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Predictive model for blastocyst stage in in vitro fertilization

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Abstract

This study aims to determine predictive model for blastocyst stage development in In Vitro Fertilization (IVF). We retrieved embryos upon day five in January 2012 to October 2012. Categorical variables were compared using chi-square test and continues variables were analyzed using independent t-test, while $p<0.05$ was considered statistically significant. To identify the relationship between statistically significant variables with occurrence of blastocyst, multivariate logistic regression analysis was done. We identified 302 embryos from 37 subjects whom at least one embryo achieved blastocyst stage. Among which, we found 154 blastocysts (51%) and 148 embryos (49%) which did not achieve blastocyst stage. Two variables were statistically significant in influencing the formation of blastocyst, which are symmetrical pro nucleotide morphology with OR 4.08 (95% CI 2.30-7.25, $p<0.001$) and number of cells in day-2 with OR 2.20 (95%CI 1.33-3.64, $p=0.001$). Logistic regression analysis revealed that symmetrical pro nucleotide morphology and four cells number gave good chance of blastocyst prediction. Predictive model was Z = -1.236 + 1.203 (pro nucleotide score) + 0.732 (four cells score). This study reveals the symmetrical pro nucleotide morphology and four cells score as predictive model for blastocyst stage in IVF.

Keywords: Blastocyst stage, Embryo, IVF, Morphology

1. Introduction

The main issues facing clinician regarding embryo transfer (ET) in In Vitro Fertilization (IVF) program is to have single embryo transfer which has the best quality thus can have a better pregnancy rate outcome by increasing the implantation opportunity. Clinicians believe that embryos which can survive into blastocyst stage have going through natural selection thus ET is done in day five after ovum pick-up.

It is proven by Van der Auwera that blastocyst transfer yields clinical pregnancy outcome up to 60% when it compares with traditional day three cleavage transfer of 35% ($p<0.01$) [1]. Neonatal outcomes after cleavage versus blastocyst transfer increases likelihood of preterm birth (OR 1.35; 95% CI, 1.07-1.71) [2]. For these reasons, embryologist tried to improve the quality of embryos developing in the laboratory, such as by making a better media culture and making a close monitoring of embryos which can advance into blastocyst.

In order to guarantee the quality of the embryos development, every minute are crucial thus it makes embryologist often has to take out embryos from incubator to see its development under the microscope. However, this technique is believed to make a stressor towards the embryos and can have a greater impact towards the further development inside the uterus. Nowadays, the introduction of embryoscope into the laboratory gives a promising outcome towards monitoring the development of the embryos each day without the need to taken out embryos from its incubator. However, not every laboratory is equipped with this tool due to high cost supply and maintenance. Thus, in order to have single blastocyst transfer,

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embryologists need to do a conventional ways to closely monitor the development under the microscope each time. Blastocyst can be achieved, but will it affect the implantation, pregnancy rate, and perinatal outcomes when it compared to cleavage day three transfer rates? The answer is yes. Randomized Controlled Trial (RCT) done by Gardner whom compares transfer blastocyst versus cleavage stage did not suggest superiority in terms of births per oocyte pick-up. These techniques resulting in the same pregnancy rate [3]. Newest meta-analysis shows that among 23 RCTs conducted, there is no difference found in the clinical pregnancy outcome of blastocyst transfer compared with cleavage transfer (OR 1.14, 95%CI, 0.99-1.32) [4].

Due to the same outcome, there is a need to change the attitude to have a blastocyst transfer when there is no time-lapse monitor into the traditional cleavage stage. But the thought of blastocyst will yield into a better outcome, thus identification of embryos with the highest developmental potential into blastocyst is crucial. This study aims to determine morphological predictive model for blastocyst stage development in IVF to improve outcome for transfer.

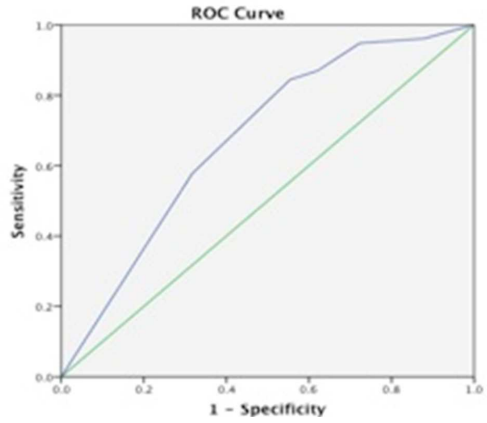

Figure 1. AUC in predicting the model for blastocyst stage in IVF. The AUC is 0.75, concluded as quite strong predictability, with 82.2% sensitivity and 64.2% specificity.

2. Materials and methods

2.1. Study design

The design of this study is retrospective cohort study. Embryos had been retrieved in January 2012 until October 2012, which was resulted in blastocyst development upon day five. All data were obtained from the database of *Yasmin* Reproductive Clinic *Dr. Cipto Mangunkusumo* National Hospital.

2.2. Statistical analysis

Statistical Package for the Social Sciences (SPSS) Software version 20.0 was being used to evaluate the relationship between morphological criteria of embryos such as pro nucleotide cells, number of cells, and fragmentation rate with the achievement of blastocyst stage in IVF. For bivariate analysis, chi-square analysis of 2x2 tables was performed. Independent t-test was then used for continuous variables meanwhile $p<0.05$ was considered statistically significant. To identify the relationship between statistically significant clinical variables with the occurrence of blastocyst, multivariate logistic regression analysis was done.

3. Results

We identified 302 embryos from 37 cases whom at least one embryo achieved blastocyst stage. Among which, it was found 154 blastocyst (51%), and 148 embryo (49%) did not achieved blastocyst stage as shown in baseline characteristics of the subjects. Symmetrical pro nucleotide morphology contributes to 226 embryos (74.83%), good fragmentation rate are seen in 222 embryos, and four cells can be seen in 163 embryos (53.97%) (see Table 1).

Table 1. Baseline characteristics of the subjects

Variables	N	%
Blastocysts		
Yes	154	50.99%
No	148	49.01%
Pro nucleotide morphology		
Symmetrical	226	74.83%
Non-symmetrical	76	25.27%
Fragmentation rate		
Good (<10%)	222	73.51%
Fair (10-25%)	60	19.87%
Poor (>50%)	20	6.62%
Number of cells in day 2		
2-3 cells	38	12.58%
4 cells	163	53.97%
5-8 cells	101	33.44%

Within bivariate analysis, symmetrical pro nucleotide morphology gives significant influence to the formation of blastocyst with OR 4.08 (95% CI 2.30-7.25, $p<0.001$) (see Table 2). When compares with four cells stage, 5-8 cells gives more ratios to the development of blastocyst OR 2.20 (95% CI 1.33-

3.64). On the other hand, 2-3 cells gives less ratios OR 0.18 (95% CI 0.07-0.44). Fragmentation rate did not give statistically significance, thus it did not being calculated in the multivariate analysis.

Variables which had p<0.25 from bivariate analysis were subjected to multivariate analysis using logistic regression of Backward Stepwise with the factors affecting the occurrence of development of blastocyst in the following order: pronucleotid morphology and four cells score with OR 3.3; (95% CI 1.82-6.10) and OR 1.08; (95% CI 1.24-3.50) respectively. Thus, equation obtained to predict development of blastocyst is Z = -1.236 + 1.203 (symmetrical pro nuclei) + 0.732 (four cells score) (see Table 3).

In measuring the prediction strength, the area under the receiver operating characteristic (ROC) curve was analyzed. The ROC curve analyzed had the area under the curve (AUC) of 0.75, which showed as quite strong predictable factors (see Figure 1). An AUC value of 0.5 indicates random performance while an AUC value of 1.0 indicates perfect conditions. The sensitivity was 82.2% and specificity was 64.2%. It reveals that factors mentioned are accurate enough as predictive models of blastocyst development further.

Table 2. Demographics morphological characteristics

	Blastocyst					
	Yes		**No**		**p**	**OR (CI)**
	n	**%**	**N**	**%**		
Number of cells in day 2						
2-3 cells	14	32.60%	29	67.40%	0.001	0.176(0.070-0.444)
4 cells	76	51.70%	71	48.30%		Reference
5-8 cells	58	48.30%	62	51.70%	0.001	2.20 (1.33-3.64)
Fragmentation rate						
Good	119	53.60%	103	46.40%	0.212	
Fair	28	46.70%	32	53.30%		
Poor	7	35.00%	13	65.00%		
Pro nucleotide morphology						
Symmetrical	128	56.60%	98	43.40%	0	4.08 (2.30-7.25)
Non-Symmetrical	15	19.70%	61	80.30%		

Abbreviations: OR: Odds Ratio, CI: Confidence Interval

Table 3. Coefficient results

	Coefficient	**p**	**OR (CI)**
Number of cells in day 2			
4 cells	0.732	0.001	1.08(1.24-3.50)
Pro nucleotide morphology			
Symmetrical	1.203	0.001	3.3 (1.82-6.10)
Constant	-1.236		

Abbreviations: OR: Odds Ratio, CI: Confidence Interval

4. Discussion

This study conducted to assess morphological characteristics, such as pro nucleotide morphology, number of cells, and fragmentation rate in determining the morphological predictive model for blastocyst stage development in IVF. In the limited resources, this approach is quite simple yet reliable method compared to the other methods aiming to produce better outcome of IVF. These characteristics were seen upon day two development but agreement upon classification which can identified good morphological characteristics are hard to describe, and the observer themselves might influence the outcome of the predictor characteristic.

To date, IVF clinics tend to select embryos for further transfer based on their development rate and morphological features as assessed by light microscopy. Having an international consensus on embryo assessment would help to validate the use of embryo morphology as an end-point in studies in IVF.

As a result, the Istanbul consensus workshop on embryo assessment has developed points to define the minimum criteria for oocyte and embryo morphology assessment. It was expected that the definition of common terminology and standardization of laboratory practice related to embryo morphology assessment would result in more effective comparisons of treatment outcome. Consensus scoring systems, which had developed, include scoring system for pronuclei, cleavage-stage embryos, day four embryos, and blastocyst. The scoring system for blastocyst, as numerical interpretation of the Gardner scale, is a combination of the stage of development, and of the grade of the inner cell mass (ICM) and trophectoderm (TE) [5].

The first result obtained in this study revealed the significance of symmetrical pro nucleotide morphology in predicting the quality of blastocyst, which is in accordance with studies conducted before. Studies regarding morphological characteristic found day two morphology as the common basis for embryo grading and usually comprise five aspects: appearance of the cytoplasm, pro nuclei, nucleoli, cytoplasmic deficit, and developmental rate, which showed the strongest association with blastocyst development [6]. In addition, the cell number, degree of fragmentation, symmetry of blastomeres, and degree of compaction, clarity and texture are also important factors in selecting the best day 3 embryos [7].

The nucleolus is a distinct nuclear construct where ribosomal nucleic acids (RNAs) are produced. The nucleoli in human embryos are thought to be inactive in the sense of ribosomal RNA synthesis before the third division of blastomere, but chromatin infiltration may occur at the time of nucleolar precursor bodies (NPB) formation and for the proper assembly of NPB also early RNA synthesis takes place. It has been suggested that DNA encoding ribosomal genes of early human embryo is incapable of initiating the transcription unless it is associated with protein matric of NPB [8].

The migration process begins when the two pro nuclears (PNs) normally appear within a short interval and rapidly migrate to center of the cytoplasm meanwhile nucleoli, or nucleolar precursor bodies are scattered, coalesce, then becomes polarized at adjacent poles of the positioned PNs.

Failure to migrate and asynchronous nucleolar dynamics are associated with a lower developmental potential, which related with epigenetic sperm dysfunction as a cause of the failure of PNs to migrate in combination with abnormal PN formation. Those explain why PN and nucleolar morphology seemed influencing the protein synthesis mechanism and to be strong determinants of blastocyst development [6]. Study conducted by Senn also showed that the dynamic sequences of morphological changes occurring in the pronuclear zygote plays a critical role for subsequent embryo development and may be used as markers of implantation potential of the embryo [9].

Other result indicates the importance of the cleavage rate of the embryos, which showed that compares with four cells stage, 5-8 cells gives more ratios to the development of blastocyst while 2-3 cells gives less ratios. Studies referred to cleavage rate as a major determinant of the development. Sjoblom revealed that embryos at the four cells stage even at 42 hours after insemination have a better developmental capacity than slower or faster embryos. Early cleavage or entry into syngamy at 25-29 hours after insemination has also been shown to correlate well with developmental potential [6].

Reference used as the normal cleavage rate is being in four cells in day two. However, another study appointed that early-cleavage embryos on day two were reported to achieve higher implantation rates compared to non-early-cleavage embryos. Furthermore, early-cleavage and cell number on day 2 were the most powerful parameters to predict the development of a good morphology blastocyst at day five. The reason may be speculated that such embryos derive from oocytes with adequately synchronized cytoplasmic and nuclear maturation, so they should be less likely to be exposed to a critical minimal level of maternal mRNA before activation of the embryonic genome [10]. Suggestions made regarding studies about cleavage rate is observing the embryos development in using real-time approach, so that the cleavage mechanism could be observed in detail and be discovered whether it is still in accordance or not with the normal cleavage rate.

In our study, fragmentation rate did not show statistically significance, even though it indicated the trend of better blastocyst development in association with good fragmentation rate. It has been hypothesized that the fragmentation is a part of variation in cleavage mechanism. However, studies showed the similar results explained that fragments might physically impede cell-cell interactions, interfering with compaction, cavitation, and blastocyst formation. Fragments also might release toxic substances damaging nearby cells and reduce the volume of cytoplasm, which then deplete the embryos of essential organelles or polarized domains [10]. Other study appointed to cytoplasmic fragmentation related with mosaicism and originated in spindle errors [11].

Sjoblom also found that a cytoplasmic fragment is commonly used in embryo scoring and it is probably more appropriate to evaluate the fragmentation patterns [6].

To date, there are many studies are using embryo scoring to predict the outcome of pregnancy rather than blastocyst development rate. The reason for this is that scientists believe the use of cleavage stage as a prediction of the development of blastocyst is poor [7, 12]. Hence, clinicians are more interested in improving the pregnancy rate rather than the other variables influencing it. But, both of clinicians and embryologist should be aware that in order to improve pregnancy rate, it is all started with the quality of the embryo to begin with. At present study, we have seen strong correlation between pro nucleotide morphology with blastocyst stage, which is consistent with the study of Sjoblom ($r = 0.13$; $p < 0.01$) [6]. In addition, the division reaching four cells at day two after ovum pick-up gave a good prediction upon the development of blastocyst stage.

Authors are aware that present study only sees the morphological features with assumption it can predict the development of the blastocyst after it being transferred and not other factors such as quality of sperm. Thus, further study is needed in order to see the real correlation between several factors, which can predict the outcome of blastocyst stage and pregnancy rate.

5. Conclusion

To summarise, we have identified morphological characteristics such as pro nucleotide morphology and four cells score, which can predict the outcome of blastocyst. With the equation given above, we hope that they can give good prediction of embryo which can reach blastocyst stage, thus it will resulted in better implantation and pregnancy rate.

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References:

1. Van der Auwera I, Debrock S, Spiessens C, et al. A prospective randomized study: day 2 versus day 5 embryo transfer. Hum Reprod. 2002; 17(6): 1507-1512.
2. Fernando D, Halliday JL, Breheny S, Healy DL. Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology. Fertil Steril. 2012; 97(3): 579-584.
3. Gardner DK, Lane M, Calderon I, Leeton J. Environment of the preimplantation human embryo in vivo: metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. Fertil Steril. 1996; 65(2): 349-353.
4. McLernon DJ, Harrild K, Bergh C, Davies M J, et al. Clinical effectiveness of elective single versus double embryo transfer: meta-analysis of individual patient data from randomised trials. BMJ. 2010; 341: c6945.
5. Alpha Scientists in Reproductive, Medicine, & Embryology, Eshre Special Interest Group of. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011; 26(6): 1270-1283.
6. Sjoblom P, Menezes J, Cummins L, et al. Prediction of embryo developmental potential and pregnancy based on early stage morphological characteristics. Fertil Steril. 2006; 86(4): 848-861.
7. Milki AA, Hinckley MD, Gebhardt J, et al. Accuracy of day 3 criteria for selecting the best embryos. Fertil Steril. 2002; 77(6): 1191-1195.
8. Salumets A, Hyden-Granskog C, Suikkari AM, et al. The predictive value of pronuclear morphology of zygotes in the assessment of human embryo quality. Hum Reprod. 2001; 16(10): 2177-2181.
9. Senn A, Urner F, Chanson A, Primi MP, et al. Morphological scoring of human pronuclear zygotes for prediction of pregnancy outcome. Hum Reprod. 2006; 21(1): 234-239.
10. Guerif F, Le Gouge A, Giraudeau B, et al. Limited value of morphological assessment at days 1 and 2 to predict blastocyst development potential: a prospective study based on 4042 embryos. Hum Reprod. 2007; 22(7): 1973-1981.
11. Arroyo G, Santalo J, Parriego M, et al. Pronuclear morphology, embryo development and chromosome constitution. Reprod Biomed Online. 2010; 20(5): 649-655.
12. Rijnders PM, Jansen CA The predictive value of day 3 embryo morphology regarding blastocyst formation, pregnancy and implantation rate after day 5 transfer following in-vitro fertilization or intracytoplasmic sperm injection. Hum Reprod. 1998; 13(10): 2869-2873.