Research Report

Embryo Quality: The Most Critical Factor for Pregnancy Rates after day-2, day-3, and day-5 of Embryo Transfer

Kualitas Embrio: Faktor Terpenting pada Keberhasilan Kehamilan setelah Transfer Embrio Hari ke-2, 3 dan 5

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Abstract

Objective: To determine the most critical factor on day-2, day-3, and day-5 of embryo transfer in correlation with pregnancy rates.

Method: This research is a retrospective study in Aster Fertility Clinic, IVF program- Dr. Hasan Sadikin Hospital, Bandung, Indonesia. One hundred ninety five women enrolled in an IVF program conducted from March 2006 through November 2009 at the Aster Fertility Clinic. Effect of embryo-transfer day and any other factors (including quality of embryo, oocyte quantity, difficulty of embryo transfer technique, and blood or mucus contamination on the catheter) on pregnancy rate in IVF.

Results: The mean age of the pregnant group was 34.65 (SD = 3.91), and the mean of the infertility period was 7.25 years (SD = 3.54). There were no siginificant differences in pregnancy rates in the day-2, day-3, and day-5 groups. The most critical factor influencing pregnancy was the total score for the quality of embryos [p = 0.001; OR (CI 95%) = 1.94 (0.91 - 4.08)]. Otherwise, the day of embryo transfer, oocyte quantity, and difficulties in embryo transfer did not affect the pregnancy rate (p > 0.05).

Conclusion: Our study suggests that the total score for the quality of the embryos was the most critical factor for the success rate of pregnancy rather than the day of embryo transfer, oocyte quantity, difficulty of embryo transfer technique, or contamination of blood and mucus on the catheter.

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Keywords: day of embryo transfer, in vitro fertilization, quality of embryo total score, oocytes quantity, catheter contamination

Abstrak

***Tujuan**: Untuk menentukan faktor yang terpenting pada keberhasilan kehamilan setelah transfer embrio hari ke-2, ke-3 dan ke-5.*

***Metode**: Penelitian ini adalah studi retrospektif di klinik Fertilitas Aster, Program IVF- Rumah Sakit Dr. Hasan Sadikin, Bandung, Indonesia. Penelitian ini melibatkan seratus sembilan puluh lima perempuan yang mengikuti program IVF sejak Maret 2006 hingga Nopember 2009. Efek dari waktu (hari) transfer embrio dan faktor-faktor lainnya (termasuk kualitas embrio, jumlah oosit, kesulitan dalam teknik transfer embrio, dan kontaminasi darah atau mukus dalam kateter) terhadap angka keberhasilan kehamilan pada IVF.*

***Hasil**: Rata-rata usia perempuan hamil pada penelitian ini 34,65 (SD = 3,91), dan rata-rata periode infertilitas 7,25 (SD = 3,54). Tidak terdapat perbedaan bermakna pada angka kebehasilan kehamilan setelah transfer embrio baik pada hari ke-2, -3 dan -5. Faktor terpenting yang mempengaruhi keberhasilan kehamilan adalah skor total dari kualitas embrio [p = 0,001; OR (CI 95%) = 1,94 (0,91 - 4,08)]. Dilain fihak, waktu transfer embrio, jumlah oosit, kesulitan dalam teknik transfer embrio tidak mempengaruhi kebehasilan kehamilan (p > 0,05).*

***Kesimpulan**: Hasil studi kami menunjukkan bahwa faktor terpenting yang mempengaruhi keberhasilan kehamilan adalah skor total dari kualitas embrio dibandingkan dengan waktu transfer embrio, jumlah oosit, kesulitan dalam teknik transfer embrio, atau kontaminasi darah dan mukus dalam kateter.*

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***Kata kunci**: hari setelah transfer embrio, fertilisasi in vitro, skor total dari kualitas embrio, jumlah oosit, kontaminasi kateter*

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INTRODUCTION

Infertility still remains a problem in the area of reproductive medicine. Infertility is not only become a problem for particular families and couples, but also for clinicians to meet the expectations of their patients. In 2009, it was estimated 10 to 15% of the West Java population were having infertility problems. Therefore, an effort is needed in order to improve the quality of services in promoting assisted reproductive technology.

In vitro fertilization (IVF) is one of the last options in treating infertile couples, particularly when conventional methods fail to achieve pregnancy. However, the average of clinical pregnancy rate remains low, ranging from 20 to 30%; moreover, the survival rate of the babies is 10 - 15%.[1]

Currently, Aster clinic is the only official reproductive clinic in West Java Province. It was established at Dr. Hasan Sadikin Hospital, Bandung, in May 2005. Aster clinic currently performs day-2 and day-3 embryo transfer, and blastocyst stage transfer. The decision regarding to the time of embryo transfer is usually made on day-2 after the embryos have gone through one division and are somewhere between the 2 - 8 cell stage (on day-2 or day-3). By day-5, an embryo should consist of 64 - 128 cells and should

have reached more advanced embryonal stage called the blastocyst stage (embryo transfer is performed on day-5).

Determining the transfer day is being considered essential to give the best chances of having a full-term healthy baby.[2,3] Several studies have been performed to determine when is the best time to transfer the embryo. Unfortunately, the current study results still remain controversial. Bungum et al[4] reported that there are no significant differences between day-3 or day-5 embryo transfer in terms of the number of biochemical or clinical pregnancy rates, implantation rates, or abortion events. Despite those conflicting findings, Racowsky et al[5] and Ragione et al[6] reported that successful in achieving pregnancy was not only being determined by the day of embryo transfer but, rather, by the quality of embryos obtained.

The purpose of this study was to identify the crucial factors that play essential factor in the success rate of day-2, day-3, and day-5 embryo transfer. There are variables that have been considered such as the quality of embryos, number of oocytes, technical difficulty of embryo transfer, and blood or mucus contamination on the catheter tip.

MATERIALS AND METHODS

Patients

This is a retrospective analysis study involving IVF patients from Aster Clinic, Dr. Hasan Sadikin Hospital, Bandung (2006 - 2009). 195 patients who met the inclusion criteria for the IVF program were being recruited. All patients have signed informed consent forms according to our institutional guidelines. Patients were being excluded if no significant follicle growth during ovarian stimulation, no oocytes can be collected during ovum pick up procedure, or fertilization at day-1 was observed 24 - 25 hours after fertilization.

Controlled-ovarian hyperstimulation

All patients underwent controlled-ovarian hyperstimulation using GnRH agonist (Busereline® 500 iu sc/day), from day-21 of periods until complete menstruation suppression occurs (estradiol < 80 pg/ml, no follicular diameter > 10 mm and thin endometrium). After the occurrence of complete suppression, patients received recombinant FSH (r-FSH) (Gonal-F®) starting from initial dose (75 - 300 IU) based on the patient's age, cause of infertility, and individual response on r-FSH. Follicle growth is monitored by ultrasound (Hitachi EUB-525 real-time follicle-scanner). Follicle is measured on day-3, day-5, and day-7 to day-12, followed by examination of serum estradiol. Follicles with diameter of more than 2 mm were measured as day-7. Moreover, 250 μg of r-hCG (recombinant human chorionic gonadotrophin) were administered after follicles achieve 18 mm.

Monitoring of embryo development

Approximately 36 hours later after hCG stimulation, the ova were picked up under ultrasound guidance. Fertilization was conducted six hours later. A simple medium was used for the embryo culture on day-1 to day-3. Monitoring of the embryo development was carried out at 18 - 20 hours after fertilization to observe the number of pro-nuclei (PN) and polar-body (PB). The embryonic development was monitored on day-1, at 24 - 25 hours after fertilization in order to assess the quantity, quality and scoring of the embryo during the stage of two-cell or early division. Embryo monitoring on day-2 (48 hours after fertilization) and day 3 (72 hours after fertilization) were done at 10 a.m. to assess the quantity, quality, and scoring of the embryo. A complex medium was used as the embryo culture reach day-3 to day-5. The embryo transfer (ET) was conducted on day-2 if fewer than four fertilized embryos were present on day-1 (24 - 25 hours after fertilization). Embryo transfer was performed on day-5 if more than four embryos with eight cells were observed on day-3, but if fewer were observed, the transfer was performed on day-3. The types of catheter used for ET are Cook U1603094 Fr/12, 6.8 cm in size (Cook Spencer, Indiana, USA) and the K-type needle Cook 1-0 ATS in 1000, nontoxic (Queensland, Australia).

Assessment of a successful pregnancy

After ET, patients received 250 mg hydroxy progesterone caproate intramuscularly (im) (Proluton Depot®) or 1500-3000 IU β-hCG im at four-day intervals for 16 days for luteal phase support. The chemical pregnancy was determined by checking β-hCG serum level on day-16 after ET (> 80 IU); further transvaginal ultrasound examination was performed on day-30 to identify the presence of gestational sac.

Statistical analysis

All statistical analyses were conducted by SPSS® for Windows®' 13th version. Continuous data were described as mean and standard deviation (SD). All p values were two-sided, and the statistical significance of differences was considered significant when p value was ≤ 0.05.

RESULTS

There were 195 patients have been recruited for this study, with total pregnancy rate was 31.8%. (Table 1)

Table 1. Characteristic of the patients (n=195).

	Day of Embryo Transfer		
	Day-2	**Day-3**	**Day-5**
Age (in year) *X* (SD)	35.5 (4.83)	34.7 (4.18)	34.9 (4.59)
Period of infertility (in year) *X* (SD)	7.52 (3.62)	6.85 (4.03)	6.37 (2.93)
Quantity of oocyte *X* (SD)	5.12 (3.38)	8.95 (4.72)	14.57 (4.27)
Quality of embryo total score: *X* (SD)	7 (3.97)	8.7 (3.53)	10.29 (1.49)
Pregnancy rates (%)	28.57	30.92	57.1
Total pregnancy rates (%)	31.8		
Technical difficulties in embryo transfer (%)	9.23		
Blood contamination (%)	12.82		
Mucus contamination (%)	22.56		

The pregnancy rate was not affected by the day of embryo transfer and the duration of infertility (Table 2; p > 0.05).

Table 2. Patient's characteristic based on age & period of infertility with pregnancy rates (n=195).

	Pregnancy Rates		p value
	Pregnant	Not Pregnant	
Age (in year) *X* (SD)	34.65 (3.91)	35.05 (4.82)	0.796
< 30	12	27	
31 - 35	18	44	
36 - 40	32	62	
Total	62 (31.8%)	133 (68.2%)	
Period of Infertility (in year) *X* (SD)	7.25 (3.54)	6.95 (3.92)	0.436
1 - 5	20	54	
5 - 10	30	52	
> 10	12	27	
Total	62 (31.8%)	133 (68.2%)	

Note: Pearson chi-square.

However the pregnancy rate was significantly influenced by the quality of the embryos [Table 3; p = 0.001; OR (CI 95%) = 1.94 (0.91 - 4.08)], and it was not affected by the day of embryo transfer, oocyte quantity, ET technical difficulties, or ET catheter contamination by blood or mucus (p > 0.05).

Table 3. The effect of day embryo transfer, oocyte quantity, quality of embryo total score, embryo transfer technique, catheter contamination by blood and mucus on pregnancy rates.

	Pregnancy Rates		p value	OR (CI 95%)
	Pregnant	Not Pregnant		
Day of embryo transfer				
Day 2	24	60	0.101	1
Day 3	30	67		0.893 (0.47 - 1.69)
Day 5	8	6		0.3 (0.94 - 0.957)
Quantity of oocyte X (SD)	8.00 (4.57)	7.56 (5.09)	0.356	1.45 (0.66 - 3.02)
Quantity of oocyte:				
≥ 4	52	104		
< 4	10	29		
Quality of embryo X (SD)	9.68 (3.98)	7.38 (3.45)	0.001	1.94 (0.91 - 4.08)
- Skor total:				
≥ 5.5	51	94		
< 5.5	11	39		
ET technical Difficulties level			0.082	0.43 (0.16 - 1.14)
Easy	53	124		
Difficult	9	9		
Existance of Blood on ET catheter			0.370	0.64 (0.24 - 1.69)
None	56	114		
Exists	6	19		
Existance of Mucus on ET catheter			0.271	0.65 (0.30 - 1.39)
None	51	100		
Exists	11	33		

Note: Pearson chi-square

A model of multiple logistic regression shows that the total score for the quality of the embryo is an independent variable that will determine the pregnancy rate (p = 0.0001) with β-coefficient value -0.243 (OR 0.79). That means by having higher total score for embryo quality, it might have 0.79 times greater chance to get successful pregnancy. This is more accurate indicator compared to other factors (oocyte quantity, difficulty level of embryo transfer, and contamination of blood or mucus on the catheter).

DISCUSSION

Based on our knowledge, this is the first study that has been done using data from day-5 embryo transfer in Indonesia. Despite the results are considered satisfactory, further study should be needed in order to observe more closely the effect of day-5 embryo transfer in terms of the pregnancy rate.

The subjects of this study were coming from Aster Clinic at Dr. Hasan Sadikin Hospital, Bandung (2005 -2009). Rapid progress in the development of assisted reproductive technology (ART) brings us more satisfactory results. However, the decision to transfer an embryo on day-5 or earlier is still being influenced by thea patient's age at the time of the procedure.[7-10]

The Aster Clinic is currently using a guideline for the time of ET based on the quality of the embryo; the data represented here is influenced by the age of the patient and the number of oocytes. Therefore, our results does support the previous studies by Sjoblom et al[11] and Borini et al[12].

Although six variables are compared, the pregnancy rates are mostly being influenced by the quality of the embryos [p = 0.0001, OR (CI 95%) = 0.79 (0.70 - 0.87)], as shown in Table 4. However, the pregnancy rate is not being affected by oocyte quantity, the day of embryo transfer, the level of technical difficulties in ET, or the contamination of blood or mucus on the ET catheter.

Independent analyses on each group for time of embryo transfer are performed, and the results indicates that embryo quality is more crucial for pregnancy rate after ET in day-2 and day-3 groups. On the contrary, there are no dominant factors can determine the pregnancy rate for day-5 ET. These results indicate that only high-quality embryos that could be transferred on day-5 at Aster Clinic. These results are remain consistent after being evaluated by logistic regression analysis. The analysis shows that the crucial factor for a successful pregnancy is the quality of the embryo.

Our study does support a prospective randomized study done by Bungum et al.[4] regarding to the association between embryo transfer and pregnancy rate., The study shows no significant implantation rate associated between day-3 and day-5 embryo transfer, despite the pregnancy rate after embryo transfer on day-5 is quantitatively increase. This study is trying to used more strict criteria than other studies with a higher confidence level.

On the contrary, a similar retrospective analysis study by Mangalraj et al.[13] with 135 patients suggests to do ET on day-5 if at least there are four or more

Table 4. Multiple logistic regression analysis on day embryo transfer, quality of embryo total score, oocyte quantity, difficulty of embryo transfer technique, catheter contamination by blood and mucus with pregnancy rates.

Model	β	SE	p value	OR (CI 95%)
Initial				
Quality of embryo total score	- 0.257	0.058	0.0001	0.77 (0.69 - 0.86)
Day of embryo transfer	- 0.594	0.253	0.019	0.55 (0.33 - 0.91)
Quantity of oocyte	0.169	0.057	0.003	1.18 (1.06 - 1.32)
ET Technical difficulties	- 1.475	0.587	0.012	0.22 (0.72 - 0.72)
ET Catheter contamination by blood	- 0.664	0.583	0.255	0.52 (0.16 - 1.61)
Catheter contamination by mucus	- 0.643	0.460	0.162	0.53 (0.21 - 1.29)
Constants	7.309	1.736		
Final				
Quality of embryo total score	- 0.243	0.056	0.0001	0.79 (0.70 - 0.87)
Day of embryo transfer	- 0.573	0.249	0.021	0.56 (0.35 - 0.91)
Quantity of oocyte	0.149	0.054	0.006	1.16 (1.04 - 1.29)
Difficulty of embryo transfer technique	- 1.093	0.953	0.041	0.33 (0.12 - 0.95)
Constants	4.45	0.983		

embryos classified as grade-one on day-3. They noted higher success in achieving pregnancy and embryo implantation rates on day-5 compared to day-3, although no significant difference was observed on the incidence of twins or other multiple pregnancies. Similar results were obtained in a study by Papanikolaou et al.[14] who found that embryo transfer on day-5 increased the success of pregnancy more than that on day-3 [52.5% vs. 32.1%, OR (CI 95%) = 2.33]. Therefore, both of studies suggest, if the number of embryos with good quality exceeds more than four, the embryo culture can be continued after day-3 and the embryo transfer finally can be performed on day-5. However, the results of these studies show no decrease in the incidence of multiple pregnancies, even though the Papanikolaou studies suggest that day-5 embryo transfer might reduces the complications of pregnancy, such as multiple pregnancies. Finally, neither of these studies addresses the effects of multiple pregnancies.

Kovacic et al.[15] issued a controversial report in 2002 with regard to the best time for embryo transfer either on day-2, day-3, or day-5. He concluded that the lengthening of the embryo culture in the laboratory up to day-5 did not significantly increase the success rate of pregnancy. Extension of the embryo culture until day-5 has been considered can increase the total cost and has potential of psychological burden if ET is fail to be done.

The success in IVF cycle is to have pregnancy with a single fetus without obstetric complications, with lower cost.[16-18] However, the results from many studies have not determined which transfer day would give the best result. Further study should be needed in order to determine which parameters influence the success rate of pregnancy in IVF cycle.

The weaknesses of this retrospective study are due to its long interval, more restricted criteria on day-5 embryo transfer, and unequal number of patients in each embryo transfer group.

CONCLUSION AND SUGGESTION

In conclusion, success in achieving pregnancy is strongly influenced by the total score of the quality of embryos and not by the day of embryo transfer, oocyte quantity, technical difficulty during ET, or contamination of blood and mucus on the ET catheter.

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