result of the low follicular response at the time of the controlled ovarian hyperstimulation - would diminish the possible success of IVF.¹⁵ The higher pregnancy rates were found in patients with ≥ 6 oocytes with sensitivity of 61.5%, and specificity of 60.3%.

Variables which had p value of < 0.25 from bivariate analysis were subjected to multivariate analysis with logistical regression. Multivariate analysis was performed with logistical regression of Backward Stepwise with the factors affecting the occurrence of pregnancy in the following order: endometrial thickness, patient's age, and the number of mature oocytes.

The equation obtained was:
 $y = \text{constant} + a_1 \times 1 + a_2 \times 2 + a_3 \times 3$
 $y = -2.621 - 0.594 (\text{age}) + 0.677 (\text{oocyte}) + 0.926$
(endometrial thickness) + 0.818 (type of infertility)

In order to predict the probability of a patient in obtaining pregnancy, the following formula was used:
 $p = 1/(1+e^{-y})$
 where
 p = probability of the occurrence of an event
 e = natural coefficient = 2.7

Table 3. Comparison between groups in pregnancy.

Variable	Pregnant (n=78)	Not-pregnant (n=270)	RR	CI	AUC
Endometrial thickness					
< 10.95 mm	28	164	2.543	1.49 - 4.34	0.671
≥ 10.95 mm	50	106			
Mature oocytes > 6 oocytes					
1 - 6 oocytes	30	164	0.481	0.280 - 0.824	0.683
≥ 6 oocytes	48	106			
Patient's age					
< 35 years	44	101	0.563	0.330 - 0.962	0.644
≥ 35 years	34	169			
Basal FSH					
< 10 mIU/ml	72	224	0.594	0.236 - 1.54	0.577
≥ 10 mIU/ml	6	46			
Mature follicles					
1 - 8 follicles	35	173	1.146	0.477 - 2.75	0.666
≥ 8 follicles	43	97			
Type of infertility					
Primary	68	247	2.490	1.017 - 6.099	0.524
Secondary	10	22			
Length of infertility					
≤ 7.5 years	45	146	0.989	0.909 - 1.075	0.460
> 7.5 years	33	124			
Causes of infertility due to female factor					
Tubal factor	15	35	0.989	0.785 - 1.245	0.493
Endometriosis	6	51			
Ovulation dysfunction	9	20			
Other factors	8	39			
Idiopathic	40	125			
Stimulation protocol					
Long-term protocol	60	187	0.523	0.238 - 1.148	0.478
Short-term protocol	18	66			
Natural cycle	0	14			
Estradiol level on the day of hCG injection					
≥ 2000 pg/ml	36	151	0.994	0.986-1.002	0.430
< 2000 pg/m	42	119			

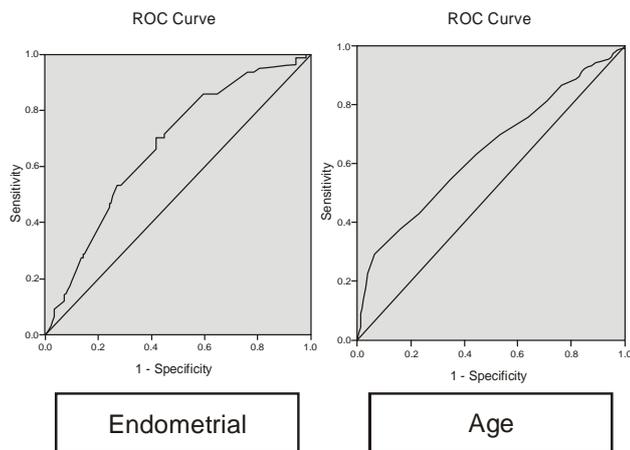


Figure 1. ROC curve of pregnancy rates based on patient's age, endometrial thickness, and number of mature oocytes.

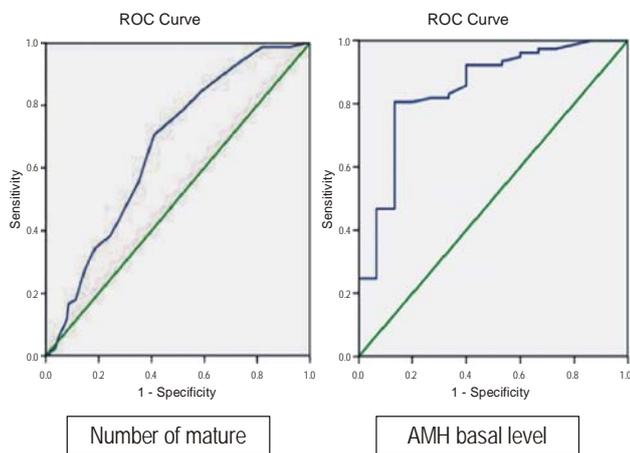


Figure 2. ROC curve of the number of mature oocytes based on basal AMH level.

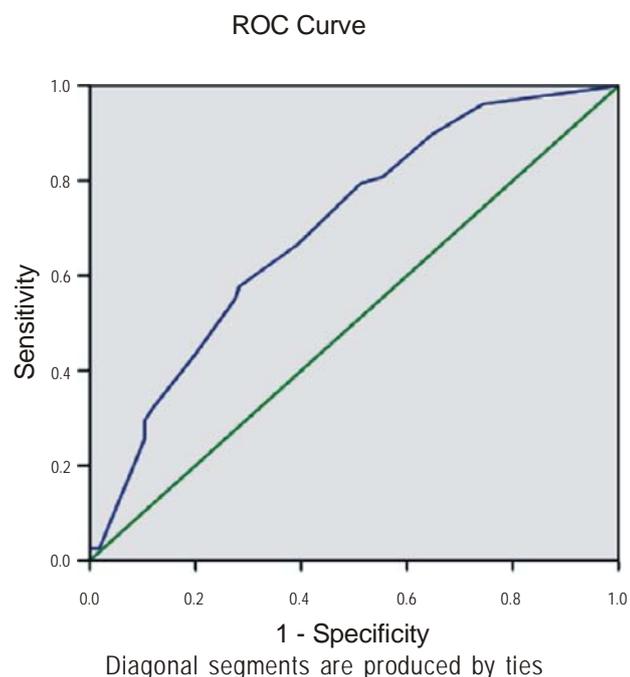


Figure 3. ROC curve of pregnancy rates after multivariate analysis. (AUC = 0.7)

CONCLUSION

Factors which can be used to predict the occurrence of pregnancy in the cycles of IVF were endometrial thickness ≥ 10.95 mm, patient's age < 35 years, and the number of mature oocytes ≥ 6 .

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