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APPENDIX

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

A. Compositions of Modified T6 and HEPES-T6 medium

Component	Modified T6 (mM)	HEPES- T6 (mM)
NaCl	99.4	99.4
KCl	1.42	1.42
MgCl ₂ . 6H ₂ O	0.47	0.47
Na ₂ HPO ₄	0.36	0.36
Glucose	5.56	5.56
NaHCO ₃	25.00	4.00
Na pyruvate	0.47	0.47
Na lactate (60% syrup)	24.9	24.9
CaCl ₂ .2H ₂ O	1.78	1.78
Penicillin G	100 IU /ml	100 IU /ml
Streptomycin sulfate	50 µg/ml	50 µg/ml
HEPES	-	21.00
Phenol red	0.001%	0.001%
BSA	5 mg/ml	5 mg/ml

BSA = bovine serum albumin

Final osmolality 288-292 mOsm/Kg H₂O

pH 7.2-7.4

B. Embryo biopsy medium

Component	Concentration (mM)
NaCl	106.00
KCl	2.70
KH_2PO_4	1.50
Na_2HPO_4	8.10
Glucose	5.60
Na lactate	25.00
Na pyruvate	0.33
EDTA	2.00
Sucrose	100.00
BSA	3.00 mg/ml

EDTA = Ethylenediaminetetraacetic acid

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

C. Acidic Tyrode's solution for removing the zona pellucida

Component	g/100 ml
NaCl	0.800
KCl	0.020
CaCl ₂ . 2H ₂ O	0.024
MgCl ₂ . 6H ₂ O	0.010
Glucose	0.100
Polyvinylpyrrolidone (PVP)	0.400

Adjust to pH 2.5 with Analar HCl

สถาบันวิทยบริการ
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D. TE buffer

1 M Tris- HCl ,pH 8.0	1.0 ml
0.5 M Na ₂ EDTA, pH 8.0	0.2 ml

E. Lysis buffer

50 mM KCl
10 mM Tris-HCl
2.5 mM MgCl ₂
0.5 % tween 20
100 µg/ml Proteinase K

F. 10X TBE buffer stock solution

Tris-Base	108 g
Boric acid	55 g
Na ₂ EDTA	9.3 g
Water to	1 liter
(pH = 8.3)	

G. Control group of the 4-cell stage embryo biopsy

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	31	24	21	17/21
2	29	23	20	17/20
3	30	24	21	20/21
4	34	29	24	20/24
5	25	22	17	15/17
6	22	19	16	13/16
7	30	25	22	20/22
8	26	22	19	16/19
Total	227	189	159	138/159

H. Solution control group of the 4-cell stage embryo biopsy

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	30	24	21	17/21
2	25	21	19	16/19
3	25	19	15	13/15
4	34	27	22	19/22
5	20	17	15	13/15
6	26	21	18	15/18
7	30	24	21	18/21
8	24	21	20	18/20
Total	214	174	151	129/151

สภามหาวิทยาลัย
จุฬาลงกรณ์มหาวิทยาลัย

I. Embryo biopsy at the 4-cell stage by PZD-push technique

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched
		early blastocyst	hatching blastocyst	blastocyst
1	30	24	20	13/20
2	32	24	22	16/22
3	27	22	21	18/21
4	26	21	19	15/19
5	27	21	19	16/19
6	20	16	16	12/16
7	18	14	12	10/12
8	25	20	19	16/19
Total	205	162	148	116/148

J. Embryo biopsy at the 4-cell stage by direct aspiration technique

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	23	16	17	11/17
2	25	18	16	11/16
3	18	13	11	7/11
4	25	19	17	12/17
5	26	20	18	12/18
6	26	21	19	13/19
7	27	22	19	12/19
8	27	23	21	15/21
Total	197	152	138	93/138

K. Control group of the 8-cell stage embryo biopsy

experiment	No. of embryo	No. of embryo developed to		Rate of complete hatched
		early blastocyst	hatching blastocyst	blastocyst
1	23	20	17	15/17
2	30	26	23	19/23
3	42	36	31	27/31
4	35	31	26	23/26
5	20	17	15	13/15
6	43	38	32	27/32
7	27	23	20	17/20
8	36	31	27	24/27
Total	256	222	191	165/191

L. Solution control group of the 8-cell stage embryo biopsy

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	20	16	14	13/14
2	26	22	19	14/19
3	34	29	24	22/24
4	20	17	15	12/15
5	20	17	14	11/14
6	36	30	27	23/27
7	20	17	14	12/14
8	30	26	22	20/22
Total	206	174	149	127/149

จุฬาลงกรณ์มหาวิทยาลัย

M. Embryo biopsy at the 8-cell stage by PZD-push technique

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	27	23	23	19/23
2	36	28	26	21/26
3	50	40	40	31/40
4	40	34	32	27/32
5	28	23	22	18/22
6	50	42	40	33/40
7	28	21	19	14/19
8	35	29	27	21/27
Total	294	240	229	184/229

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

N. Embryo biopsy at the 8-cell stage by direct aspiration technique

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	25	20	18	13/18
2	37	29	25	19/25
3	45	36	33	23/33
4	35	27	26	18/26
5	26	21	19	14/19
6	45	39	31	24/31
7	23	19	18	14/18
8	35	28	25	17/25
Total	271	219	195	142/195

จุฬาลงกรณ์มหาวิทยาลัย

O. Control group of the morula stage embryo biopsy

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	25	21	17	13/17
2	30	25	22	20/22
3	20	18	16	14/16
4	35	32	29	26/29
5	30	26	24	21/24
6	25	22	20	18/20
7	30	27	24	21/24
8	25	22	18	16/18
Total	220	193	170	149/170

P. Solution control group of the morula stage embryo biopsy

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	25	22	20	17/20
2	30	26	22	20/22
3	20	17	14	11/14
4	30	25	23	19/23
5	24	21	20	17/20
6	20	18	16	14/16
7	25	21	19	16/19
8	20	17	15	13/15
Total	194	167	149	127/149

สงวนลิขสิทธิ์
จุฬาลงกรณ์มหาวิทยาลัย

Q. Embryo biopsy at the morula stage by PZD-push technique

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	27	19	17	10/17
2	36	27	27	22/27
3	25	19	18	14/18
4	36	29	28	22/28
5	34	28	28	23/28
6	27	22	21	18/21
7	36	29	29	27/29
8	26	21	19	16/19
Total	238	194	187	1152/187

R. Embryo biopsy at the morula stage by direct aspiration technique

experiment	No. of biopsied embryo	No. of embryo developed to		Rate of complete hatched blastocyst
		early blastocyst	hatching blastocyst	
1	21	17	14	9/14
2	27	21	20	13/20
3	25	19	17	12/17
4	33	28	23	17/23
5	27	22	22	14/22
6	24	20	18	14/18
7	35	29	28	20/28
8	26	21	21	17/21
Total	218	177	163	116/163

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S. Body weight of mouse pups derived from control embryos

Embryo No.	Body weight (gm) at		
	24 hours	3 weeks	6 weeks
1	1.6	25.0	35.8
2	1.5	23.0	36.4
3	1.9	26.0	39.0
4	1.4	22.2	32.5
5	1.5	23.4	35.3
6	1.5	22.5	35.0
7	1.7	25.5	40.0
8	2.1	28.5	41.4
9	1.5	22.5	36.0
10	1.6	24.0	37.4
11	2.0	27.5	40.5
12	1.5	22.0	34.5
mean \pm S.D.	1.65 \pm 0.23	24.3 \pm 2.16	36.98 \pm 2.71

T. Body weight of mouse pups derived from biopsied embryos

Embryo No.	Body weight (gm) at		
	24 hours	3 weeks	6 weeks
1	1.7	24.5	36.5
2	2.0	26.0	40.0
3	1.6	22.5	35.0
4	1.5	22.7	35.5
5	1.5	21.5	33.1
6	1.9	26.5	39.5
7	1.7	26.4	40.2
8	1.6	23.6	35.5
9	1.5	25.5	37.0
10	1.8	28.2	40.0
11	1.5	22.5	34.5
12	1.6	24.5	36.0
mean \pm S.D.	1.66 \pm 0.17	24.50 \pm 2.03	36.90 \pm 2.44

U. Reproductive capacity of mice derived from biopsied embryo

Group	couple	Litter size
1. Control x control	1	12
	2	11
	3	9
2. Control x biopsied	1	8
	2	10
	3	11
	4	13
	5	11
3. Biopsied x biopsied	1	11
	2	10
	3	9
	4	14
	5	10

V. Nucleotide of mouse Y-linked sequence ,Sry

stop

* * *

Sry 1

1. AGA TCT TGA TTT TTA GTG TTC AGC CCT ACA GCC ACA TGA TAT CTTAAA

CTC TGA AGA AGA GAC AA GTT TTG GGA CTG GTG ACA ATT GTC TAG AGA GCA

Met 101

ATG GAG GGC CAT GTC AAG CGC CCC ATG AAT GCA TTT ATG GTG TGG TCC CGT

Sry 2

GG TGA GAG GCA CAA GTT GGC CCA GCA GAA TCC CAG CAT GCA AAA TAC AGA

201

GA TCA GCA AGC AGC TGG GAT GCA GGT GGA AAA GCC TTA CAG AAG CCG AAA

Sry 3

AA AGG CCC TTT TTC CAG GAG GCA CAG AGA TTG AAG ATC CTA CAC AGA GAG

301

AA ATA CCC AAA CTA TAA ATA TCA GCC TCA TCG GAG GGC TAA AGT GTC ACA

GA GGA GTG GCA TTT TAC AGC CTG CAG TTG CCT CAA CAA AAC TGT ACA ACC TT

401

Sry 4

CTG CAG TGG GAC AGG AAC CCA CAT GCC ATC ACA TAC AGG CAA GAC TCC AG

TAG AGC TGC ACA CCT GTA CTC

SRY 1 5' TCT TAA ACT CTG AAG AAG AGA C 3'

SRY 2 5' GTG AGA GGC ACA AGT TGG C 3'

SRY 3 5' GAT TGA AGA TCC TAC ACA GAG 3'

SRY 4 5' CC ATC ACA TAC AGG CAA GAC 3'



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W. Sequence of cDNA for murine Zfy-1

1. ATG GAT GAA GAT GAA ATT GAA TTG ACC CCA GAA GAA GAA AAG TCA TTA

TTT GAT GGA ATA GGA GCT GAT GCA GTA CAC ATG GAT AGT GAC CAG ATT TCT

121

GTA GAA GTA CAA GAA ACT GTT TTT TTA TCT AAT TCA GAT GTA ACT GTG CAC

AAT TTT GTT CCT GAT GAT CCA GAC GCA GTT ATA ATT CAA GAT GTT ATT GAA

241

AAT GTT CTT ATT GAA GAT GTT CAC GGT TCA CAT ATT TTA GAA GAA ACA GAT

ATA TCT GAC AAT GTC ATT ATT CCT GAG CAA GTT CTC GAT TTA GAT ACA GCT

GAA GAA GTG TCT TTA GCA CAG TTC TTA ATT CCA GAC ATT TTA ACT TCC AGT

361

ATT ACA TCA ACC TCA TTG ACT ATG CCT GAA CAT GTC TTG ATG AGT GAA GCT

ATA CAT GTG TCT AAT GTA GGA CAT TTT GAA CAA GTG ATT CAT GAT AGC CTA

481

GTA GAA AGA GAA GTC ACC ACT GAT CCT TTG ACA GCC GAC ATT TCA GAT ATA

CTG GTA GCA GAT TGG GCT TCT GAA GCA GTC TTA GAT TCC AGT GGG ATG CCT

601

CTG GAG CAG CAA GAT GAT GCC AGA ATC AAC TGT GAG GAT TAT CTA ATG ATG

TCT TTG GAT GAG CCT AGC AAA ACA GAT CAT GAA GGT TCC TCT GAA GTT ACC

ATG AAT GCA GAG TCA GAA ACT GAT TCT TCT AAA TTG GAT GAA GCA TCT CCA

721

GAA GTT ATC AAG GTG TGC ATT CTT AAA GCT GAC TCA GAA GTG GAT GAT GTA

GGA GAA ACT ATA CAA GCA GTA GAG AGT GAG ACC GAC AAT GGC AAT GAA GCT

841

GAA GTC ACT GAT CAG AGG ACT AGC ATT CAT GTT CCC AAA GTC AAC ATT TAT

ATG TTA GCC AGT GAT TCG CAA AAG GAA GAA GAA GAT ACT AAA GTA ATT GTA

961

GGA GAT GAA GAT GCT GGT GGC ACA GCT GCA GAT ACT CCT GAG CAT GAG CAA

CAG ATG GAT GTC AGT GAA ATA AAA GCA GCT TTC CTA CCT ATT GCA TGG ACA

GCA GCT TAT GAT AAT AAT TCT GAT GAA ATT GAA GTG CAG AAT GCC ACT GCC

1081

AGT GCT ATG TTA CAC CAT GAT GAG TCT GGT GGC CTT GAC AGA GTA CCA AAA

CAA AAA TCA AAA AAG AAA AAA AGA CCT GAA TCC AAA CAG TAC CAG TCA

1201

GCA ATA TTT GTT GCT CCT GAT GGA CAA ACT TTA CGT GTC TAT CCT TGC ATG

TTT TGT GGG AAA AAA TTT AAG ACC AAA AGG TTT TTG AAA AGA CAC ATA AAA

AAC CAT CCT GAA TAC CTT GCT AAT AAA AAA TAT CAC TGT ACT GAG TGT GAT

1321

Zfy1

TAC AGT ACC AAC AAG AAGATAAGC.TTA.CAT.AAT.CAC.ATG.GAG.AGC.CAC.

Zfy2

AAG CTA ACC ATT AAG ACA GAA AAG ACC ACC GAA TGT GAT GAC TGT.AGG

1441

AAG AAT CTT TCT CAT GCT GGG ACT TTG TGT ACT CAC AAA ACA ATG CAT ACA

GAA AAA GGA GTC AAC AAA ACA TGT AAG TGT AAG TTC TGT GAC TAT GAA ACA

1561

GCT GAA CAG ACA TTA TTG AAT CAC CAC CTT TTG GTG GTC CAC AGG AAG AAA

Zfy 3

TTT CCT CAC ATT TGT GGA GAA TGT GGT AAA GGT TTC CGT CAC CCA TCA GCA

CTC AAA AAG CAC ATA CGA GTT CAC ACA GGA GAG AAG CCC TAT GAA TGT CAG

1681

TAT TGT GAG TAC AAG TCT GCA GAC TCT TCC AAC TTG AAA ACT CAT ATA AAA

TCT AAG CAT AGT AAA GAG ATA CCA CTG AAG TGT GAC ATC TGT CTC CTG ACT

1801

TTC TCA GAT ACC AAA GAG GCT CAG CAA CAT GCC GTT CTG CAC CAA GAA AGC

AGA ACA CAT CAA TGT TCA CAT TGC AAC CAT AAG AGT TCA AAC TCA AGT GAT

2021

TTA AAG CGA CAC ATA ATT TCC GTT CAC ACA AAG GCG TAT CCT CAT AAA TGT

Zfy4

GAC ATG TGC AGC AAA GGA TTT CAT AGG CCT TCA GAA CTC AAG AAG CAT

GTG GCT ACC CAT AAA AGT AAA AAA ATG CAC CAA TGT AGA CAC TGT GAC TTT

2041

AAT AGT CCA GAT CCA TTT CTG CTT AGT CAC CAT ATT CTC TCA GCT CAC ACA

AAG AAT GTT CCA TTC AAG TGT AAG AGA TGT AAA AAG GAA TTT CAA CAA CAG

2161

TGT GAG CTT CAA ACG CAT ATG AAG ACC CAC AGT AGC CGA AAA GTC TAT CAG

TGT GAG TAC TGT GAA TAT AGC ACC AAA GAT GCC TCA GGT TTT AAG CGT CAC

GTT ATC TCC ATT CAT ACG AAA GAC TAT CCT CAC CGC TGT GAC TTC TGC AAG

2281

AAA GGA TTC CGG AGA CCC TCG GAA AAG AAT CAA CAC ATA ATG CGA CAT CAT

AAA GAA GTT GGC CTG CTC TAA GTG TGG AAT CCT AAC ATA TGG GGA CAT TGG

2401

CAT TCA GGC AGG AAA TTC ATT TTA AAA GTA GGC ATC CTT GTT CAC GTT CAA

TGT CAT GAA TTT GTC AAG TGA ATA TAA ATA TGA TAT TGC TTC AAA AAA AAA

AAA AAA AAA AAA AAA AAA AAA AA 2507

ZFY1 5' AAG ATA AGC TTA CAT AAT CAC ATG GA 3'

ZFY2 5' GTA GGA AGA ATC TTT CTC ATG CTG G 3'

ZFY3 5' CCG TCA CCC ATC AGC ACT CAA AAA 3'

ZFY4 5' AC ATG TGC AGC AAA GGA TTT CAT AGG 3'

X. DNA Sequence of the enhancer-containing fragment: DXNds 3

1. GCT GCA GGT CGA GGG ATC CAG CAA CAC CTG GGC CCA AGA CAA GCA ACG
 GGG AGT CAC TAA AGC CCA CTT CTA GGT GTT TAC AGA CTA GAG CAG GAG CTG
 100
 GAA AGT CCC AAT GCT GCC TGT AGC CCA CAG TCC TTG CAT TTC CCT GGA TTT
 200
 GGG TCC TCA GGA ACC GGA AGT AGG GCC TGC TTG CCC CCA CAC CAC CGC TTT
 CTT CCC CAA ACC ACC AGA TCT TTC TGG TAG CCA GGC ACA TAT TTC CCG AAG
 300
 AAT GAA GTC TAG AGT GGG ACA GGA GAC CCT TGC CTC CCC CTG CCA CCT CGC
 CCA CAA AGA CAT ACA CAC ACA CAC ACA CAC ACA CAG TAG CAG TAG
 Nds1 400
 TCA AAA CAA AGG GTT CCT TAT AAG AGT GCC TCA TCT ATA CTT ACA GAA TTC
 Nds2
 ATG TCA GAC CCA ACA CCT AGC CCT ATG CTT ERC CAG TGT ACA TAG AGG GTT
 500
 GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG
 Nds3
 TGT GTG TGT TGT CTC CAA TGG CTG CTT TCC GGA ACC CCC AAA GCA GCA TAA
 Nds4
 ATT GAG TTT AGG ATG GAC ACA GAA ATT TGG AAA GAG GAA GCA ACT AAT CAA
 600
CAA TGA ACT AGA GAT GGG ACC TTG TCC ACA GAG CAT TGC CTG GGC TCT ACA
 700
 GCA GAA GTC TAA GGG GAA AAG CTT AGG GAG GAA TGC CTG GCT AAG GCC CTC

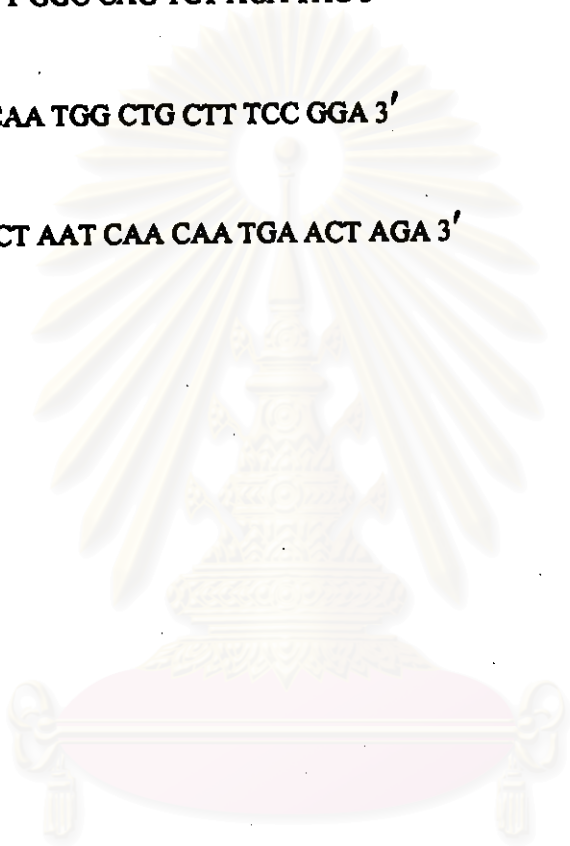
ATT ACT TAA GAA ACA AGT CCT GAC TTC AAA GTG AGC TCC 750

Nds 1 5' GAG TGC CTC ATC TAT ACT TAC AG 3'

Nds 2 5' ATG CTT GGC CAG TGT ACA TAG 3'

Nds 3 5' TCTC CAA TGG CTG CTT TCC GGA 3'

Nds 4 5' GCA ACT AAT CAA CAA TGA ACT AGA 3'



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Comparison of in vitro development between biopsied and control embryo at the 4-cell stage

Blastocyst formation

Hatching blastocyst

	Control	Aspiration	total
	189	152	341
	38	45	83
total	227	197	424
$\chi^2 =$	2.49487		

	Control	Aspiration	total
	159	138	297
	68	59	127
total	227	197	424
$\chi^2 =$	0.0000		

	Solution	Aspiration	total
	174	152	326
	40	45	85
total	214	197	411
$\chi^2 =$	1.0775		

	Solution	Aspiration	total
	151	138	289
	63	59	122
total	214	197	411
$\chi^2 =$	0.0128		

	Control	PZD	total
	189	162	351
	38	43	81
total	227	205	432
$\chi^2 =$	1.2685		

	Control	PZD	total
	159	148	307
	68	57	125
total	227	205	432
$\chi^2 =$	0.2424		

	Solution	PZD	total
	174	162	336
	40	43	83
total	214	205	419
$\chi^2 =$	0.3438		

	Solution	PZD	total
	151	148	299
	63	57	120
total	214	205	419
$\chi^2 =$	0.1368		

	PZD	Aspiration	total
	162	152	314
	43	45	88
total	205	197	402
$\chi^2 =$	0.2048		

	PZD	Aspiration	total
	148	138	286
	57	59	116
total	205	197	402
$\chi^2 =$	0.2250		

Complete Hatching

	Control	Aspiration	total		Solution	PZD	total
	138	93	231		129	116	245
	21	45	66		22	32	54
total	159	138	297	total	151	148	299

$$\chi^2 = 16.0891$$

$$\chi^2 = 2.5118$$

	Solution	Aspiration	total		PZD	Aspiration	total
	129	93	222		116	93	209
	22	45	67		32	45	77
total	151	138	289	total	148	138	286

$$\chi^2 = 13.1752$$

$$\chi^2 = 4.3816$$

	Control	PZD	
	138	116	254
	21	32	53
total	159	148	307

$$\chi^2 = 3.7993$$

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จุฬาลงกรณ์มหาวิทยาลัย

Comparison of in vitro development between biopsied and control embryo at the 8-cell stage

Blastocyst formation

Hatching blastocyst

	Control	Aspiration	total
	222	219	441
	34	52	86
total	256	271	527
$\chi^2 =$	3.36363		

	Control	Aspiration	total
	191	195	386
	65	76	141
total	256	271	527
$\chi^2 =$	0.4730		

	Solution	Aspiration	total
	174	219	393
	32	52	84
total	206	271	477
$\chi^2 =$	1.0771		

	Solution	Aspiration	total
	149	195	344
	57	76	133
total	206	271	477
$\chi^2 =$	0.0082		

	Control	PZD	total
	222	240	462
	34	54	88
total	256	294	550
$\chi^2 =$	2.6339		

	Control	PZD	total
	191	229	420
	65	65	130
total	256	294	550
$\chi^2 =$	0.8165		

	Solution	PZD	total
	174	240	414
	32	54	86
total	206	294	500
$\chi^2 =$	0.6828		

	Solution	PZD	total
	149	229	378
	57	65	122
total	206	294	500
$\chi^2 =$	2.0307		

	PZD	Aspiration	total
	240	219	459
	54	52	106
total	294	271	565
$\chi^2 =$	0.0623		

	PZD	Aspiration	total
	229	195	424
	65	76	141
total	294	271	565
$\chi^2 =$	2.6527		

Complete Hatching

	Control	Aspiration	total		Solution	PZD	total
	165	142	307		127	184	311
	26	53	79		22	45	67
total	191	195	386	total	149	229	378
$\chi^2 =$	10.9107			$\chi^2 =$	0.0000		

	Solution	Aspiration	total		PZD	Aspiration	total
	127	142	269		184	142	326
	22	53	75		45	53	98
total	149	195	344	total	229	195	424
$\chi^2 =$	7.6351			$\chi^2 =$	0.0000		

	Control	PZD	
	165	184	349
	26	46	72
total	191	230	421
$\chi^2 =$	3.0029		

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Comparison of in vitro development between biopsied and control embryo at the morula stage

Blastocyst formation

Hatching blastocyst

	Control	Aspiration	total
	193	177	370
	27	41	68
total	220	218	438
$\chi^2 =$	3.56519		

	Control	Aspiration	total
	170	163	333
	50	55	105
total	220	218	438
$\chi^2 =$	0.37612		

	Solution	Aspiration	total
	167	172	339
	27	46	73
total	194	218	412
$\chi^2 =$	3.6332		

	Solution	Aspiration	total
	149	163	312
	45	55	100
total	194	218	412
$\chi^2 =$	0.23093		

	Control	PZD	total
	193	194	387
	27	44	71
total	220	238	458
$\chi^2 =$	3.3708		

	Control	PZD	total
	170	187	357
	50	51	101
total	220	238	458
$\chi^2 =$	0.11217		

	Solution	PZD	total
	167	194	361
	27	44	71
total	194	238	432
$\chi^2 =$	1.6252		

	Solution	PZD	total
	149	187	336
	45	51	96
total	194	238	432
$\chi^2 =$	0.19314		

	PZD	Aspiration	total
	194	172	366
	44	46	90
total	238	218	456
$\chi^2 =$	0.4906		

	PZD	Aspiration	total
	187	163	350
	51	55	106
total	238	218	456
$\chi^2 =$	0.92124		

Complete Hatching

	Control	Aspiration	total		Solution	PZD	total
	149	116	265		127	152	279
	21	47	68		22	35	57
total	170	163	333	total	149	187	336
$\chi^2 =$	13.9096			$\chi^2 =$	0.91919		

	Solution	Aspiration	total		PZD	Aspiration	total
	127	116	243		152	116	268
	22	47	69		35	47	82
total	149	163	312	total	187	163	350
$\chi^2 =$	8.9457			$\chi^2 =$	4.96957		

	Control	PZD	total
	149	152	301
	21	35	56
total	170	187	357
$\chi^2 =$	2.7266		

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Comparison of PZD-push between different stage of embryo

Blastocyst formation

	4-cell	8-cell	total		8-cell	morula	total
	162	240	402		229	187	416
	43	54	97		65	51	116
total	205	294	499	total	294	238	532
$\chi^2 =$	0.52469			$\chi^2 =$	0.0357		

	4-cell	morula	total	<u>Complete hatching</u>			
	162	194	356	4-cell	8-cell	total	
	43	44	87	116	184	300	
total	205	238	443	32	45	77	
$\chi^2 =$	0.4321			total	148	229	377

	8-cell	morula	total	$\chi^2 =$	0.2149		
	240	194	434	4-cell	morula	total	
	54	44	98	116	152	268	
total	294	238	532	32	35	67	
$\chi^2 =$	0.0013			total	148	187	335
				$\chi^2 =$	0.4358		

Hatching blastocyst

	4-cell	8-cell	total		8-cell	morula	total
	148	229	377		184	152	336
	57	65	122		45	35	80
total	205	294	499	total	229	187	416
$\chi^2 =$	2.1215			$\chi^2 =$	0.0578		

	4-cell	morula	total
	148	187	335
	57	51	108
total	205	238	443
$\chi^2 =$	2.4289		

Comparison of direct Aspiration between different stage of embryo

Blastocyst formation

	4-cell	8-cell	total		8-cell	morula	total
	152	219	371		195	163	358
	45	52	97		76	55	131
total	197	271	468	total	271	218	489
$\chi^2 =$	0.92721			$\chi^2 =$	0.4881		

	4-cell	morula	total	<u>Complete hatching</u>			
	152	177	329	4-cell	8-cell	total	
	45	41	86	93	142	235	
total	197	218	415	45	53	98	
$\chi^2 =$	1.0257			total	138	195	333

	8-cell	morula	total	$\chi^2 =$	1.1469			
	219	177	396	4-cell	morula	total		
	52	41	93	93	116	209		
total	271	218	489	45	47	92		
$\chi^2 =$	0.0114			total	138	163	301	
				$\chi^2 =$	0.5016			

Hatching blastocyst

	4-cell	8-cell	total		8-cell	morula	total
	138	195	333		142	116	258
	59	76	135		53	47	100
total	197	271	468	total	195	163	358
$\chi^2 =$	0.2017			$\chi^2 =$	0.1208		

	4-cell	morula	total
	138	163	301
	59	55	114
total	197	218	415
$\chi^2 =$	1.1571		

Comparison of implantation between biopsied and control embryo at the 4-cell stage

	Control	Aspiration	total
	9	5	14
	21	29	50
total	30	34	64

$$\chi^2 = 2.18138$$

	Control	PZD	total
	9	8	17
	21	28	49
total	30	36	66

$$\chi^2 = 0.5176$$

	PZD	Aspiration	total
	8	5	13
	28	29	57
total	36	34	70

$$\chi^2 = 0.6532$$

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Comparison of implantation between biopsied and control embryo at the 8-cell stage

	Control	Aspiration	total
	15	6	21
	21	24	45
total	36	30	66

$$\chi^2 = 3.54095$$

	Control	PZD	total
	15	11	26
	21	21	42
total	36	32	68

$$\chi^2 = 0.3814$$

	PZD	Aspiration	total
	6	11	17
	24	21	45
total	30	32	62

$$\chi^2 = 1.6077$$

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Comparison of implantation between biopsied and control embryo at the morula stage

	Control	Aspiration	total
	14	9	23
	26	27	53
total	40	36	76

$$\chi^2 = 0.89779$$

	Control	PZD	total
	14	13	27
	26	29	55
total	40	42	82

$$\chi^2 = 0.1520$$

	PZD	Aspiration	total
	13	9	22
	29	27	56
total	42	36	78

$$\chi^2 = 0.3392$$

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Comparison of total live-births between biopsied embryo at the 4-cell stage

	Control	Aspiration	total
	18	6	24
	48	46	94
total	66	52	118

$$\chi^2 = 4.4441$$

	Control	PZD	total
	18	12	30
	48	54	102
total	66	66	132

$$\chi^2 = 1.5529$$

	PZD	Aspiration	total
	12	6	18
	54	46	100
total	66	52	118

$$\chi^2 = 0.9930$$

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Comparison of total live-births between biopsied embryo at the 8-cell stage

	Control	Aspiration	total
	28	16	44
	40	50	90
total	68	66	134
$\chi^2 =$	4.3550		

	Control	PZD	total
	28	23	51
	40	45	85
total	68	68	136
$\chi^2 =$	0.7843		

	PZD	Aspiration	total
	23	16	39
	45	50	95
total	68	66	134
$\chi^2 =$	1.4900		

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Comparison of total live-births between biopsied embryo at the morula stage

	Control	Aspiration	total
	22	11	33
	44	43	87
total	66	54	120

$$\chi^2 = 2.5032$$

	Control	PZD	total
	22	18	40
	44	42	86
total	66	60	126

$$\chi^2 = 0.1512$$

	PZD	Aspiration	total
	18	11	29
	42	43	85
total	60	54	114

$$\chi^2 = 1.3895$$

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Comparison of total live-births between biopsied embryo at three different stage

	4-cell	8-cell	total
	36	67	103
	148	135	283
total	184	202	386

$$\chi^2 = 9.1077$$

	4-cell	morula	total
	36	51	87
	148	129	277
total	184	180	364

$$\chi^2 = 3.8460$$

	8-cell	morula	total
	67	51	118
	135	129	264
total	202	180	382

$$\chi^2 = 1.0423$$

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Comparison of total implantation between biopsied and control embryo at the 4-cell

	4-cell	8-cell	total
	22	32	54
	78	66	144
total	100	98	198

$$\chi^2 = 2.8319$$

	4-cell	morula	total
	22	36	58
	78	82	160
total	100	118	218

$$\chi^2 = 2.0068$$

	8-cell	morula	total
	32	36	68
	66	82	148
total	98	118	216

$$\chi^2 = 0.1142$$

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Curriculum Vitae

I was born in Bangkok, the capital of Thailand, on October, 29 1960. I obtained the degree of Bachelor in Nursing and Midwifery (1979-1983) from the Faculty of Nursing, Siriraj Hospital, Mahidol University. I finished my Master in Physiology (1986-1988) from the interdepartment of Physiology, Chulalongkorn University. At present, I work in the department of Physiology, Faculty of Medicine, Chaing Mai University as an instructor of physiology. My interesting research field was in the assisted reproductive technology.



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