

4) Results – Original Data

Experiment 1 – Preliminary batch 1 – 23/6/03

Treatment 1 and 2=120 embryos	250 oocytes to mature (23/6/03)	240 oocytes to fertilize (24/6/03)
Treatment 3 = 115 embryos	235 into culture (25/6/03)	Cleaved: 191 (81%)

32 hrs post fertil.									
Treatment	Uncleaved	Cleaved	3-4 cells	Medium					
Tr. 1 & 2	80	40		SOF-					
Tr. 3	71	42	2	SOF-					
44 hrs post fertil.									
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	Medium				
Tr. 1 & 2	24	47	46	3	SOF-				
Tr. 3	24	35	53	3	SOF-				
56 hrs post-fertil									
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	Medium	Total embryos			
Tr. 1	3	22	19	21	SOF-	65			
Tr. 2	4	15	19	28	SOF- (2%)	66			
Tr. 3	1	20	20	19	SOF-	60			
68 hrs post-fertil									
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Medium	Total embryos		
Tr. 1	3	16	18	27	1	SOF-	65		
Tr. 2	3	15	20	28		SOF- (2%)	66		
Tr. 3	1	10	15	32	2	SOF-	60		
80 hrs post-fertil									
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Medium	Total embryos		
Tr. 1	6	18	8	14	19	SOF-	65		
Tr. 2	10	13	16	12	15	SOF- (2%)	66		
Tr. 3	1	14	13	12	20	SOF-	60		
92 hrs post-fertil									
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Medium	Total embryos		
Tr. 1	7	13	12	14	19	SOF-	65		
Tr. 2	8	13	16	12	17	SOF- (2%)	66		
Tr. 3	5	10	14	13	18	SOF-	60		
NOTE: Incubator door left open for 12 hrs approx									
104 hrs post-fertil									
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Morula	Medium	Total embryos	
Tr. 1	14		2	6	23	20	SOF-	65	
Tr. 2	17			8	26	15	SOF- (2%)	66	
Tr. 3	18			9	25	8	SOF+	60	

116 hrs post-fertil										
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Morula	Blast	Medium	Total embr	
Tr. 1	40			3		19	3	SOF-	65	
Tr. 2	44				3	15	4	SOF- (2%)	66	
Tr. 3	32					25	3	SOF+	60	
128 hrs post-fertil										
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Morula	Blast	Exg blast	Medium	Total embr
Tr. 1	24	9	3	3	5	18	3		SOF-	65
Tr. 2	26	8	3	9	1	15	4		SOF- (2%)	66
Tr. 3	34	2		5		15	2	2	SOF+	60
140 hrs post-fertil										
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Morula	Blast	Exg blast	Medium	Total embr
Tr. 1	22	9	8		5	18	3		SOF-	65
Tr. 2	30	8	1	2	3	17	5		SOF- (2%)	66
Tr. 3	29	2	1	2	5	17	2	2	SOF+	60
152 hrs post-fertil										
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Morula	Blast	Expg blast	Medium	Total embr
Tr. 1	20	4	10	2	6	19	4		SOF-	65
Tr. 2	30	8	1		3	18	6		SOF- (2%)	66
Tr. 3	24	3	2		1	16	12	2	SOF+	60
DAY 7 164 hrs post-fertil										
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Mor	Blast	Expg blast	Medium	Total embr
Tr. 1	26		10	1	1	13	14		SOF-	65
Tr. 2	21		3	7		11	18	6	SOF- (2%)	66
Tr. 3	18	1	2			9	26	4	SOF+	60

GRADING OF SOME BLASTOCYSTS-Treatment 3 (For practice purposes) – Data not included in thesis

Stage	Grade	Diameter (Gr. Units)	Diameter (Micrometers)	Cell Count (Average)
Blast	3	48	162.7	22
Blast	3	47	159.3	45
Blast	2.5	55	186.4	105
Blast	2.5	52	176.2	56
Blast	2.5	51	172.8	61
Blast	2.5	50	169.5	25
Blast	2.5	45	152.5	61
Blast	2.5	46	155.9	22
Blast	2.5	47	159.3	31
Blast	2.5	52	176.2	63
Blast	2.5	50	169.5	19
Blast	2.5	48	162.7	67
Expg blast	2.5	65	220.3	121
Blast	3	46	155.9	35

Note: Treatments and their numbers correspond to those described in the Material and Methods Section.

Experiment 1 – Preliminary batch 2 – 3/7/03

210 oocytes to mature (3/7/03)	200 oocytes to fertilize (4/7/03)
185 into culture (5/7/03)	Cleaved: 138 (75%)

32 hrs post fertil.							
Uncleaved	Cleaved	3-4 cells					
62	120	3					
44 hrs post fertil.							
Uncleaved	2 cell	3-4 cell	5-8 cell	Medium			
43	47	82	13	SOF-			
56 hrs post-fertil							
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Medium	Total embrs
Tr. 1	2	3	17	15	4	SOF-	41
Tr. 2	4	12	8	14	2	SOF- (2%)	40
Tr. 3	3	14	16	24		SOF-	57
68 hrs post-fertil							
Treatment	Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Medium	Total embrs
Tr. 1		4	10	18	9	SOF-	41
Tr. 2		4	11	14	11	SOF- (2%)	40
Tr. 3		10	15	24	8	SOF-	57

GRADING OF BLASTOCYSTS (Treatment 3) – Data not included in thesis				
Stage	Grade	Diameter (Gr. Units)	Diameter (Micrometers)	Cell Count (Average)
Exg blast	1.5	49	166.061	119
Blast	2	50	169.45	69
Blast	2.5	51	172.839	57
Blast	3	52	176.228	38
Blast	3	53	179.617	35
Blast	2.5	54	183.006	58
Blast	2.5	55	186.395	76
Blast	2.5	56	189.784	93
Exg blast	2	57	193.173	113
Exg blast	2.5	58	196.562	133
Exg blast	2.5	59	199.951	119
Exg blast	3	60	203.34	98
Blast	3	61	206.729	72
Blast	3	62	210.118	53

Note: Treatments and their numbers correspond to those described in the Material and Methods Section.

Experiment 2 – Replicate 1 – 7/7/03

N=50	200 oocytes to mature (7/7/03)	183 oocytes to fertilize (8/7/03)
	174 into culture (9/7/03)	Cleaved: 136 (78%)

Day 2	Grades					Total embryos
Dish 1 sel	6c1	5c1	4c1	4c1	3c1	5
Dish 1 unsel	3c1	3c2	2c1	2c1	2c2	5
Dish 2 sel	6c1	5c1	4c1	4c2	4c2	5
Dish 2 unsel	4c2	2c1	2c1	2c2	2c3	5
Dish 3 sel	6c1	6c1	4c1	4c1	4c1	5
Dish 3 unsel	3c2	3c3	2c2	2c2	2c2	5
Dish 4 sel	6c1	6c1	6c2	4c2	3c1	5
Dish 4 unsel	4c2	3c2	2c1	2c1	2c2	5
Dish 5 sel	6c1	4c2	4c2	4c2	4c2	5
Dish 5 unsel	2c2	2c2	2c2	2c2	2c3	5
Day 4						
Day 4	Grades					Total embryos
Dish 1 sel	16c	16c	12c	4c	6c	5
Dish 1 unsel	8c uneq	4c	8c	deg	deg	5
Dish 2 sel	16c	8c	8c	16c	deg	5
Dish 2 unsel	8c comp	4c dark	2c	2c patchy	deg	5
Dish 3 sel	16c	16c	16c	16c	6c	5
Dish 3 unsel	6c	4c	4c	2c	2c	5
Dish 4 sel	8c	4c uneq	4c uneq	4c uneq	deg	5
Dish 4 unsel	4c	3c	2c	2c	deg	5
Dish 5 sel	16c	16c	8c	8c	comp	5
Dish 5 unsel	16c	8c	2c	2c	3c uneq	5
Day 6						
Day 6	Grades					Total embryos
Dish 1 sel	8c	6c	EB	comp	6c comp	5
Dish 1 unsel	8c comp	8c	4c	4c comp	2c	5
Dish 2 sel	Mor-blast	Mor-blast	comp	8c	deg	5
Dish 2 unsel	8c uneq	2c	2c	2c patchy	deg	5
Dish 3 sel	mor	mor	comp	deg	deg	5
Dish 3 unsel	EB	4c	4c	2c	2c	5
Dish 4 sel	EB	mor	4c deg	deg	deg	5
Dish 4 unsel	4c	mor	3c	2c	deg	5
Dish 5 sel	EB	mor	Mor 8c	Mor	comp	5
Dish 5 unsel	EB	EB	Mor 8c	comp	comp	5
Day 7						
Day 7	Grades					Total embryos
Dish 1 sel	Mor-blast	Mor-blast	Blast	6c comp	deg	5
Dish 1 unsel	8c comp	8c	8c	2c	deg	5
Dish 2 sel	Mor-blast	Mor-blast	Mor-blast	8c	deg	5
Dish 2 unsel	mor	2c	deg	deg	deg	5
Dish 3 sel	Blast	Blast	Blast	deg	deg	5
Dish 3 unsel	Mor-blast	4c	4c	2c	2c	5

Dish 4 sel	Exg blast	Mor-blast	4c deg	deg	deg	5
Dish 4 unsel	deg	deg	deg	deg	deg	5
Dish 5 sel	Blast	mor	mor	Mor	mor	5
Dish 5 unsel	Exg blast	blast	Mor-blast	mor	mor	5

GRADING OF BLASTOCYSTS					
Stage	Grade	Diameter (Gr. Units)	Diameter (Micrometers)	Dish	Cell Count (Average)
Blast	2	52	176.228	1-S	63
Blast	2	54	183.006	3-S	98
Blast	2.5	49	166.061	3-S	88
Blast	3	49	166.061	3-S	41
Exg blast	2	58	196.562	4-S	133
Blast	3	50	169.45	5-S	39
Exg blast	2	57	193.173	5-U	129
Blast	3	46	155.894	5-U	34
Exg blast	2.5	53	179.617	1-S	82
Exg blast	2.5	60	203.34	2-S	144
Blast	2.5	52	176.228	3-S	43

Note: Treatments correspond to those described in the Material and Methods Section.

Experiment 2 – Replicate 2 – 15/7/03

N=40	190 oocytes to mature (15/7/03)	190 oocytes to fertilize (16/7/03)
	180 into culture (17/7/03)	Cleaved: 140 (78%)

Day 2	Grades					Total embryos
Dish 1 sel	4c1	3c1	4c1	4c1	8c2	5
Dish 1 unsel	4c2	4c2	2c1	2c1	2c2	5
Dish 2 sel	4c1	4c1	8c1	3c1	4c2	5
Dish 2 unsel	4c2	3c2	2c1	2c2	4c3	5
Dish 3 sel	8c1	6c2	6c2	8c2	4c1	5
Dish 3 unsel	4c2	4c2	2c2	3c2	2c2	5
Dish 4 sel	8c1	4c1	6c2	8c1	8c1	5
Dish 4 unsel	4c2	2c2	3c1	3c3	3c2	5
Day 4	Grades					Total embryos
Dish 1 sel	4c	8c	4c	4c	4c	5
Dish 1 unsel	8c	4c	8c	3c	2c	5
Dish 2 sel	5c	4c	4c	6c	6c uneq	5
Dish 2 unsel	4c	4c	3c	2c	2c uneq	5
Dish 3 sel	8c	8c	8c	6c	4c	5
Dish 3 unsel	8c	6c	3c	3c	2c	5
Dish 4 sel	8c	8c	6c	6c	4c	5
Dish 4 unsel	6c	6c	6c	2c	deg	5
Day 6	Grades					Total embryos
Dish 1 sel	4c	6c	mor	mor-blast	mor-blast	5
Dish 1 unsel	mor-blast	6c	4c	mor (deg)	deg	5
Dish 2 sel	6c comp	4c comp	mor-blast	mor-blast	comp (dark)	5
Dish 2 unsel	mor-blast	comp (dark)	comp (dark)	3c	2c	5
Dish 3 sel	comp	comp (dark)	6c	3c	deg	5
Dish 3 unsel	comp	comp	4c (uneq)	deg	deg	5
Dish 4 sel	comp	comp	comp	comp	comp	5
Dish 4 unsel	comp	comp	comp (4c)	deg	deg	5
Day 7	Grades					Total embryos
Dish 1 sel	blast	6c	mor	mor	deg	5
Dish 1 unsel	blast	mor	mor	mor (deg)	deg	5
Dish 2 sel	6c comp	8c comp	6c comp	blast	deg	5
Dish 2 unsel	mor	deg	deg	4c	2c	5
Dish 3 sel	blast	mor	mor	mor	deg	5
Dish 3 unsel	mor-blast	mor	mor	deg	deg	5
Dish 4 sel	mor-blast	mor	mor	mor	deg	5
Dish 4 unsel	blast	comp	mor	deg	deg	5

GRADING OF BLASTOCYSTS						
Stage	Grade	Diameter (Gr. Units)	Diameter (Micrometers)	Dish	Cell count (average)	Pyruvate (picomols)
Blast	2.5	46	155.894	1-S	83	-17.2
Blast	2	53	179.617	1-U	102	-4.6
Blast	2.5	50	169.45	2-S	115	5.5
Blast	3	46	155.894	3-S	57	-15.7
Blast	2.5	52	176.228	4-U	52	9.3

Experiment 2 – Replicate 3 – 15/7/03

N=60	220 oocytes to mature (15/7/03)	210 oocytes to fertilize (16/7/03)
	204 into culture (17/7/03)	Cleaved: 176 (86%)

Day 2	Grades					Total embryos
Dish 1 sel	4c1	6c2	6c1	5c2	5c1	5
Dish 1 unsel	2c1	2c1	4c2	4c1	4c2	5
Dish 2 sel	6c1	8c1	8c1	4c1	5c2	5
Dish 2 unsel	3c3	2c3	3c2	3c3	2c3	5
Dish 3 sel	6c1	6c1	6c1	4c2	5c2	5
Dish 3 unsel	4c3	3c2	2c2	2c2	2c1	5
Dish 4 sel	4c1	6c2	4c2	4c1	3c1	5
Dish 4 unsel	3c2	2c1	2c1	2c1	2c2	5
Dish 5 sel	6c1	3c1	8c1	8c1	8c1	5
Dish 5 unsel	3c2	2c2	2c2	3c2	2c2	5
Dish 6 sel	3c1	4c1	4c2	8c1	4c1	5
Dish 6 unsel	2c1	2c1	2c2	2c2	2c2	5
Day 4	Grades					Total embryos
Dish 1 sel	8c (dark)	8c	12c	12c	8c	5
Dish 1 unsel	2c (+frs)	12c (+frs)	2c	3c	2c	5
Dish 2 sel	8c	12c	12c	8c	8c comp	5
Dish 2 unsel	2c	2c	3c	2c	4c	5
Dish 3 sel	2c	6c	8c	6c	8c comp	5
Dish 3 unsel	2c	2c	4c	3c	2c	5
Dish 4 sel	5c	4c	2c	3c	3c	5
Dish 4 unsel	3c	3c	2c	2c	2c	5
Dish 5 sel	16c	16c	6c	6c	6c	5
Dish 5 unsel	4c	3c	2c	2c	deg	5
Dish 6 sel	16c	8c	8c	4c	3c	5
Dish 6 unsel	6c (dark)	3c	3c	2c	deg	5
Day 6	Grades					Total embryos
Dish 1 sel	8c	8c	12c	comp	mor-blast	5
Dish 1 unsel	Mor	2c	2c	3c	deg	5
Dish 2 sel	6c comp	mor	mor	mor-blast	comp	5
Dish 2 unsel	3c	2c	2c	deg	deg	5
Dish 3 sel	mor-blast	8c	6c	6c	deg	5
Dish 3 unsel	2c	2c	3c	deg	deg	5
Dish 4 sel	4c	3c	3c	2c	deg	5
Dish 4 unsel	3c	2c	2c	2c	deg	5
Dish 5 sel	mor-blast	mor-blast	6c	6c	6c	5
Dish 5 unsel	4c	6c	2c	deg	deg	5
Dish 6 sel	mor-blast	16c	comp	4c	4c	5
Dish 6 unsel	6c	2c	deg	deg	deg	5

Day 7	Grades					Total embryos
Dish 1 sel	Blast	8c	mor (dark)	mor-blast	mor-blast	5
Dish 1 unsel	Mor	2c	deg	deg	deg	5
Dish 2 sel	Blast	blast	blast	blast	deg	5
Dish 2 unsel	4c	2c	deg	deg	deg	5
Dish 3 sel	exg blast	blast	mor	mor	deg	5
Dish 3 unsel	2c	2c	3c	deg	deg	5
Dish 4 sel	8c	3c	2c	deg	deg	5
Dish 4 unsel	3c	2c	2c	2c	deg	5
Dish 5 sel	exg blast	blast	mor	12c	6c	5
Dish 5 unsel	4c	6c	2c	deg	deg	5
Dish 6 sel	Blast	mor	mor	mor	4c	5
Dish 6 unsel	8c	3c	deg	deg	deg	5

GRADING OF BLASTOCYSTS						
Stage	Grade	Diameter (Gr. Units)	Diameter (Micrometers)	Dish	Cell count (average)	Pyruvate (picomols)
Blast	2.5	50	169.45	1-S	52	-5.6
Early Blast	3	47	159.283	1-S	22	-1.4
Early Blast	3	50	169.45	1-S	73	-0.5
Blast	2	52	176.228	2-S	63	-1.2
Blast	3	49	166.061	2-S	31	0
Blast	3	50	169.45	2-S	51	-5.9
Blast	2.5	48	162.672	2-S	15	-5.1
Exg Blast	2	60	203.34	3-S	63	-21.3
Blast	3	46	155.894	3-S	38	-56.4
Exg Blast	2	59	199.951	5-S	131	20.2
Blast	3	51	172.839	5-S	119	38
Blast	3	49	166.061	6-S	72	18.2

Experiment 2 – Replicate 4 – 23/7/03

N=40	210 oocytes to mature (23/7/03)	180 oocytes to fertilize (24/7/03)
	170 into culture (25/7/03)	Cleaved: 126 (74%)

Day 2	Grades					Total embryos
Dish 1 sel	5c1	6c2	4c1	4c1	4c1	5
Dish 1 unsel	2c2	3c2	4c3	2c2	2c2	5
Dish 2 sel	6c1	6c1	4c1	3c1	4c2	5
Dish 2 unsel	2c3	3c2	2c1	3c2	2c2	5
Dish 3 sel	6c1	6c1	4c1	6c1	4c1	5
Dish 3 unsel	2c2	3c2	3c2	3c2	3c3	5
Dish 4 sel	3c2	3c2	5c2	4c1	2c1	5
Dish 4 unsel	2c2	2c3	2c3	2c1	2c2	5
Day 4	Grades					Total embryos
Dish 1 sel	12c	12c	8c	4c	4c	5
Dish 1 unsel	3c	4c	6c	2c	deg	5
Dish 2 sel	8c	6c	4c	4c	deg	5
Dish 2 unsel	6c	2c	4c	2c	2c uneq	5
Dish 3 sel	16c	16c	16c	8c	2c	5
Dish 3 unsel	8c	4c	2c	2c	deg	5
Dish 4 sel	8c	3c	3c	4c	8c	5
Dish 4 unsel	4c	3c	2c	deg	deg	5
Day 6	Grades					Total embryos
Dish 1 sel	5c	6c	6c	mor	mor-blast	5
Dish 1 unsel	5c	3c	2c	8c	deg	5
Dish 2 sel	4c	3c	deg	deg	deg	5
Dish 2 unsel	mor-blast	6c	4c	3c	deg	5
Dish 3 sel	16c	8c	mor	mor	comp	5
Dish 3 unsel	mor	4c	3c	2c	2c	5
Dish 4 sel	mor-blast	mor	mor	2c	deg	5
Dish 4 unsel	deg	deg	deg	deg	deg	5
Day 7	Grades					Total embryos
Dish 1 sel	blast	blast	6c	8c	8c	5
Dish 1 unsel	8c	3c	6c	3c	deg	5
Dish 2 sel	deg	deg	deg	deg	deg	5
Dish 2 unsel	blast	6c	deg	deg	deg	5
Dish 3 sel	blast	mor	comp	8c	deg	5
Dish 3 unsel	blast	mor	mor	deg	2c	5
Dish 4 sel	blast	mor	mor	2c	deg	5
Dish 4 unsel	deg	deg	deg	deg	deg	5

GRADING OF BLASTOCYSTS						
Stage	Grade	Diameter (Gr. Units)	Diameter (Micrometers)	Dish	Cell count (average)	Pyruvate (picomols)
Blast	2	53	179.617	1-S	93	10.4
Blast	3	43	145.727	1-S	68	-5.7
Blast	2	50	169.45	2-U	89	3.9
Blast	2.5	49	166.061	3-U	29	13.5
Blast	2	49	166.061	3-S	75	1
Blast	2	54	183.006	4-S	84	7

Experiment 2 – Replicate 5 – 24/7/03

N=30	210 oocytes to mature (24/7/03)	160 oocytes to fertilize (25/7/03)
	142 into culture (26/7/03)	Cleaved: 109 (77%)

Day 2	Grades					Total embryos
Dish 1 sel	4c1	4c1	4c1	6c1	8c1	5
Dish 1 unsel	3c2	3c2	2c2	3c3	4c2	5
Dish 2 sel	6c1	6c2	6c2	6c1	5c2	5
Dish 2 unsel	3c2	3c2	2c2	4c1	4c2	5
Dish 3 sel	6c2	6c2	6c1	6c1	4c1	5
Dish 3 unsel	3c2	2c2	2c2	2c3	3c2	5

Day 4	Grades					Total embryos
Dish 1 sel	8c	8c	4c	4c	6c	5
Dish 1 unsel	6c	4c (+frs)	4c (dark)	2c	deg	5
Dish 2 sel	8c	8c	8c (+frs)	3c + frs	3c + frs	5
Dish 2 unsel	3c	3c	4c	4c	2c	5
Dish 3 sel	16c	6c	8c (+frs)	6c	4c	5
Dish 3 unsel	8c	3c	3c (dark)	deg	deg	5

Day 6	Grades					Total embryos
Dish 1 sel	8c	4c	2c	deg	deg	5
Dish 1 unsel	6c	4c	deg	deg	deg	5
Dish 2 sel	mor-blast	deg	deg	4c	2c	5
Dish 2 unsel	8c	4c	4c	deg	deg	5
Dish 3 sel	mor	6c	4c	deg	deg	5
Dish 3 unsel	mor-blast	4c	5c	deg	deg	5

Day 7	Grades					Total embryos
Dish 1 sel	mor	8c	deg	deg	deg	5
Dish 1 unsel	6c	4c	deg	deg	deg	5
Dish 2 sel	blast	deg	deg	4c	deg	5
Dish 2 unsel	mor	4c	deg	deg	deg	5
Dish 3 sel	blast	8c	4c	deg	deg	5
Dish 3 unsel	blast	6c	5c	deg	deg	5

GRADING OF BLASTOCYSTS						
Stage	Grade	Diameter (Gr. Units)	Diameter (Micrometers)	Dish	Cell count (average)	Pyruvate (picomols)
Blast	2	48	162.672	2-S	82	16.7
Blast	2	55	186.395	3-S	120	-2.8
Blast	2.5	45	152.505	3-U	53	3.8