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ภาคผนวก

วิธีการเตรียมกรดอะมิโนในแต่ละจำพวกเพื่อทดสอบผลของกรดอะมิโนต่อการเจริญของเอ็มบริโอในระยะ 72 - 192 ชั่วโมงหลังผสม

1. เตรียมน้ำยาเพาะเลี้ยง mHECM-3 หรือ BM-3 ที่ไม่มีกลูตามีนจำนวน 1 ลิตร (ตามในตารางที่ 2.1) คำนวมน้ำหนักสารต่าง ๆ ตามตารางสารละลายใน 1000 มิลลิลิตร ซึ่งสารตามน้ำหนักที่คำนวณได้ลงในแก้วตวงขนาด 500 มิลลิลิตร เติมน้ำ Milli Q water 400 มิลลิลิตร ละลายสารต่าง ๆ ให้เข้ากัน นำไปเทลงในขวดตวงขนาด 1000 มิลลิลิตร เติมน้ำ Milli Q water จนมีปริมาตรของสารละลาย 1000 มิลลิลิตร เขย่าให้เข้ากัน นำไปวัด pH และ ออสโมลาริตี ซึ่งควรมีค่าประมาณ 7.2 - 7.4 และ 275 ± 5 mOsmol ตามลำดับ

2. เตรียมกรดอะมิโนชนิดไม่จำเป็น (NEA) ซึ่งน้ำหนักของกรดอะมิโนในแต่ละตัว (ตามตารางที่ 2.2) ตามขั้นตอนต่อไปนี้

- Gly + Glu = หลอดที่ 1
- GIN + SER = หลอดที่ 2
- AIA + ASP = หลอดที่ 3
- Cystine = หลอดที่ 4
- HyPro + Tyr + Pro = หลอดที่ 5

ใส่ในหลอดทดสอบขนาด 10 มิลลิลิตร จำนวน 6 หลอด เติมน้ำยาเพาะเลี้ยง BM-3 จำนวน 10 มิลลิลิตร ในแต่ละหลอดทดสอบจนครบ 6 หลอด เขย่าให้กรดอะมิโนละลายให้หมด เติมกรดอะมิโนที่ละลายหมดแล้วในแต่ละหลอด จำนวน 1 มิลลิลิตร ลงในหลอดทดสอบที่มี BM-3 ในข้อ 1 อยู่ 5 มิลลิลิตร พร้อมทั้งเติม 100 ไมโครลิตรของ Cysteine (1000X) และเติม PSA 100 ไมโครลิตร เขย่าให้เข้ากัน กรองสารละลายที่ได้ ซึ่งก็คือทรีเมนต์

3. เตรียมกรดอะมิโนชนิดจำเป็น (EA) ซึ่งน้ำหนักของกรดอะมิโนชนิดจำเป็นในแต่ละตัว (ในตารางที่ 2.3) ตามขั้นตอนต่อไปนี้

- LEU + ILE
- TRP + VAL
- ARG + HIS
- LYS + MET
- PHE + THR

ใส่ในหลอดทดสอบขนาด 10 มิลลิลิตร จำนวน 5 หลอด เติมน้ำยาเพาะเลี้ยง BM-3 10 มิลลิลิตร ในแต่ละหลอดทดสอบจนครบจำนวน 5 หลอดทดสอบ

เขย่าให้กรดอะมิโนละลายให้หมด เติมกรดอะมิโนที่ละลายหมดแล้วในแต่ละหลอดจำนวน 1 มิลลิลิตร ลงไปในหลอดทดสอบขนาด 10 มิลลิลิตรที่มี BM-3 อยู่ 5 มิลลิลิตร พร้อมทั้งเติม PSA 100 ไมโครลิตร เขย่าให้เข้ากัน กรองสารละลายที่ได้ จึงเป็นทรีเมนต์

4. เตรียมกรดอะมิโนชนิดไม่จำเป็น (NEA) และกรดอะมิโนชนิดจำเป็น (EA) นำกรดอะมิโนชนิดไม่จำเป็นและชนิดจำเป็น เติมลงในหลอดทดสอบขนาด 10 มิลลิลิตร ลงไปอย่างละ 1 มิลลิลิตร จนครบได้ปริมาณ 10 มิลลิลิตร พร้อมทั้งเติม 100 ไมโครลิตรของ Cysteine (1000X) และเติมสารละลาย PSA 100 ไมโครลิตร กรองสารละลายที่ได้ จึงเป็นทรีเมนต์ 3

5. ดูด stock solution จากข้อ 1 จำนวน 9 มิลลิลิตร เติม Bovine Calf Serum จำนวน 1 มิลลิลิตร เติม 100 ไมโครลิตรของ PSA นำมากรองด้วย millipore filter จึงเป็นทรีเมนต์ 4

6. ดูด stock solution ของ TCM-199 จำนวน 9 มิลลิลิตร เติม Bovine Calf Serum จำนวน 1 มิลลิลิตร เติม 100 ไมโครลิตรของ PSA นำมากรองด้วย millipore filter จึงเป็นทรีเมนต์ 4

จุฬาลงกรณ์มหาวิทยาลัย

**การเตรียมน้ำยาเพาะเลี้ยง เพื่อทดสอบผลของกลูโคสและฟอสเฟตต่อการเจริญของเอ็มบริโอ
ในระยะ 72 - 192 ชั่วโมงหลังผสม**

1. เตรียมน้ำยา BM-3 + กรดอะมิโนชนิด NEA + EA จำนวน 40 มิลลิลิตร ที่มี PSA จำนวน 40 ไมโครลิตร ไว้เป็น stock solution โดยผ่านการกรองด้วย Millipore filter
2. ชั่งกลูโคส 0.0036 กรัม ใส่ในหลอดใหม่ แล้วดูด stock solution จากข้อที่ 1 มา 10 มิลลิลิตร ใส่ลงไป เขย่าให้ละลาย จะได้เป็นทริเมนต์ 2 (2.0 mM Glucose)
3. ดูดทริเมนต์ 2 มา 1 มิลลิลิตร ใส่ในหลอดใหม่ ดูดเอา stock solution จากข้อที่ 1 มา 9 มิลลิลิตร ผสมลงไป จะได้ทริเมนต์ 1 (0.2 mM Glucose)
4. ชั่งกลูโคสมา 0.1002 กรัม ใส่ลงในหลอดเล็กขนาด 5 มิลลิลิตร แล้วดูด stock solution จากข้อที่ 1 มา 1 มิลลิลิตร ผสมลงไป เขย่าให้ละลาย จะได้ความเข้มข้น 100X ของ 5.56 mM Glucose
ตั้งนั้นดูด Stock solution จากข้อ 1 มาจำนวน 10 มิลลิลิตร ใส่ลงในหลอดทดสอบขนาด 10 มิลลิลิตร แล้วดูด 100 X ของ 5.56 mM Glucose มาจำนวน 100 ไมโครลิตร ผสมลงไปจะได้ทริเมนต์ 3
5. ชั่ง NaH_2PO_4 มา 0.0048 กรัม ใส่ลงในหลอดทดสอบขนาด 5 มิลลิลิตร ดูด stock solution จากข้อ 1 มา 1 มิลลิลิตร ผสมลงไป เขย่าให้ละลายจนได้ 100X ของ 0.35 mM NaH_2PO_4
6. ดูดทริเมนต์ 1 มาจำนวน 5 มิลลิลิตร ใส่ลงในหลอดทดสอบขนาด 10 มิลลิลิตร และดูด 100X NaH_2PO_4 มาจำนวน 50 ไมโครลิตร ผสมลงไป จะได้ ทริเมนต์ 4
7. ดูดทริเมนต์ 2 มาจำนวน 5 มิลลิลิตร ใส่ลงในหลอดทดสอบขนาด 10 มิลลิลิตร และดูด 100X NaH_2PO_4 มาอีก 50 ไมโครลิตร ผสมลงไป จะได้ ทริเมนต์ 5
8. ดูดทริเมนต์ 3 มาจำนวน 5 มิลลิลิตร ใส่ลงในหลอดทดสอบขนาด 10 มิลลิลิตร และดูด 100X NaH_2PO_4 มาจำนวน 50 ไมโครลิตร ผสมลงไป จะได้ ทริเมนต์ 6
9. ดูด stock solution ของ BM-3 จำนวน 9 มิลลิลิตร ใส่ลงในหลอดทดสอบขนาด 10 มิลลิลิตร และดูด Bovine calf serum จำนวน 1 มิลลิลิตร ผสมลงไป จะได้ ทริเมนต์ 7

10. คุก stock solution ของ TCM-199 จำนวน 9 มิลลิลิตร ใต้งไปในหลอดทดสอบขนาด 10 มิลลิลิตร และคุก Bovine calf serum จำนวน 1 มิลลิลิตร ผสมลงไป จะได้ ปริมาตร 8

สารเคมีที่ใช้ในการเตรียม DNA จากเซลล์เม็ดเลือดขาวและเม็ดเลือดแดง

1. liquid nitrogen
2. Digestion buffer ได้แก่
 - 100 mM NaCl
 - 10 mM TrisCl, pH8
 - 25 mM EDTA, pH8
 - 0.5% sodium dodecyl sulfate
 - 0.1 mg/ml proteinase K:
 ใส่ในน้ำยาเมื่อเตรียมเสร็จและใช้ทันที
3. Ice-cold phosphate buffered saline (PBS)
4. 25: 24: 1 phenol/ chloroform / isoamyl alcohol
5. 7.5 M ammonium acetate
6. 100% และ 70% ethanol
7. TE buffer, pH8
8. 0.1% sodium dodecyl sulfate (SDS)
9. 1 mg/ml Dnase - free Rnase

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

**ผลการวิเคราะห์ทางสถิติของการทดสอบกรดอะมิโนชนิดต่าง ๆ ต่อการเจริญของ
เอ็มบริโอโค ในระยะการเพาะเลี้ยงที่ 2 ในหลอดทดลอง**

Experiment 1

Anova: Single Factor of Experiment 1, Blast
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	16	0.0932	0.0583	0.0048
Treatment 2	16	0.2538	0.0159	0.0012
Treatment 3	16	0.5456	0.0341	0.0031
Treatment 4	16	0.2000	0.0125	0.0011
Treatment 5	16	0.3889	0.0243	0.0045

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.0216	4	0.0054	1.8300	0.1319	2.4937
Within Groups	0.2213	75	0.0030			
Total	0.2429	79				

Anova: Single Factor of Experiment 1, Exp Blast
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	16	1.3500	0.0844	0.0146
Treatment 2	16	1	0.0625	0.0118
Treatment 3	16	2.7461	0.1716	0.0119
Treatment 4	16	4.4210	0.2763	0.0098
Treatment 5	16	3.8551	0.2409	0.0135

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.5630	4	0.1407	11.4234	2.71E-07	2.4937
Within Groups	0.9241	75	0.0123			
Total	1.4870	79				

Anova: Single Factor of Experiment 1, Hatch Blast
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	16	0	0	0
Treatment 2	16	0.0769	0.0048	0.0004
Treatment 3	16	0	0	0
Treatment 4	16	1.7520	0.1095	0.0094
Treatment 5	16	1.5816	0.0989	0.0240

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.2032	4	0.0508	7.5279	3.74E-05	2.4937
Within Groups	0.5060	75	0.0067			
Total	0.7092	79				

Anova: Single Factor of Experiment 1, Total Blast
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	16	2.2825	0.1427	0.0144
Treatment 2	16	1.3308	0.0832	0.0126
Treatment 3	16	3.2917	0.2057	0.0125
Treatment 4	16	6.3730	0.3983	0.0144
Treatment 5	16	5.8256	0.3641	0.0337

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.2112	4	0.3028	17.2979	4.30E-10	2.4937
Within Groups	1.3129	75	0.0175			
Total	2.5242	79				

ผลการวิเคราะห์ทางสถิติของการทดสอบกลูโคสและฟอสเฟตต่อการเจริญของเอ็มบริโอโค ในระยะการเพาะเลี้ยงที่ 2 ในหลอดทดลอง

Experiment 2

Anova: Single Factor of Experiment 2, Less than Morulas
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	4	5	1.25	0.9167
Treatment 2	4	0	0	0
Treatment 3	4	2	0.5	0.3333
Treatment 4	4	3	0.75	0.25
Treatment 5	4	0	0	0
Treatment 6	4	0	0	0
Treatment 7	4	0	0	0
Treatment 8	4	0	0	0

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	6.375	7	0.9107	4.8571	0.0016	2.4226
Within Groups	4.5	24	0.1875			
Total	10.88	31				

Anova: Single Factor of Experiment 2, Morulas
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	4	6	1.5	5.6667
Treatment 2	4	2	0.5	1
Treatment 3	4	6	1.5	1.6667
Treatment 4	4	6	1.5	1.6667
Treatment 5	4	4	1	1.6667
Treatment 6	4	6	1.5	1.6667
Treatment 7	4	2	0.5	1
Treatment 8	4	2	0.5	0.3333

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	6.875	7	0.9821	0.5749	0.7691	2.4226
Within Groups	41	24	1.7083			
Total	47.88	31				

Anova: Single Factor of Experiment 2, Early Blast
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	4	0.1	0.025	0.0025
Treatment 2	4	0.1	0.025	0.0025
Treatment 3	4	0.2	0.05	0.0033
Treatment 4	4	0.1	0.025	0.0025
Treatment 5	4	0.2	0.05	0.0033
Treatment 6	4	0.1	0.025	0.0025
Treatment 7	4	0.1	0.025	0.0025
Treatment 8	4	0.1	0.025	0.0025

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.004	7	0.0005	0.1978	0.9829	2.4226
Within Groups	0.065	24	0.0027			
Total	0.069	31				

Anova: Single Factor of Experiment 2, Blast
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	4	0	0	0
Treatment 2	4	0	0	0
Treatment 3	4	0	0	0
Treatment 4	4	0.1	0.025	0.0025
Treatment 5	4	0	0	0
Treatment 6	4	0.1	0.025	0.0025
Treatment 7	4	0	0	0
Treatment 8	4	0	0	0

Anova: Single Factor of Experiment 2, Exp Blast
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	4	1.2	0.3	0.0133
Treatment 2	4	0.4	0.1	0.0067
Treatment 3	4	0.5	0.125	0.0225
Treatment 4	4	0.8	0.2	0.0333
Treatment 5	4	0.4	0.1	0.0067
Treatment 6	4	0.8	0.2	0.02
Treatment 7	4	0.9	0.225	0.0292
Treatment 8	4	1	0.25	0.01

Anova: Single Factor of Experiment 2, Hatch Blast
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	4	0.2	0.05	0.0033
Treatment 2	4	0.5	0.125	0.0225
Treatment 3	4	0.1	0.025	0.0025
Treatment 4	4	0	0	0
Treatment 5	4	0	0	0
Treatment 6	4	0.1	0.025	0.0025
Treatment 7	4	0.3	0.075	0.0025
Treatment 8	4	0.7	0.175	0.0158

Anova: Single Factor of Experiment 2, Total Blast
SUMMARY

Groups	Count	Sum	Average	Variance
Treatment 1	4	1.5	0.375	0.0425
Treatment 2	4	1	0.25	0.0167
Treatment 3	4	0.8	0.2	0.06
Treatment 4	4	1	0.25	0.0367
Treatment 5	4	0.6	0.15	0.0033
Treatment 6	4	1.1	0.275	0.0158
Treatment 7	4	1.3	0.325	0.0492
Treatment 8	4	1.8	0.45	0.03

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.004	7	0.0005	0.8571	0.553	2.4226
Within Groups	0.015	24	0.0006			
Total	0.019	31				

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.15	7	0.0214	1.2101	0.3352	2.4226
Within Groups	0.425	24	0.0177			
Total	0.575	31				

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.11	7	0.0157	2.5496	0.041	2.4226
Within Groups	0.148	24	0.0061			
Total	0.257	31				

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.26	7	0.0371	1.1677	0.3571	2.4226
Within Groups	0.763	24	0.0318			
Total	1.022	31				

สารเคมีที่ใช้เตรียมกรดอะมิโนชนิดต่าง ๆ

mHECM-3 NON-ESSENTIAL AMINO ACIDS (NEA)

	M.W.	mM	10 x (mM)	g/10 ml
Alanine	89.09	0.56	5.6	0.0050
Aspartate* (acid)	133.1	0.45	4.5	0.0060
Cysteine HCl.H ₂ O	175.6	0.0006	0.6 (1000x)	0.0011
Glutamate* (acid)	169.1	0.89	8.9	0.0150
Glutamine	146.1	0.68	6.8	0.0099
Glycine	75.07	0.67	6.7	0.0050
Proline	115.1	0.35	3.5	0.0040
Serine	105.1	0.48	4.8	0.0050
Tyrosine 2 Na H ₂ O	225.2	0.26	2.6	0.0059
Cystine 2HCl	313.2	0.08	0.8	0.0025
Hydroxy proline	131.1	0.08	0.8	0.0010

mHECM-3 ESSENTIAL AMINO ACIDS (EA)

	M.W.	mM	10 x (mM)	g/10 ml
Arginine HCl	210.7	0.33	3.3	0.0070
Histidine HCl.H ₂ O	209.6	0.1	1	0.0021
Isoleucine	131.2	0.31	3.1	0.0041
Leucine	131.2	0.92	9.2	0.0121
Lysine HCl	182.6	0.38	3.8	0.0069
Methionin	149.2	0.2	2	0.0030
Phenylalanine	165.2	0.3	3	0.0050
Threonine	119.1	0.5	5	0.0060
Tryptophan	204.2	0.1	1	0.0020
Valine	117.1	0.43	4.3	0.0050

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ข้อมูลการเพาะเลี้ยงไข่โคในจานทดลอง เป็นระยะเวลา 72 ชั่วโมงหลังผสม

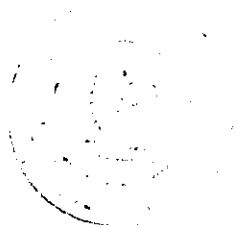
Treatment	Replicate	Duplicate	Number of egg		Balst		Exp. Balst		Hatch Balst		Total Balst	
			No.	%	No.	%	No.	%	No.	%		
1	1	1	12	1	8.33%	3	25.00%	0	0.00%	4	33.33%	
1	1	2	13	1	7.69%	0	0.00%	0	0.00%	1	7.69%	
1	2	1	12	1	8.33%	0	0.00%	0	0.00%	1	8.33%	
1	2	2	12	2	16.67%	0	0.00%	0	0.00%	2	16.67%	
1	3	1	10	1	10.00%	0	0.00%	0	0.00%	1	10.00%	
1	3	2	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
1	4	1	10	1	10.00%	1	10.00%	0	0.00%	2	20.00%	
1	4	2	9	2	22.22%	0	0.00%	0	0.00%	2	22.22%	
1	5	1	10	0	0.00%	2	20.00%	0	0.00%	2	20.00%	
1	5	2	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
1	6	1	10	0	0.00%	1	10.00%	0	0.00%	1	10.00%	
1	6	2	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
1	7	1	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
1	7	2	10	1	10.00%	1	10.00%	0	0.00%	2	20.00%	
1	8	1	10	0	0.00%	4	40.00%	0	0.00%	4	40.00%	
1	8	2	10	0	0.00%	2	20.00%	0	0.00%	2	20.00%	
2	1	1	13	1	7.69%	0	0.00%	1	7.69%	2	15.38%	
2	1	2	13	1	7.69%	0	0.00%	0	0.00%	1	7.69%	
2	2	1	13	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
2	2	2	12	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
2	3	1	10	0	0.00%	4	40.00%	0	0.00%	4	40.00%	
2	3	2	10	1	10.00%	1	10.00%	0	0.00%	2	20.00%	
2	4	1	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
2	4	2	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
2	5	1	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
2	5	2	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
2	6	1	10	0	0.00%	1	10.00%	0	0.00%	1	10.00%	
2	6	2	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
2	7	1	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
2	7	2	10	0	0.00%	1	10.00%	0	0.00%	1	10.00%	
2	8	1	10	0	0.00%	1	10.00%	0	0.00%	1	10.00%	
2	8	2	10	0	0.00%	2	20.00%	0	0.00%	2	20.00%	
3	1	1	11	1	9.09%	4	36.36%	0	0.00%	5	45.45%	
3	1	2	13	1	7.69%	1	7.69%	0	0.00%	2	15.38%	
3	2	1	12	2	16.67%	1	8.33%	0	0.00%	3	25.00%	
3	2	2	12	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
3	3	1	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	
3	3	2	10	0	0.00%	1	10.00%	0	0.00%	1	10.00%	
3	4	1	9	0	0.00%	1	11.11%	0	0.00%	1	11.11%	
3	4	2	9	1	11.11%	1	11.11%	0	0.00%	2	22.22%	
3	5	1	10	0	0.00%	2	20.00%	0	0.00%	2	20.00%	
3	5	2	10	0	0.00%	1	10.00%	0	0.00%	1	10.00%	
3	6	1	10	0	0.00%	1	10.00%	0	0.00%	1	10.00%	
3	6	2	10	1	10.00%	1	10.00%	0	0.00%	2	20.00%	
3	7	1	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	
3	7	2	10	0	0.00%	2	20.00%	0	0.00%	2	20.00%	
3	8	1	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	
3	8	2	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	

Treatment	Replicate	Duplicate	Number		Balst		Exp. Balst		Hatch Balst		Total Balst	
			of egg	No.	%	No.	%	No.	%	No.	%	
4	1	1	13	0	0.00%	2	15.38%	0	0.00%	2	15.38%	
4	1	2	10	0	0.00%	2	20.00%	1	10.00%	3	30.00%	
4	2	1	12	0	0.00%	3	25.00%	3	25.00%	6	50.00%	
4	2	2	11	0	0.00%	3	27.27%	1	9.09%	4	36.36%	
4	3	1	10	1	10.00%	3	30.00%	2	20.00%	6	60.00%	
4	3	2	10	1	10.00%	2	20.00%	0	0.00%	3	30.00%	
4	4	1	9	0	0.00%	4	44.44%	1	11.11%	5	55.56%	
4	4	2	10	0	0.00%	4	40.00%	1	10.00%	5	50.00%	
4	5	1	10	0	0.00%	2	20.00%	1	10.00%	3	30.00%	
4	5	2	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	
4	6	1	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	
4	6	2	10	0	0.00%	2	20.00%	2	20.00%	4	40.00%	
4	7	1	10	0	0.00%	5	50.00%	0	0.00%	5	50.00%	
4	7	2	10	0	0.00%	2	20.00%	2	20.00%	4	40.00%	
4	8	1	10	0	0.00%	2	20.00%	3	30.00%	5	50.00%	
4	8	2	10	0	0.00%	3	30.00%	1	10.00%	4	40.00%	
5	1	1	13	0	0.00%	5	38.46%	1	7.69%	6	46.15%	
5	1	2	13	0	0.00%	2	15.38%	1	7.69%	3	23.08%	
5	2	1	12	2	16.67%	2	16.67%	4	33.33%	8	66.67%	
5	2	2	12	0	0.00%	3	25.00%	7	58.33%	10	83.33%	
5	3	1	10	0	0.00%	2	20.00%	1	10.00%	3	30.00%	
5	3	2	10	0	0.00%	3	30.00%	1	10.00%	4	40.00%	
5	4	1	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	
5	4	2	10	0	0.00%	1	10.00%	1	10.00%	2	20.00%	
5	5	1	9	2	22.22%	0	0.00%	1	11.11%	3	33.33%	
5	5	2	10	0	0.00%	2	20.00%	0	0.00%	2	20.00%	
5	6	1	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	
5	6	2	10	0	0.00%	1	10.00%	0	0.00%	1	10.00%	
5	7	1	10	0	0.00%	4	40.00%	0	0.00%	4	40.00%	
5	7	2	10	0	0.00%	4	40.00%	1	10.00%	5	50.00%	
5	8	1	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	
5	8	2	10	0	0.00%	3	30.00%	0	0.00%	3	30.00%	

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Experiment 2

Treatment	Replicate	Number of egg	Number							≥7 cell	< Morula	Early Blast		Blast		Exp Blast		Hatch Blast		Total Blast	
			1 cell	2 cell	3 - 6 cell	cell	Morula	No.	%			No.	%	No.	%	No.	%	No.	%	No.	%
1	1	10	0	0	3	7	2	0	0	1	10.00%	0	0.00%	4	40.00%	1	10.00%	4	40.00%	4	33.33%
1	2	10	0	0	2	8	1	1	0	0	0.00%	0	0.00%	4	40.00%	1	10.00%	1	10.00%	1	7.69%
1	3	10	1	3	1	5	0	0	0	0	0.00%	0	0.00%	2	20.00%	0	0.00%	0	0.00%	1	8.33%
1	4	10	1	1	3	5	2	5	0	0	0.00%	0	0.00%	2	20.00%	0	0.00%	2	20.00%	2	16.67%
2	1	10	0	1	3	6	0	0	0	0	0.00%	0	0.00%	1	10.00%	2	20.00%	1	10.00%	1	10.00%
2	2	10	0	0	6	4	0	2	0	0	0.00%	0	0.00%	1	10.00%	3	30.00%	0	0.00%	0	0.00%
2	3	10	2	2	2	4	0	0	1	10.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	2	20.00%	
2	4	10	2	2	1	5	0	0	0	0.00%	0	0.00%	2	20.00%	2	20.00%	0	0.00%	2	22.22%	
3	1	10	0	2	2	6	0	3	1	10.00%	0	0.00%	2	20.00%	0	0.00%	0	0.00%	2	20.00%	
3	2	10	0	0	2	8	0	0	1	10.00%	0	0.00%	3	30.00%	1	10.00%	0	0.00%	0	0.00%	
3	3	10	2	2	5	1	1	1	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	10.00%	
3	4	10	2	0	4	4	1	2	0	0.00%	0	0.00%	0	0.00%	4	40.00%	0	0.00%	0	0.00%	
4	1	10	0	0	1	9	0	1	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
4	2	10	0	0	4	6	0	3	0	0.00%	1	10.00%	1	10.00%	3	30.00%	0	0.00%	2	20.00%	
4	3	10	1	2	5	4	1	2	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	4	40.00%	
4	4	10	2	2	2	4	1	1	1	10.00%	0	0.00%	0	0.00%	1	10.00%	0	0.00%	2	20.00%	
5	1	10	0	3	3	4	0	1	0	0.00%	0	0.00%	2	20.00%	0	0.00%	2	20.00%	2	15.38%	
5	2	10	1	0	3	5	0	2	0	0.00%	0	0.00%	1	10.00%	0	0.00%	0	0.00%	1	7.69%	
5	3	10	2	0	5	3	0	0	1	10.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
5	4	10	2	2	0	6	0	1	1	10.00%	0	0.00%	1	10.00%	1	10.00%	0	0.00%	0	0.00%	
6	1	10	0	0	5	5	0	1	0	0.00%	0	0.00%	0	0.00%	2	20.00%	1	10.00%	4	40.00%	
6	2	10	0	0	4	6	0	3	0	0.00%	1	10.00%	1	10.00%	3	30.00%	0	0.00%	2	20.00%	
6	3	10	2	0	8	0	0	0	1	10.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	
6	4	10	0	0	4	6	0	2	0	0.00%	0	0.00%	3	30.00%	0	0.00%	0	0.00%	0	0.00%	
7	1	10	0	3	6	1	0	0	1	10.00%	0	0.00%	2	20.00%	1	10.00%	0	0.00%	0	0.00%	
7	2	10	0	0	6	4	0	2	0	0.00%	0	0.00%	3	30.00%	1	10.00%	0	0.00%	0	0.00%	
7	3	10	3	2	5	0	0	0	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	10.00%	
7	4	10	0	2	3	5	0	0	0	0.00%	0	0.00%	4	40.00%	1	10.00%	0	0.00%	0	0.00%	
8	1	10	0	1	4	5	0	0	0	0.00%	0	0.00%	3	30.00%	2	20.00%	0	0.00%	0	0.00%	
8	2	10	0	0	3	7	0	1	0	0.00%	0	0.00%	3	30.00%	2	20.00%	2	20.00%	1	10.00%	
8	3	10	3	0	4	3	0	1	1	10.00%	0	0.00%	1	10.00%	1	10.00%	0	0.00%	1	10.00%	
8	4	10	1	0	2	7	0	0	0	0.00%	0	0.00%	3	30.00%	3	30.00%	3	30.00%	2	20.00%	



ประวัติผู้เขียน

นางสาวสุนันทา สักดิ์ทวีกุลกิจ เกิดเมื่อวันที่ 9 มีนาคม 2499 เป็นชาว กรุงเทพมหานคร ได้รับการศึกษาระดับปริญญาตรี จากคณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ เมื่อปีการศึกษา 2523 ต่อมาได้รับการศึกษาต่อในระดับปริญญาโท จากคณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ เมื่อปีการศึกษา 2527 และเริ่มเข้ารับราชการในตำแหน่งอาจารย์ ระดับ 4 ตั้งกักภาควิชาวิทยาศาสตร์ คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตปัตตานี เมื่อปี พ.ศ. 2533 จนถึงปัจจุบัน



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