Prediction of Blastocyst Quality from Morphokinetic Criteria

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Background: *The assisted reproductive technologies continue to improve in both techniques and results. The goal to achieve better clinical pregnancy rates by using time-lapse video cinematography as a non-invasive selected embryo transfer method, now under investigation.*

Objective: *This study aims to verify the morphokinetic criteria to discriminate good morphological blastocyst (group I) from poor morphological blastocyst (group II) by using Primo vision® (Vitrolife, Sweden).*

Material and Method: *The prospective study was performed in infertile couples treated with assisted reproductive technology at Thammasat Fertility Center, Thailand, in 2015. The morphokinetic study was compared between the two groups by t-test and Mann-Whitney U test with $p<0.05$ as statistical significance.*

Results: *Total of 63 blastocysts from 20 infertile couples was included. Thirty-three good quality blastocysts (52.38%) in group I and 30 poor quality blastocysts (47.62%) in group II were under morphokinetic study. T5 and CC3 were longer in group I compared to those in group II (mean = 51.85 and 15.04 vs. 47.98 and 12.42 hours, p = 0.03 and 0.04, respectively). Tm of group I was shorter than group II (mean = 88.16 vs. 96.64 hours, p = 0.00), Tb and S2 were also shorter in group I compared to those in group II (median = 109.50 and 1.00 vs. 118.19 and 1.95 hours, p = 0.00 and 0.02, respectively).*

Conclusion: *T5, CC3 and S2 of cleavage stage could be used to predict the quality of blastocyst. Tm and Tb of blastocyst stage are also useful in good quality blastocyst selection.*

Keywords: *Morphokinetic criteria, Cleavage stage embryo, Blastocyst stage embryo, Time-lapse embryoscope, Assisted reproductive technology*

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Selection of good quality embryo with high implantation potential is very important in the assisted reproductive technology treatment of infertility. However, the embryo quality evaluation method using morphological criteria is still not adequately effective. At present, Time-lapse embryoscope has been developed to follow through the whole process of embryo development, timing of each stage of development covering the development information up to blastocyst stage, called "morphokinetic criteria". Meseguer M, et al[1] showed the relationship between embryo grading by morphokinetic criteria and morphological criteria including embryo implantation potential in 247 embryos transferred on day 3 after fertilization. Meseguer M, et al[2] compared embryo culture with conventional method to that with time-lapse embryoscope. They found that using time-lapse embryoscope system could increase clinical pregnancy rate. Campbell[3] also found early compaction and shortened time to blastocyst stage (Tb) in euploid blastocysts. This technology is simple, easy, non-invasive and without disturbance of embryo culture system, but costly. However, the values of optimal morphokinetic criteria of embryo quality is still in developing process. Each clinic has its own normal standard values. This study aims to develop the optimal morphokinetic values of Thammasat Fertility Center for further study and application.

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Material and Method

The prospective study was performed in infertile couples who had at least 6 oocytes collected in assisted reproductive technology treatment cycle at Thammasat Fertility Center in 2015 with the approval of Thammasat University Research Ethic Committee No. 1. The female infertile partners were treated with controlled ovarian hyperstimulation protocol using recombinant follicle stimulating hormone (Gonal-F®,

Merck Serono, Italy) and gonadotropin releasing hormone antagonist (Cetrotide®, Merck Serono, Italy) together with recombinant human chorionic gonadotropin (Ovidrel® Merck Serono, Italy) for ovulation induction. The oocyte retrieval was performed 36 hours after ovulation induction under transvaginal ultrasound guided with double lumen catheter. The oocytes were inseminated by intracytoplasmic sperm injection with prepared density gradient centrifugation sperms (Sil-Select Plus®, Fertipro, Belgium) at 4 hours after retrieval. After insemination, the embryo was cultured with Global culture media® (Life-Global, USA) under paraffin oil (Life-Global, USA) in the tri-gas (89% nitrogen, 5% oxygen and 6% carbon-dioxide) incubator under time-lapse monitoring system (Primo vision®, Vitrolife, Sweden) for 6 days. The time-lapse embryoscope monitoring system was recorded every 10 minutes from insemination up to blastocyst stage. The morphokinetic results were compared between the two groups of blastocysts derived from morphological criteria according to Gardner and Schoolcraft criteria[4]. Group I embryos with good quality blastocysts were defined as 3AA, 3AB, 3BA, 3BB, 4AA, 4AB, 4BA and 4BB and Group II: embryos with poor quality blastocysts defined as the lower grading. The morphokinetic study also included the time to 2 cells stage (T2), time to 3 cells stage (T3), time to 4 cells stage (T4), time to 5 cells stage (T5), time to 8 cells stage (T8), time to morula stage (Tm), time to blastocyst stage (Tb), second cell cycle length (CC2), third cell cycle length (CC3), synchrony of the second cell divisions (S2) and synchrony of the third cell divisions (S3) (Meseguer M, et al[1]). Those embryos which did not reach the blastocyst stage were excluded from the study. The calculated sample size was 27.8 blastocysts in each group, at $\alpha = 0.05$ and $\beta = 0.20$. The descriptive data was presented by percentage, mean and standard deviation.The means of data with normal distribution were compared by t-test, whereas the medians of data, which was not normal distribution, were compared by Mann-Whitney U test at significant level of 0.05.

Results

Twenty infertile couples were recruited in the study, with the age of 36.90±3.77 years and range of 30-44 years old. The mean of total recombinant gonadotropin dose used was 1,965±584 IU. Total of 179 oocytes (141 M II, 21 M I and 17 GV) were collected. The average number of oocytes per retrieval was 8.95±1.88 (7.05±2.19 MII, 1.05±0.83 MI, and 0.85±0.79 GV). One hundred and nineteen oocytes were fertilized (84%). Eighty four cleavage stage on day 3 (70.59%) and 63 blastocysts on day 5 (52.94%) were obtained. There were 33 blastocysts (52.38%) in group I and 30 blastocysts (47.62%) in group II (Table 1).

Time to 5 cells stage embryo (T5) in group I was longer than that in group II (mean = 51.85 vs. 47.98 hours, $p = 0.03$). Time to morula (Tm) in group I was shorter than that in group II (mean 88.16 vs. 96.64 hours, $p = 0.00$). Time to blastocyst stage (Tb) in group I was shorter than that in group II (median 109.50 vs. 118.19 hours, $p = 0.00$). There was no difference in time to 2 cells (T2), 3 cells (T3), 4 cells (T4) and 8 cells (T8) stage embryo between the two groups (Table 2).

Third cell cycle length (CC3) was longer in group I compared to that in group II (mean 15.04 vs. 12.42 hours, $p = 0.04$). The synchrony of the second cell divisions (S2) in group I was shorter than that in group II (median 1.00 vs. 1.95 hours, $p = 0.02$). The second cell cycle length (CC2) and the synchrony of the third cell divisions (S3) were not different between

Table 1. The age of infertile female partners, number of oocytes, number of cleavage stage embryo and blastocyst

Number of infertile female partner (n)	20
Age (years)	
Mean ± SD	36.90±3.77
Range (years)	30-44
Total recombinant gonadotropin dose (IU) (mean ± SD)	1,965±584
Total oocytes (n, %)	179 (100)
MII	141 (78.77)
MI	21 (11.73)
GV	17 (9.50)
The average number of oocyte per retrieval (mean ± SD)	8.95±1.88
MII	7.05±2.19
MI	1.05±0.83
GV	0.85±0.79
The fertilized oocyte (n, %)	119 (84)
The cleavage stage embryos on day 3 (n, %)	84 (70.59)
The blastocyst on day 5 (n, %)	63 (52.94)
Group I[a]	33 (52.38)
Group II[b]	30 (47.62)

[a] Group I: Embryo with good quality blastocyst was defined as 3AA, 3AB, 3BA, 3BB, 4AA, 4AB, 4BA and 4BB.
[b] Group II: Embryo with poor quality blastocyst was defined as the lower grading.
Both a, b were grading according to Gardner and Schoolcraft criteria[4]

Table 2. Comparison of morphokinetic study between the good and poor quality blastocysts

Morphological criteria	Blastocyst group I [a] (n = 33)						Blastocyst group II[b] (n = 30)						
Morphokinetic criteria	Mean (hours)	SE (hours)	25th percentile (hours)	Median (hours)	75th percentile (hours)	Interval (hours)	Mean (hours)	SE (hours)	25th percentile (hours)	Median (hours)	75th percentile (hours)	Interval (hours)	*p*-value
T2 (Time to 2 cells)	25.55	0.48	23.48	25.32	27.29	21.42-33.04	26.29	0.58	24.21	26.36	28.32	19.28-34.41	0.32[c]
T3 (Time to 3 cells)	36.81	0.60	35.13	37.22	39.15	27.26-45.07	35.56	1.02	30.52	35.56	39.72	27.26-49.45	0.30[c]
T4 (Time to 4 cells)	38.42	0.63	37.05	38.40	40.88	28.46-46.18	40.27	1.29	36.50	39.29	43.66	28.29-55.44	0.20[c]
T5 (Time to 5 cells)	51.85	1.00	49.66	52.57	54.81	30.46-62.39	47.98	1.37	41.12	48.03	54.71	36.04-60.15	0.03[c]*
T8 (Time to 8 cells)	65.87	1.10	60.36	65.39	71.29	54.56-79.22	65.95	1.89	57.61	65.59	71.25	51.43-96.06	0.97[c]
Tm (Time to morula)	88.16	1.49	82.73	88.50	94.04	62.14-108.45	96.64	1.81	87.88	97.21	101.09	82.48-128.08	0.00[c]**
Tb (Time to blastocyst)	110.51	1.21	104.73	109.50	116.31	100.15-127.08	117.33	1.84	111.89	118.19	120.07	95.54-139.02	0.00[d]**
CC2 (2nd cell cycle length)	11.26	0.46	10.71	11.80	12.77	2.00-14.00	9.27	0.80	5.88	11.15	12.10	0.70-15.04	0.11[d]
CC3 (3rd cell cycle length)	15.04	0.64	13.27	14.25	17.56	3.20-24.74	12.42	1.14	7.86	12.52	16.18	1.00-25.30	0.04[c]*
S2 (Synchrony of the 2nd cell divisions)	1.61	0.38	0.25	1.00	1.82	0.10-10.64	4.71	0.95	0.70	1.95	10.17	0.10-15.30	0.02[d]*
S3 (Synchrony of the 3rd cell divisions)	14.02	1.34	9.19	13.20	18.39	2.15-41.68	17.97	1.57	11.99	16.97	20.63	3.10-39.77	0.06[c]

[a] Group I: embryo with good quality blastocyst was defined as 3AA, 3AB, 3BA, 3BB, 4AA, 4AB, 4BA and 4BB
[b] Group II: embryo with poor quality blastocyst was defined as the lower grading
[c] Mean values of data were compared by t-test
[d] Median values of data were compared by Mann-Whitney U test
* $p<0.05$, ** $p<0.01$

both groups (Table 2).

Discussion

This study revealed that there were 5 morphokinetic parameters (T5, CC3, S2, Tm and Tb) those could discriminate the good morphological blastocyst from the poor morphological blastocyst according to Gardner and Schoolcraft[4]. T5 and S2 obtained from this study were two important parameters which were similar to those of the other study (Meseguer M, et al)[1] in which the morphokinetic model could select the cleavage stage embryo with implantation potential. This study also showed that CC3 was different between good and poor quality blastocysts. This is similar to the study of Basile N, et al[5]. They found that if CC3 was in the optimal range, the embryo was twice likely to be euploid. Tm and Tb found in this study were shorter in good quality embryo and this finding was more or less similar to those of other study (Campbell)[3] in that they found the euploid blastocysts had shorter Tm and Tb than those of the aneuploid blastocysts. This study showed that the cleavage stage timing T5, CC3 and S2 could be used to predict the blastocyst quality and be useful for the day 3 embryo transfer protocol. This study also showed that Tm and Tb may be used to predict the good quality blastocyst that may be useful for the day 5 embryo transfer protocol and also in elective single embryo transfer (eSET) especially in the condition that pre-implantation genetic screening cannot be performed or afforded. However, this study still cannot compare the clinical results such as implantation rate or pregnancy rate because of lacking our own proper morphokinetic criteria to discriminate the good from the poor quality blastocysts. And also because of the implantation process is more complicated due to its involvement with the timing of endometrial receptivity and endometrial quality, not just only the quality of embryo. Therefore, this study aimed to develop only the criteria that could be used to discriminate the good quality embryo from the poor quality embryo for the first step. Then, in the second step we will study toward the correlation between our own established morphokinetic criteria and implantation.

Further comparative study should be conducted in prospective randomized controlled trial between the good morphological blastocyst combined with 5 morphokinetic criteria found in this study and only the good morphological blastocyst in eSET. This non-invasive embryo selection method still needs further study on the impact and outcome of the main results of assisted reproductive technology such as implantation rate, clinical pregnancy rate and live birth rate. Up to now there were only two randomized controlled trials (Kahraman S, et al[6] and Rubio I, et al[7]) comparing the time lapse monitoring culture system and standard conventional culture system. Only the Rubio I et al[7] study had adequate power to show the superiority of the time lapse monitoring culture system in ongoing pregnancy rate. However, the time lapse morphokinetic parameters used to predict the good quality embryos were still vary between studies[8-15] due to difference in patient characteristics, ovarian stimulation protocols, culture conditions and the time lapse monitoring systems. Therefore, optimal morphokinetic criteria should be developed in each IVF center before applying this method in routine service.

Conclusion

T5, CC3 and S2 of the cleavage stage of good quality embryo were different from poor quality embryo. For the blastocyst stage embryo, Tm and Tb were different between good and poor quality blastocysts.

What is already known on this topic?

Time lapse morphokinetic study of embryo is potential non-invasive method in selection of good quality embryo.

The optimal value of each parameter of morphokinetic criteria is variable and depends on many factors.

What this study adds?

Developing our own morphokinetic criteria and our optimal values to select good quality embryo by non-invasive method for further study on improving success rates in assisted reproductive technology treatment for infertile couples should be undertaken.

Ethics consideration

The present study had the approval of Thammasat University Research Ethic Committee No. 1.

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Potential conflicts of interest

None.

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การทำนายคุณภาพของตัวอ่อนระยะบลาสโตซิสต์โดยใช้ morphokinetic criteria

เจริญไชย เจียมจรรยา, พัชรา วิสุตกุล, จินดารัตน์ เจนเกรียงไกร

ภูมิหลัง: ไทม์แลปส์เอ็มบริโอสโคปเป็นเทคโนโลยีที่พัฒนาขึ้นเพื่อใช้ในการติดตามและให้ข้อมูลเกี่ยวกับระยะเวลาของ พัฒนาการของตัวอ่อนในแต่ละระยะได้อย่างต่อเนื่องซึ่งเรียกว่า "มอร์โฟไคเนติก" ได้ถูกนำมาใช้เพื่อที่จะช่วยทำให้อัตราการตั้งครรภ์ของการรักษาโดยใช้เทคโนโลยีช่วยการเจริญพันธุ์เพิ่มขึ้น การศึกษาครั้งนี้เป็นการศึกษาเปรียบเทียบความแตกต่างระหว่างค่ามอร์โฟไคเนติกของตัวอ่อนที่มีคุณภาพดีกับคุณภาพไม่ดี เพื่อใช้ในการคัดเลือกคุณภาพของตัวอ่อนระยะบลาสโตซิสต์

วัตถุประสงค์: เพื่อศึกษาหาเกณฑ์ของค่ามอร์โฟไคเนติกที่จะใช้แยกตัวอ่อนระยะบลาสโตซิสต์ที่มีรูปร่างที่ดีออกจากตัวอ่อนระยะบลาสโตซิสต์ที่มีรูปร่างไม่ดีโดยใช้เครื่องพริโมวิชั่น (บริษัทวิโทรไลฟ์ ประเทศสวีเดน)

วัสดุและวิธีการ: ทำการศึกษาในสตรีที่มีบุตรยากที่มารักษาโดยใช้เทคโนโลยีช่วยการเจริญพันธุ์ที่หน่วยผู้มีบุตรยาก โรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ ปี พ.ศ. 2558 โดยศึกษาในตัวอ่อนระยะบลาสโตซิสต์ทั้งหมด 63 ตัว จากสตรีที่มีบุตรยาก 20 ราย ซึ่งได้จากการทำการปฏิสนธิโดยวิธีฉีดอสุจิเข้าในเซลล์ไข่แล้วทำการเพาะเลี้ยงตัวอ่อนภายใต้เครื่องไทม์แลปส์เอ็มบริโอสโคปเป็นเวลา 6 วัน โดยบันทึกเวลาและช่วงของเวลาที่ตัวอ่อนมีการแบ่งเซลล์เป็น 2, 3, 4, 5, 8 เซลล์, มอรูล่า, และบลาสโตซิสต์จากนั้นแบ่งกลุ่มบลาสโตซิสต์ออกเป็น 2 กลุ่ม คือกลุ่มที่มีคุณภาพดีและกลุ่มที่คุณภาพไม่ดี โดยพิจารณาจากลักษณะรูปร่างของบลาสโตซิสต์แล้วทำการเปรียบเทียบค่ามอร์โฟไคเนติกของตัวอ่อนทั้งสองกลุ่มโดยใช้สถิติ t-test และ Mann-Whitney U test เพื่อทดสอบ ความแตกต่างที่ระดับความเชื่อมั่นเท่ากับ 0.05

ผลการศึกษา: จากการศึกษาพบความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่ามอร์โฟไคเนติก ระหว่างตัวอ่อนระยะบลาสโตซิสต์ที่มีคุณภาพดีและไม่ดีทั้งหมด 5 ค่า ได้แก่ ระยะเวลาที่ตัวอ่อนแบ่งเป็น 5 เซลล์ (T5) (ค่าเฉลี่ย 51.85 และ 47.98 ชั่วโมงหลังปฏิสนธิ, p = 0.03), เวลาที่ตัวอ่อนเป็นระยะมอรูล่า (Tm) (ค่าเฉลี่ย 88.16 และ 96.64 ชั่วโมงหลังปฏิสนธิ, p = 0.00), ระยะเวลาที่ตัวอ่อนเป็นระยะบลาสโตซิสต์ (Tb) (ค่ากลาง 109.50 และ 118.19 ชั่วโมงหลังปฏิสนธิ, p = 0.00), ระยะเวลาการแบ่งเซลล์รอบที่สาม (third cell cycle length) (CC3) (ค่าเฉลี่ย 15.04 และ 12.42 ชั่วโมงหลังปฏิสนธิ, p = 0.04), และช่วงเวลาที่ใช้ขณะแบ่งเซลล์ครั้งที่ 2 (ความยาวของช่วงเวลาที่มีการเปลี่ยนแปลงของตัวอ่อนจาก 3 เซลล์ไปเป็น 4 เซลล์) (S2) (ค่ากลาง 1.00 และ 1.95 ชั่วโมงหลังปฏิสนธิ, p = 0.02)

สรุป: การศึกษาครั้งนี้พบว่าค่า T5, CC3 และ S2 สามารถนำมาใช้เป็นเกณฑ์ของค่ามอร์โฟไคเนติก (morphokinetic criteria) ของตัวอ่อนระยะแบ่งเซลล์ (cleavage stage embryo) ในการทำนายการพัฒนาไปเป็นตัวอ่อนระยะบลาสโตซิสต์ที่มีคุณภาพดี ส่วนค่า Tm และ Tb ก็สามารถนำมาใช้เพื่อคัดเลือกตัวอ่อนระยะบลาสโตซิสต์ที่มีคุณภาพดีได้