Research Report

Not only embryo quality but also Endometrial Thickness Contributes to IVF outcome: a retrospective study of all IVF cycles in Yasmin Clinic, Jakarta, Indonesia

(Tidak hanya kualitas embrio namun ketebalan endometrium juga berperan dalam meramalkan luaran siklus FIV: Sebuah studi retrospektif terhadap pasien-pasien yang menjalani siklus FIV di klinik Yasmin, Jakarta, Indonesia)

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Abstract

Objective: To study the endometrial thickness and embryo quality during In Vitro Fertilization (IVF) cycles in predicting IVF outcome.

Methods: This retrospective study involved 206 infertile patients undergoing 218 IVF cycles in Yasmin Clinic, Dr. Cipto Mangunkusumo National Referral Hospital, Jakarta, Indonesia. All IVF cycles were performed from January 2005 until May 2009. The outcome of this study is the clinical pregnancy following IVF cycles. The endometrial thickness was measured on the day of human chorionic gonadotrophin (hCG) administration. The number of embryos that developed ≥ 8 cells on cleavage II represented as the embryo quality.

Results: There were 51 among 218 cycles (23.4%) resulted in pregnancy. The endometrial thickness on day of hCG administration was significantly higher in pregnant group compared to non-pregnant group (11.49±1.97 mm versus 10.13±1.93mm; p<0.0001). The number of embryos with ≥ 8 cells on cleavage II was higher in pregnant group than non-pregnant group {3 embryos (1-11) versus 1 embryos (0-11); p<0.0001}. From the ROC curve, the endometrial thickness cut-off value ≥ 10.95 mm was the best value to predict pregnancy outcome. This value had 64.7% sensitivity, 62.3% specificity, 34.4% positive predictive value, and 85.2% negative predictive value.

Conclusion: Both embryo quality and endometrial thickness significantly have contribution to IVF outcome prediction.

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Keywords: IVF cycles, endometrial thickness, embryo quality

Abstrak

***Tujuan**: Untuk mengetahui peranan ketebalan endometrium dan kualitas embrio dalam meramalkan keberhasilan Fertilisasi In Vitro (FIV).*

***Metode**: Penelitian dilakukan secara retrospektif dengan melibatkan 206 pasien infertilitas yang menjalani 218 siklus FIV di Klinik Yasmin, RSUPN-Cipto Mangunkusumo, Jakarta, Indonesia. Semua siklus FIV dilakukan pada bulan Januari 2005 sampai dengan Mei 2009. Luaran yang diteliti adalah terjadinya kehamilan klinis setelah siklus FIV. Ketebalan endometrium diukur pada hari penyuntikan human chorionic gonadotrophin (hCG). Kualitas embrio diukur berdasarkan adanya embrio yang telah memiliki ≥ 8 sel pada saat pembelahan tahap II.*

***Hasil**: Terdapat 51 siklus dari 218 siklus (23,4%) yang berhasil hamil. Ketebalan endometrium saat pemberian hCG secara bermakna lebih tinggi pada kelompok hamil dibandingkan kelompok tidak hamil (11,49±1,97 mm dibandingkan 10,13±1,93mm; p<0,0001). Jumlah embrio yang memiliki ≥ 8 sel pada pembelahan tahap II lebih tinggi pada kelompok hamil dibandingkan kelompok tidak hamil {3 embrio (1-11) dibandingkan 1 embrio (0-11); p<0,0001}. Dari kurva ROC, didapatkan bahwa batasan minimal ketebalan endometrium yang dapat digunakan untuk meramalkan kehamilan adalah ≥ 10,95 mm. Nilai ini memiliki sensitivitas sebesar 64,7%, spesifisitas sebesar 62,3%, nilai prediksi positif 34,4% dan nilai prediksi negatif 85,2%.*

***Kesimpulan**: Baik kualitas embrio maupun ketebalan endometrium secara bermakna berperan dalam meramalkan luaran FIV.*

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***Kata kunci**: siklus FIV, ketebalan endometrium, kualitas embrio*

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INTRODUCTION

The two most important prognostic factors for IVF success are embryo quality and endometrial receptivity. Early embryo cleavage has been reported to be the strong indicator of embryo quality in IVF. The phenomenon of good-quality-embryo implantation depends not only on the quality of embryo itself, but also on endometrial receptivity. Thus endometrial receptivity is required for successful implantation of good-quality-embryos which are represented by the early cleavage embryos.[1-2]

Endometrial receptivity parameters are endometrial thickness, pattern, volume, blood flow, partial endocrine change, secretion of growth factors, and so on. Ultrasonography measurements have been used as a noninvasive technique to evaluate endometrial receptivity parameters. One of endometrial receptivity parameter in sonography is endometrial thickness. The measurement of endometrial thickness is easy to perform, easily reproducible, and have been shown to have a good intraobserver and interobserver correlation. But many studies on endometrial thickness have come with conflicting results. Many studies reported that endometrial thickness had low pregnancy prediction value in IVF.[3-9]

This retrospective study included all IVF cycles performed from January 2005 until May 2009. From this study we evaluated many factors which might contribute to IVF cycle outcome. Embryo quality and endometrial thickness were believed to be dominantly contributing factors in IVF outcome.

METHODS

All 206 infertile patients underwent 218 IVF-embryo transfer (ET) cycles between January 2005 and May 2009 was included in this study. The IVF-ET cycles were performed at Yasmin Clinic, Infertility and Reproductive Immunoendocrinology Center, Dr. Cipto Mangunkusumo National Referral Hospital, in Jakarta, Indonesia. All patients who demonstrated a normal uterine cavity prior to starting the cycle were included. Patients with submucous myomas or polyps who had undergone resection surgery were regarded as subjects with normal uterine cavity and were included in this study. The exclusion criteria were patients who did not complete IVF cycles due to oocyte retrieval failure or cancel protocols.

The stimulation protocol was long protocol, short protocol, or natural cycle. The long protocol controlled ovarian hyperstimulation used combination of gonadotrophin-releasing hormone (GnRH) agonist (Suprefact, Buserelin) and recombinant FSH (r-FSH) (Puregon, Gonal). The short protocol controlled ovarian hyperstimulation used combination of GnRH antagonist (Cetrorelix) and r-FSH (Puregon, Gonal). The administration of GnRH agonist, r-FSH, GnRH antagonist, hCG injection, gametes disposing, embryo culture, and embryo transfer were all undergone according to our regular manipulation in Yasmin Clinic. Fourteen days after pituitary desensitization with GnRH agonist, there was evaluation of follicle maturation by ultrasonography and by serum oestradiol concentration. Then r-FSH was administered at a dose of 150-450 IU according to the ovarian reserve. The r-FSH dose was adjusted according to the degree of ovarian response, evaluated by ultrasound examination and by serum estradiol concentration. The hCG administration (5000 IU-10000 IU) was done if there was follicles of > 18 mm in diameter, usually on day 12-14 rFSH administration. Oocytes were retrieved 32-36 hours after hCG administration under transvaginal ultrasound guidance.

Embryos were transferred 2 or 3 days later after oocyte retrieval. The embryos were evaluated by embryologist on the day of fertilization, day of cleavage I, and day of cleavage II. The evaluation included the blastomeres and fragmentation. Embryos with ≥ 4 cells on cleavage I and embryos with ≥ 8 cells on cleavage II were calculated. All embryos calculated had < 20% fragmentation of the total embryonic volume.

On the day of hCG administration, the measurement of endometrial thickness was recorded using 2-Dimension-Medison SA 6000 C transvaginal ultrasound or 2-Dimension-color-Medison SA 8000 transvaginal ultrasound. Endometrial thickness was defined as the maximal distance between the echogenic interfaces of the myometrium and the endometrium measured in the plane through the central longitudinal axis of the uterus. The outcome of this study was the cycle that resulted in pregnancy. The "pregnancy" termed in this study was the clinical pregnancy which was defined as the presence of gestational sac in the uterus 3-4 weeks after day of oocyte retrieval.

For the statistical analysis, the independent samples Student's T-test was used to compare variables between cycles that resulted in pregnancy and those that did not. Mann-Whitney U-test was used when data distribution was not normal. Chi-square test was used to compare categorical variables. The area under the receiver operating characteristic (ROC) curve was calculated to assess the predictive accuracy of endometrial thickness to predict the probability of pregnancy. The model of logistic regression was gained by a stepwise procedure to analyze the independently dominant factors which influenced pregnancy outcome. Statistical analysis was done with SPSS software, 16.0 version for windows. $p < 0.05$ was considered statistically significant.

RESULT

There were 206 infertile patients aged between 22 and 48 years old underwent 218 IVF-ET cycles between January 2005 and May 2009. From 218 cycles, 201 cycles were primary infertility and 17 cycles were secondary infertility. The causes of infertility were male factor (74/218 cycles; 33.9%), tubal block (27/218 cycles; 12.4%), endometriosis (22/218 cycles; 10.1%); polycystic ovary syndrome (12/218 cycles; 5.5%); combination of male factor and female factor (20/218 cycles; 9.2%), unexplained infertility (41/218 cycles; 18.8%), and other factors (22/218 cycles; 10.1%). The insemination methods consisted of 186 ICSI (85.3%), 4 conventional methods (1.8%), and 28 Mixed between ICSI and conventional methods (12.8%). There were 163 cycles (74.8%) used long protocol ovarian stimulation, 53 cycles (24.3%) used short protocol, and 2 cycles (0.9%) used natural cycles.

The clinical pregnancy rate was 23.4% (51/218 cycles). Among 51 clinical pregnancies, 45 pregnancies (88.2%) was ongoing pregnancy defined as the pregnancy where heartbeat was identified on ultrasound at about 8-10 weeks after the day of oocyte retrieval. Baseline characteristics such as body mass index, type of infertility, duration of infertility, cause of infertility, insemination method, stimulation protocol were similar between pregnant and non-pregnant group. There was no different basal FSH level between pregnant and non-pregnant group. Total dosage of FSH used was also similar in both groups (Table 1).

In cycle that resulted in pregnancy, the patients were younger, had more basal antral follicles, had more mature follicles, had more mature oocytes, had more embryos with ≥ 8 cells on day of cleavage II. Mean (± SD) endometrial thickness on hCG day was significanty greater in pregnant cycles compared to non-pregnant cycles (11.49 ± 1.97 versus 10.13 ± 1.93 mm; $p < 0.0001$) (Table 1).

Table 1. Demographic data between pregnant group and non-pregnant group

Variables	Pregnant (n=51)	Non-pregnant (n=167)	p value
Age (years old)[a]	33.08±4.77	35.17±4.77	0.01
Body Mass Index (kg/m^2)[a]	23.80±2.36	23.34±3.58	NS
Infertility type			
Primary infertility	44 (21.9%)	157 (78.1%)	NS
Secondary infertility	7 (41.2%)	10 (58.8%)	
Duration of infertility (years)[b]	7 (1-16)	7 (1-22)	NS
Cause of infertility			
Male factor	15 (20.3%)	59 (79.7%)	
Tubal block	9 (33.3%)	18 (66.7%)	
Endometriosis	3 (13.6%)	19 (86.4%)	
PCOS	4 (33.3%)	8 (66.7%)	NS
Combination (male+female) factor	4 (20.0%)	6 (80.0%)	
nexplained infertility	13 (31.7%)	28 (68.3%)	
Other factors	3 (13.6%)	19 (86.4%)	
Insemination Method			
ICSI	43 (23.3%)	142 (76.8%)	
Conventional	2 (50.0%)	2 (50.0%0	NS
Mixed (ICSI+conventional)	6 (21.4%)	22 (78.6%)	
Stimulation protocol			
Long protocol	38 (23.3%)	125 (76.7%)	
Short protocol	13 (24.5%)	40 (75.5%)	NS
Natural cycle	0 (0%)	2 (100%)	
No. of basal anthral follicles[b]	8.5 (2-14)	7 (0-19)	0.001
Baseline FSH (IU/l)[a]	6.25±2.25	8.00±4.80	NS
Total dosage of FSH	1913	2063	NS
(IU)[b]	(375-5025)	(0-5400)	
Endometrial thickness (mm) on hCG day[a]	11.49±1.97	10.13±1.93	<0.0001
No. of follicles >17 mm on hCG day[b]	8 (1-16)	6 (0-19)	0.001
No. of metaphase II oocytes[b]	8 (1-16)	5 (0-19)	<0.0001
No. of cell with 2 pn[b]	4 (0-11)	2 (0-10)	<0.0001
No. of embryos ≥ 4 cells (cleavage I)[b]	3 (1-11)	1 (0-10)	<0.0001
No. of embryos ≥8 cells (cleavage II)[b]	3 (1-11)	1 (0-10)	<0.0001

Note: NS = not statistically significant,
[a] mean ± SD,
[b] median (value)

The ROC curve of endometrial thickness value was analyzed to show the predictive value of endometrial thickness. From the ROC curve, the endometrial thickness cut-off value ≥10.95 mm was the best profile to predict the cycle outcome. When endometrial thickness reached 10.95 mm, pregnancy rates were improved (33/96; 34.3%) compared when endometrial thickness was below 10.95 mm (18/122; 14.8%). Endometrial thickness cut-off ≥10.95 mm had 64.7% sensitivity, 62.3% specificity, 34.4% positive predictive value, and 85.2% negative predictive value.

Logistic regression analysis was evaluated to pregnancy as dependent variable versus independent variables. The variables which were statistically associated with pregnancy (shown in Table 1) included as independent variables. Those variables were patients' age, basal antral follicles, endometrial thickness, number of mature follicles, mature oocytes, cell with 2 pn, embryos with ≥ 4 cell on cleavage I, and embryos with ≥ 8 cells on cleavage II. In the logistic regression model consecutively the number of embryos with ≥ 8 cells and endometrial thickness were dominantly influenced pregnancy outcome. If the number of embryos with ≥ 8 cells exceeded 1.5 and endometrial thickness exceeded 10.95 mm, those two factors would result in greater probability of achieving pregnancy (OR 6.309 and 3.797) (Table 3).

Table 2. Endometrial thickness between pregnant and non-pregnant group

Endometrial thickness (mm)	Pregnant (n=51)	Non-pregnant (n=167)	p value	OR	95% CI	Sens (%)	Spec (%)	PPV (%)	NPV (%)
≥ 8	48	152	0.576	1.579	0.438-	94.1	9.0	24.0	83.3
< 8	3	15			5.687				
≥ 9	48	126	0.004	5.206	1.539-	94.1	24.6	27.6	93.2
< 9	3	41			17.608				
≥ 10	44	96	< 0.0001	4.649	1.978-	86.3	57.5	31.4	91.0
< 10	7	71			10.926				
≥ 11	33	63	0.001	3.026	1.574-	64.7	62.3	34.4	85.2
< 11	18	104			5.821				
≥ 12	24	38	0.001	3.018	1.562-	47.1	77.2	38.7	82.7
< 12	27	129			5.828				
≥ 13	14	18	0.003	3.132	1.428-	27.5	89.2	43.8	80.1
< 13	37	149			6.872				
≥ 14	6	7	0.083	3.048	0.975-	11.8	95.8	46.2	78.0
< 14	45	160			9.524				
Total	51	167							

Note: OR =Odds Ratio, CI = Confidence Interval, Sens = sensitivity, Spec = specificity, PPV = Positive Predictive Value, NPV = Negative Predictive Value

Table 3. Multivariate analysis of variables independently predictive of pregnancy

Variables	Range	Pregnant (n=51)	Non-pregnant (n=167)	p value	Odds Ratio	Coefficient Beta	95% CI of Beta
Number of embryos with ≥ 8 cells	> 1.5	39	50	0.0001	6.309	0.387	(0.056-0.106)
	≤ 1.5	12	117				
Endometrial thickness (mm)	≥ 11	33	63	<0.0001	3.797	0.224	(0.022-0.072)
	< 11	18	104				

DISCUSSION

The two main factors that contributed to pregnancy outcome in this study were the embryo quality and endometrial thickness. The embryo quality that can be easily determined is the embryo cleavage rate. The final mature embryo which was represented by the number of embryos with ≥ 8 cells in cleavage II in this study more dominantly contributed to pregnancy outcome, followed by endometrial thickness. Those two important factors might have protective effect for not achieving pregnancy. This study had similar result with study by Lundin et al,[2] Rinaldi et al,[3] Dietterich et al,[4] Zhang et al[5] and Kovacs et al.[6]

Many studies showed no significant association between endometrial thickness and pregnancy outcome (De Geyter et al,[7] Noyes et al[8]). Moreover, other studies reported that "increased" endometrial thickness may have detrimental effect on pregnancy outcome. Study by Weissman et al[9] showed endometrial thickness ≥ 14 mm on day of hCG administration was associated with lower implantation and pregnancy rate. However, in our study, increased endometrial thickness was associated and correlated with the pregnancy outcome. There is no tendency that increased endometrial thickness ≥ 14 mm has adverse effect on pregnancy. Many studies reported the low prognostic value of using endometrial thickness as the prediction of pregnancy possibility. In this study, the prognostic values of endometrial thickness cut-off ≥ 10.95 mm were 64.7% sensitivity, 62.3% specificity, 34.4% positive predictive value, and 85.2% negative predictive value. The endometrial thickness ≥ 10.95 mm which resulted in pregnancy and endometrial thickness < 10.95 mm which did not result in non-pregnancy was three times higher than endometrial thickness < 10.95 mm which resulted in pregnancy and endometrial thickness ≥ 10.95 mm which did not result in pregnancy (OR = 3.126; 95% CI 1.574-5.821; p = 0.001)

There are many parameters of endometrial receptivity that can be measured by ultrasound such as endometrial thickness, pattern, volume, and blood flow. The weakness of this study is we do not measure other factors than endometrial thickness as the evaluation of endometrial receptivity. However, based on the result of this study, endometrial thickness measurement has been known to be simple and easy procedure which has role in pregnancy prediction especially in small IVF center. In conclusion, our study revealed that both embryo quality and endometrial thickness might have influence the pregnancy outcome in IVF.

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