hESC derivation

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1.-Centre de Medicina Regenerativa de Barcelona

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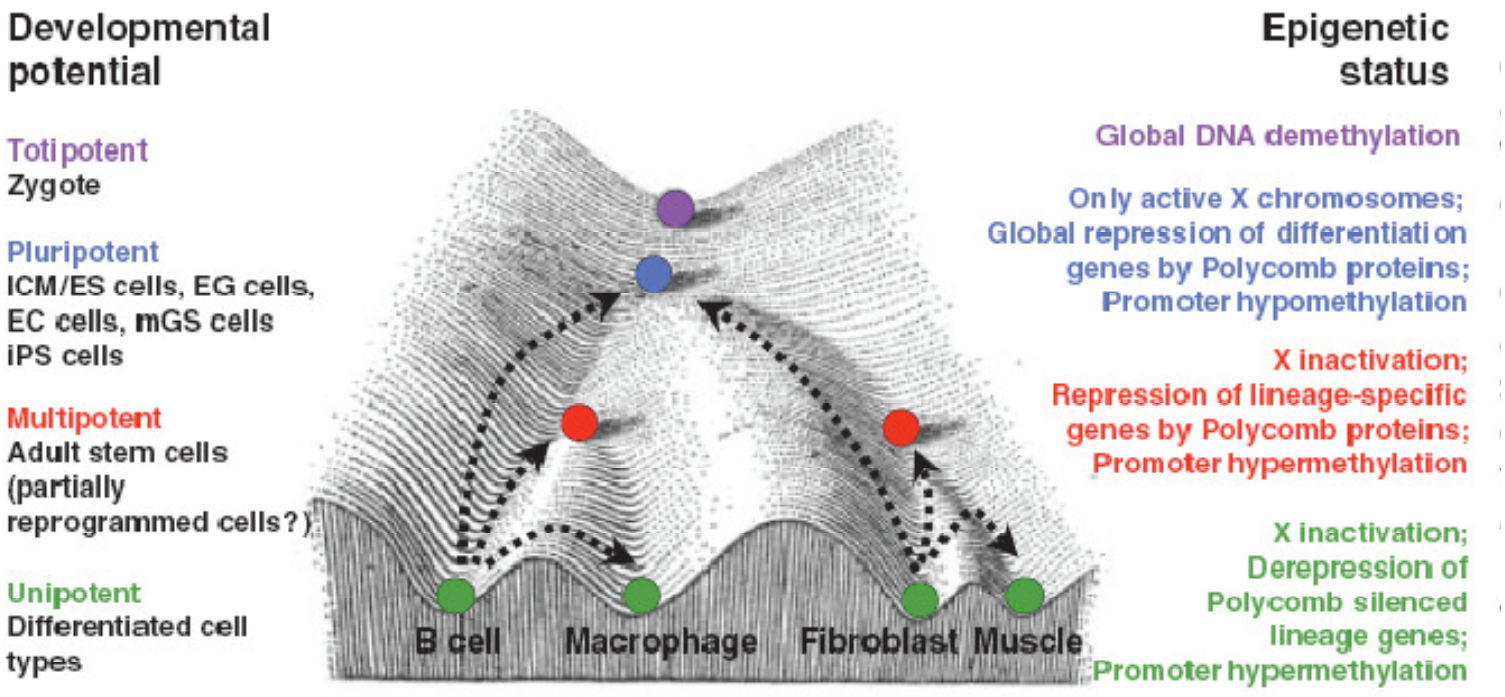
Pluripotency

Embryonic Stem Cells

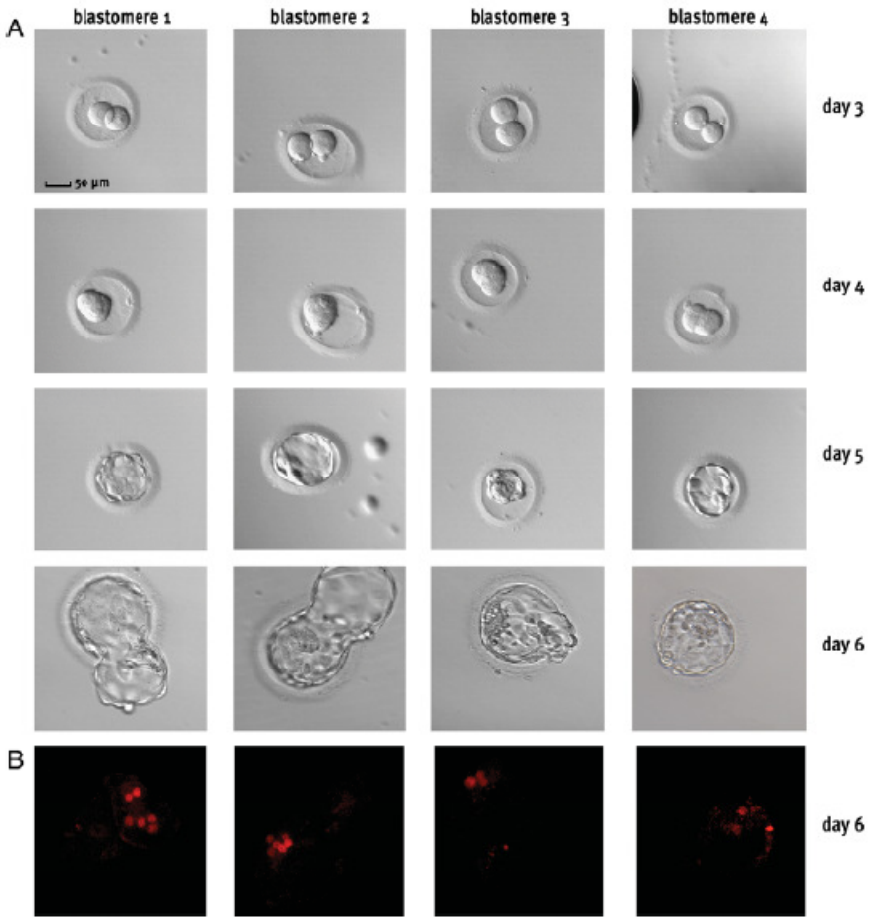
Methodology for hESC derivation

- Embryo culture. Embryo quality
- ICM isolation
- hESC derivation and culture conditions
  - Feeder cells
  - Xeno-free, feeder free and GMP derivation
- Results

Pluripotency



Hochedlinger, Development 2009 (adapted from Waddington, 1957)



Van de Velde, 2009

The four blastomeres of a 4-cell stage human embryo are able to develop individually into blastocysts with inner cell mass and trophectoderm

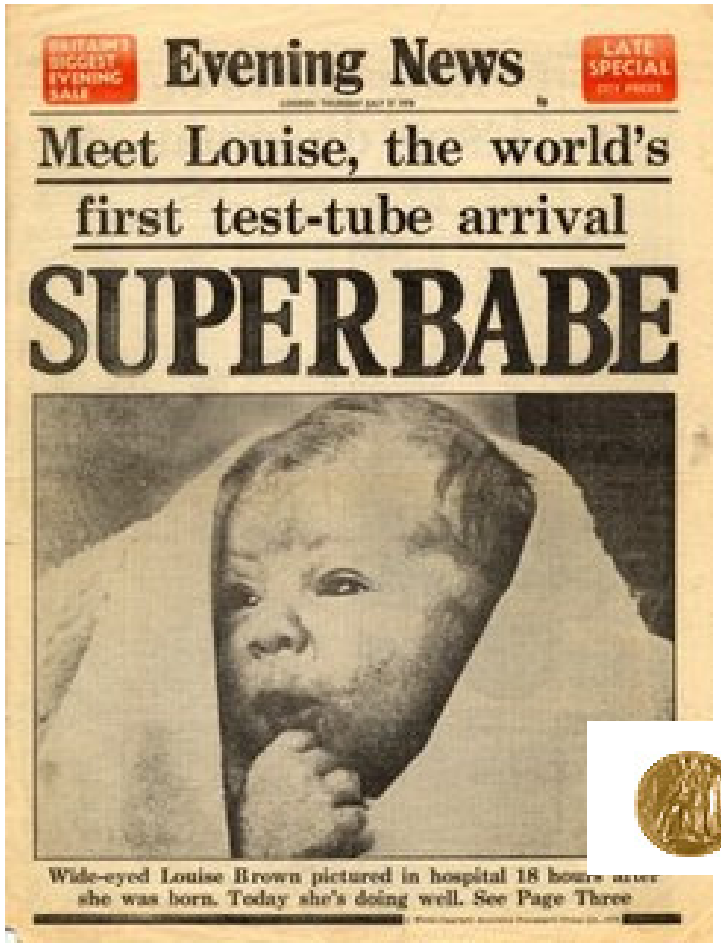
❄ Pluripotent Stem Cells can be obtained from cells located in the inner cell mass of blastocysts and from nuclear reprogramming (SCNT and iPS).

Embryonic Stem cells

ESSAY

30 years: from IVF to stem cells

Ruth Deech, former chair of Britain's Human Fertilisation and Embryology Authority, reflects on how the science that gave an infertile couple a baby has been extended to saving lives.



Evening News

LATE SPECIAL

Meet Louise, the world's first test-tube arrival

SUPERBABE

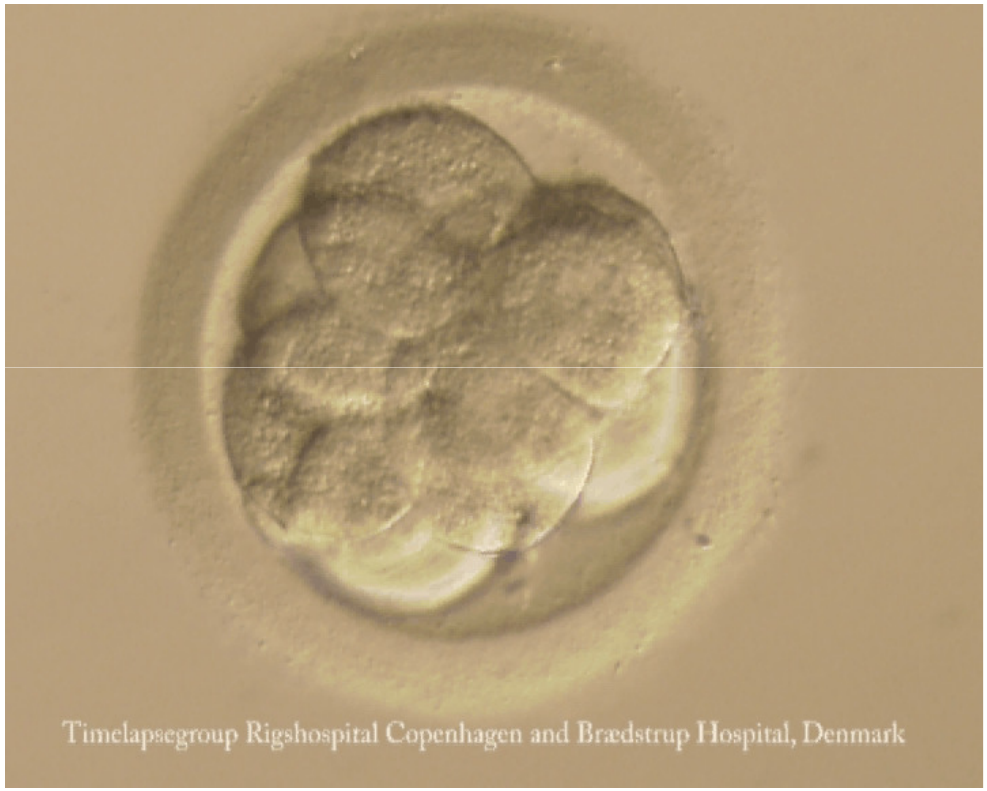
Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

The Nobel Prize in Physiology or Medicine 2010
Robert G. Edwards

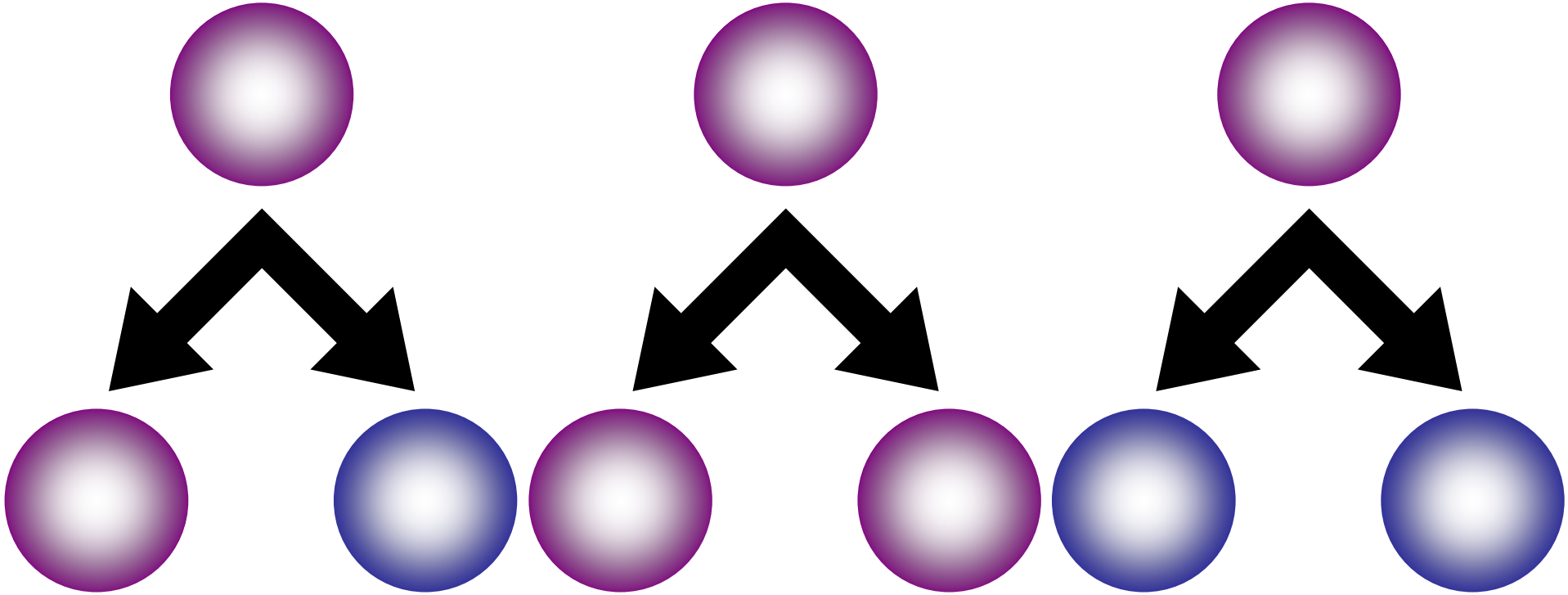
Embryonic Stem Cells

- Derived from the inner cell mass of the blastocyst (ICM) (day 5-7 of development,

  ± 150-200 cells). They give rise to the 3 germ layers: ectoderm, mesoderm and endoderm.



Embryonic Stem Cells: Self Renewal and Differentiation



Asymmetric division

Self-renewal

Differentiation

Embryonic stem cells

Embryo origin

- IVF embryos donated for research. PGD/PGS abnormal embryos
- Embryos created for research from donated gametes
- Embryos created through somatic cell nuclear transfer (SCNT)
- Other possibilities: parthenogenesis,...

Methodology for hESC derivation

- Embryo culture. Embryo quality

Establishment in culture of pluripotential cells from mouse embryos

M. J. Evans* & M. H. Kaufman†

Letters to Nature

Nature **292**, 154-156; 1981

Science

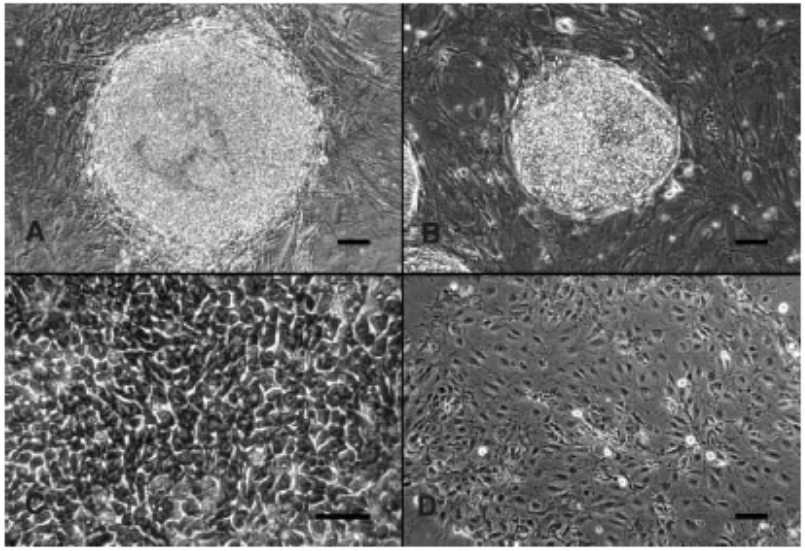
AAAS

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson, *et al.*

Science **282**, 1145 (1998);

DOI: 10.1126/science.282.5391.1145



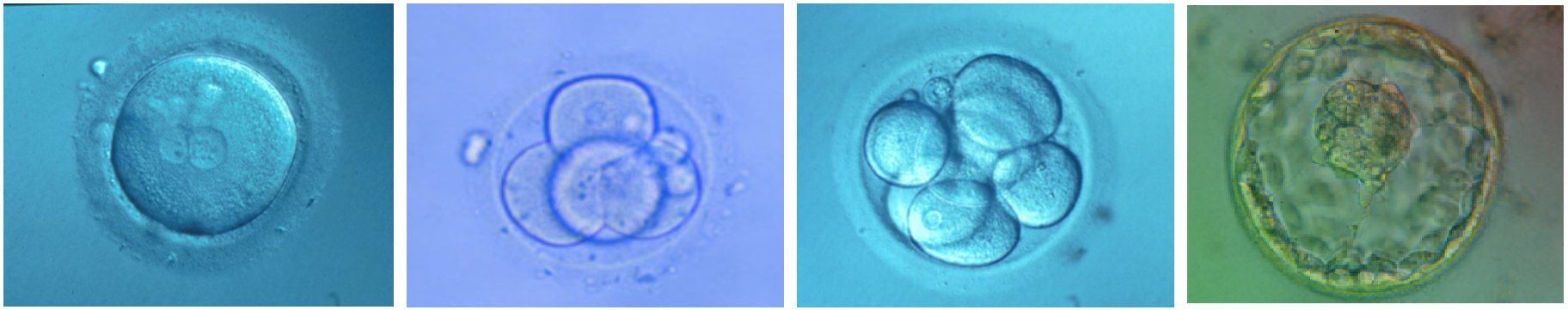
Methodology

- Embryo thawing and culture to the blastocyst stage.
- Zona pellucida removal
- ICM isolation (optional)
- Seeding

Methodology

- Embryo thawing* or warming*.
- Embryo culture*.

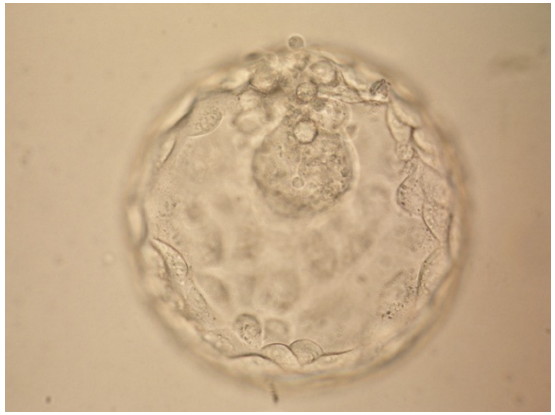
Embryos at different developmental stages (day 1, day 2 , day 3) have to be cultured to the blastocyst stage.



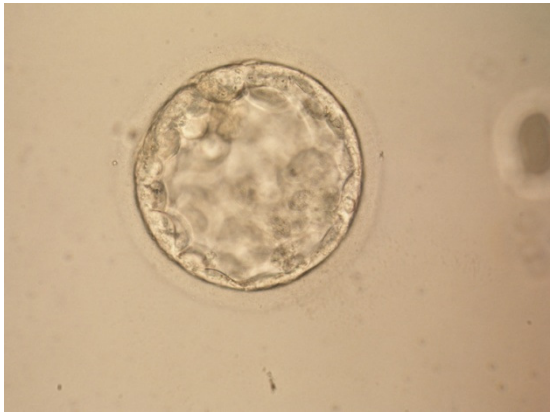
* Protocols developed at the IVF laboratory to be followed

Blastocyst classification

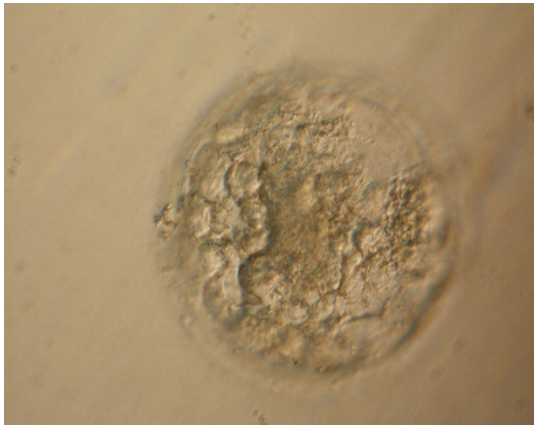
- Expansion degree
- Number of cells of the ICM
- Number of cells and trophectoderm appearance



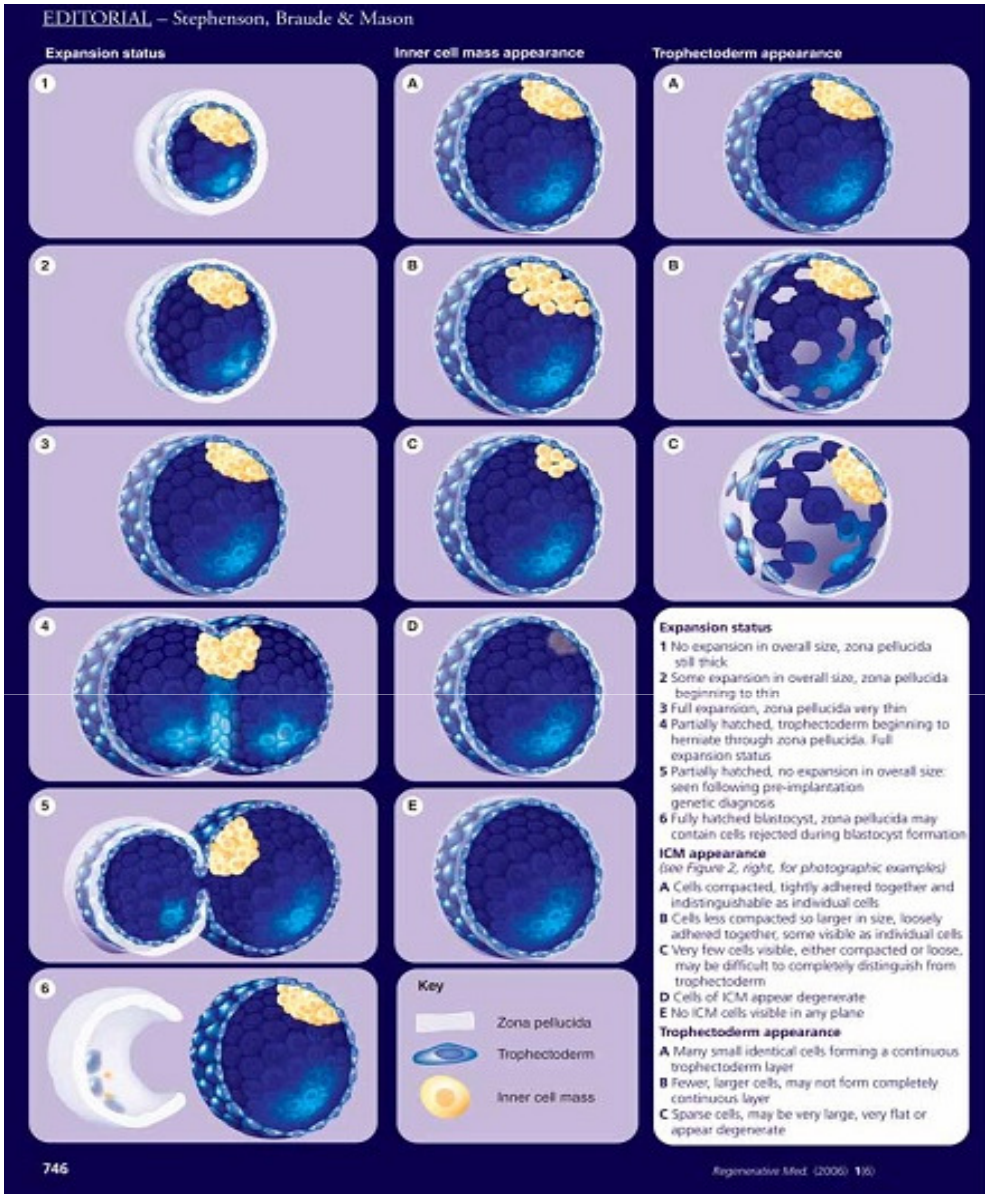
good



intermediate



poor



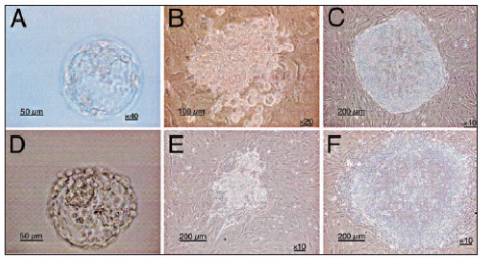
Proposal for a universal minimum information convention for the reporting on the derivation of human embryonic stem cell lines.

Emma Stephenson[1,2], Peter Braude[2,3], Chris Mason[1]

Future Medicine, Reg. Medicine 1(6), 739-750; 2006

Derivation, Characterization, and Differentiation
of Human Embryonic Stem Cells

Nico Heins,[a*] Mikael C.O. Englund,[a*] Cecilia Sjöblom,[a*] Ulf Dahl,[b] Anna Tonning,[b]
Christina Bergh,[c] Anders Lindahl,[d] Charles Hanson,[c] Henrik Semb[b]

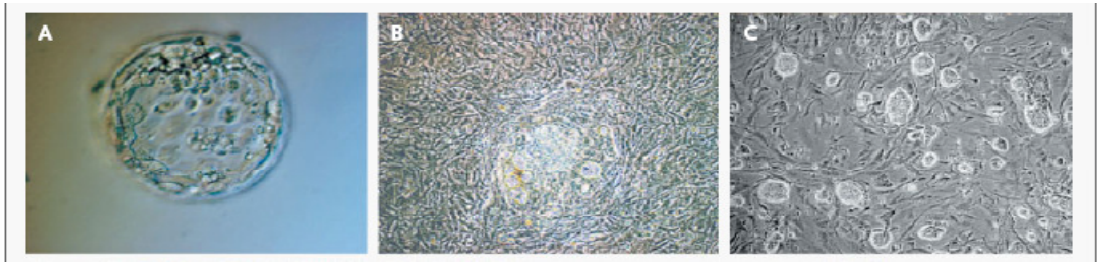


2003

Derivation of Embryonic Stem-Cell Lines from Human Blastocysts

2004

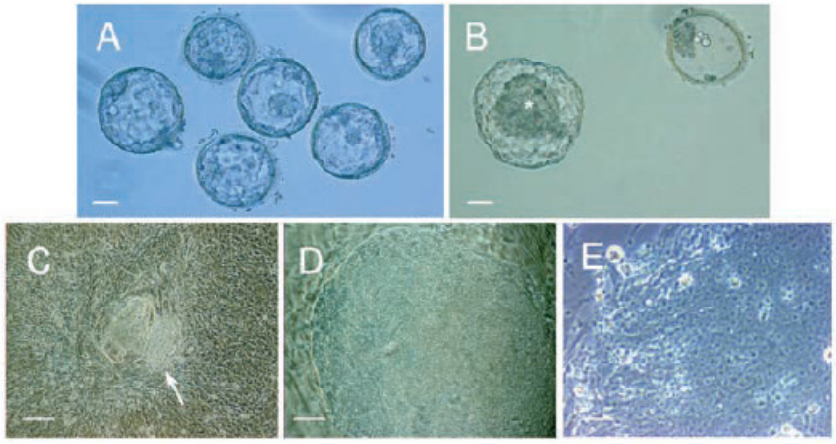
Chad A. Cowan, Ph.D., Irina Klimanskaya, Ph.D., Jill McMahon, M.S., Jocelyn Atienza, B.S.,
Jeannine Witmyer, Ph.D., Jacob P. Zucker, B.S., Shunping Wang, Ph.D., Cynthia C. Morton, Ph.D.,
Andrew P. McMahon, Ph.D., Doug Powers, Ph.D., and Douglas A. Melton, Ph.D.



Derivation of Human Embryonic Stem Cells from Day-8
Blastocysts Recovered after Three-Step In Vitro Culture

Miodrag Stojkovic,[a] Majlinda Lako,[a] Petra Stojkovic,[a] Rebecca Stewart,[b]
Stefan Przyborski,[b] Lyle Armstrong,[b] Jerry Evans,[a] Mary Herbert,[c] Louise Hyslop,[a]
Sajjad Ahmad,[a] Alison Murdoch,[c] Tom Strachan[a]

2004



Optimal Timing of Inner Cell Mass Isolation Increases the Efficiency of Human Embryonic Stem Cell Derivation and Allows Generation of Sibling Cell Lines

Alice E. Chen,[4,8] Dieter Egli,[4,8] Kathy Niakan,[4] Jie Deng,[7] Hidenori Akutsu,[6] Mariko Yamaki,[4] Chad Cowan,[1,2,6] Claire Fitz-Gerald,[4] Kun Zhang,[7] Douglas A. Melton,[2,3,4,*] and Kevin Eggan[1,2,4,*]

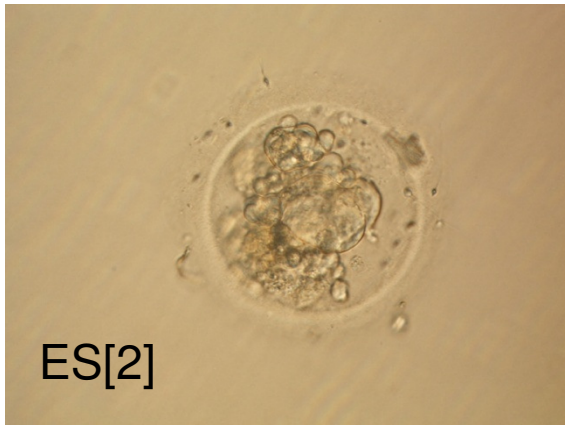
Table 1. Derivation Is Most Efficient on Day 6 Postfertilization

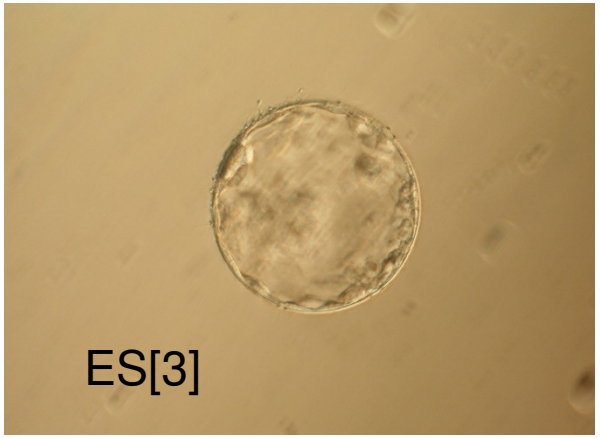
Day of ICM Isolation	Number of Blastocysts Used for Derivation	Number of Attachment Sites (Percent of Blastocysts)	Number of ICM Outgrowths	Number of Cell Lines	Percent Derivation Efficiency
5	19	6 (31)	1	1	5
6	27	22 (81)	15	14	52[a]
7	27	22 (81)	9	9	33
8	19	17 (90)	5	5	26
9	11	10 (91)	4	4	36
No isolation	10	10 (100)	1	1	10
Unknown[b]	27	ND	ND	11	40

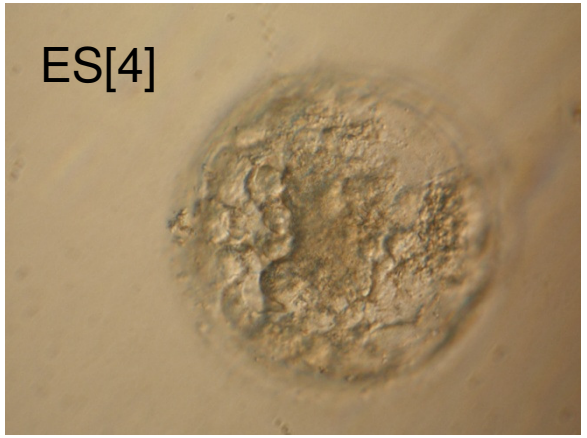
2009

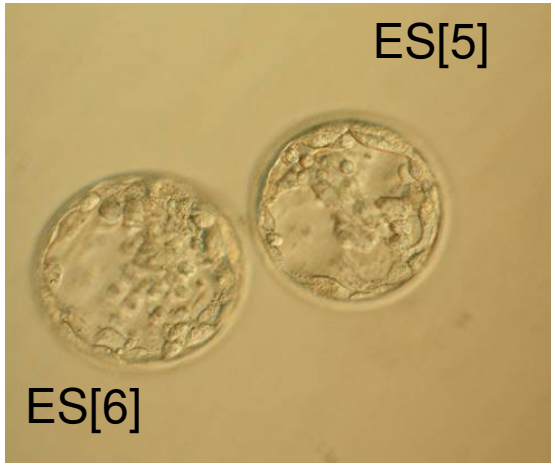
Embryo quality and derivation efficiency

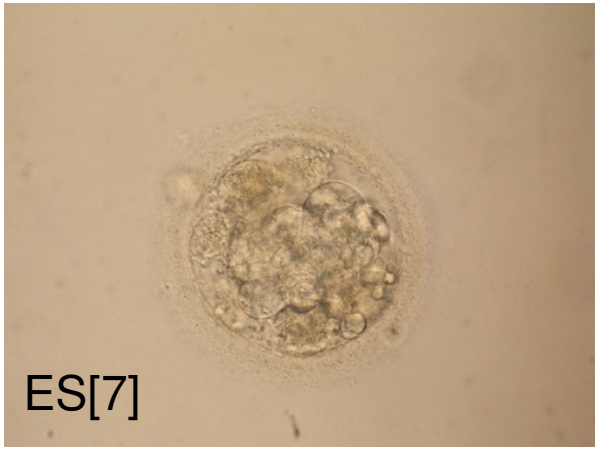
- Discarded embryos; *Mitalipova, 2003*
- Poor quality blastocysts*; Cowan, 2004*
- Day 3 low score embryos*; Chen 2005*
- Arrested embyos*; Zhang, 2007;Feki 2008*
- Poor quality embryos*; Lerou, 2008*
- Poor quality blastocysts*; Raya, 2008*
- Poor quality blastocysts; *Aran, 2010*

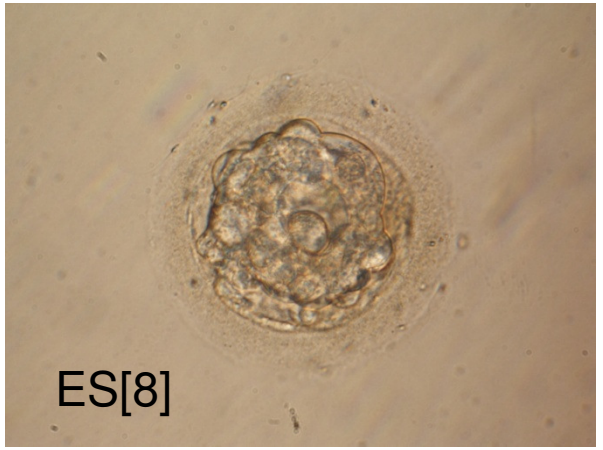

ES[2]

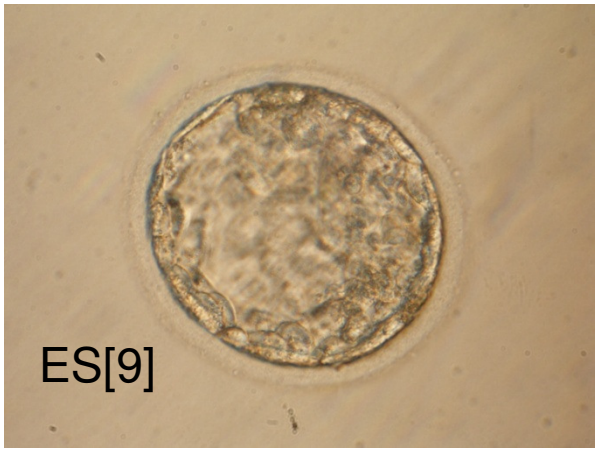

ES[3]

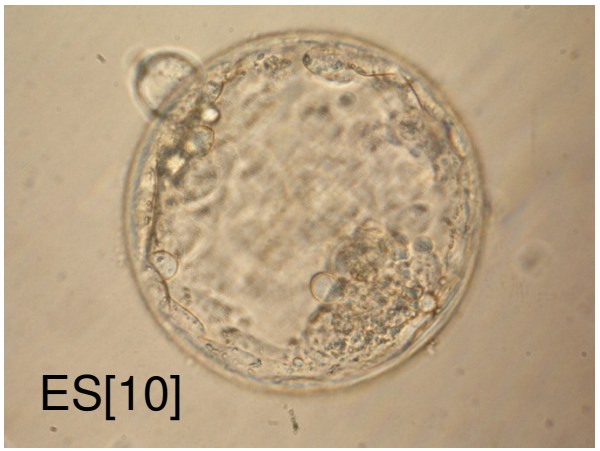

ES[4]

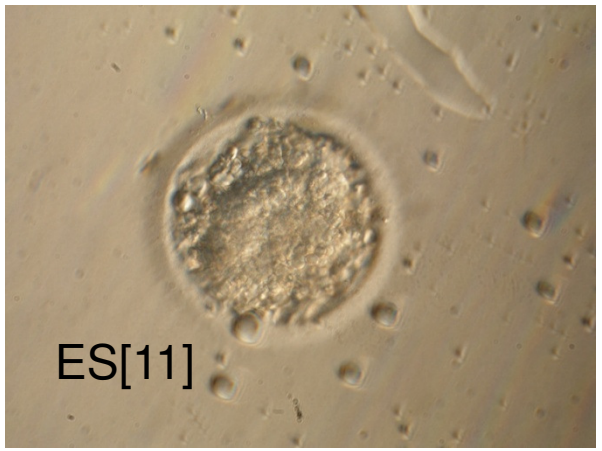

ES[5]
ES[6]


ES[7]


ES[8]

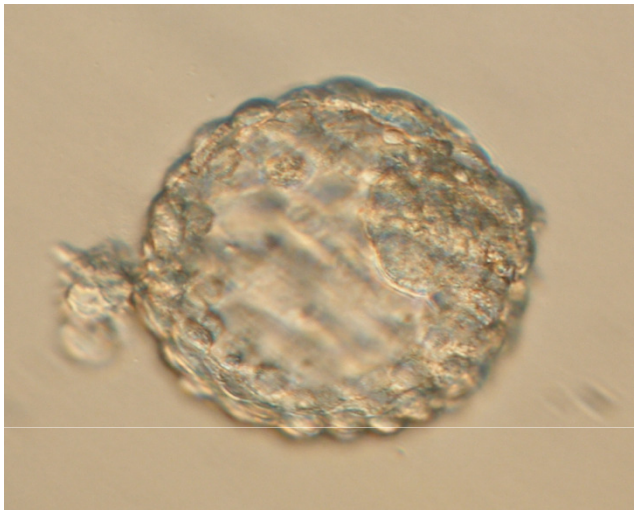

ES[9]


ES[10]


ES[11]

Methodology

Zona pellucida removal



- Pronase: xenobiotic, less agressive
- Acid Tyrode’s: xenofree, more agressive

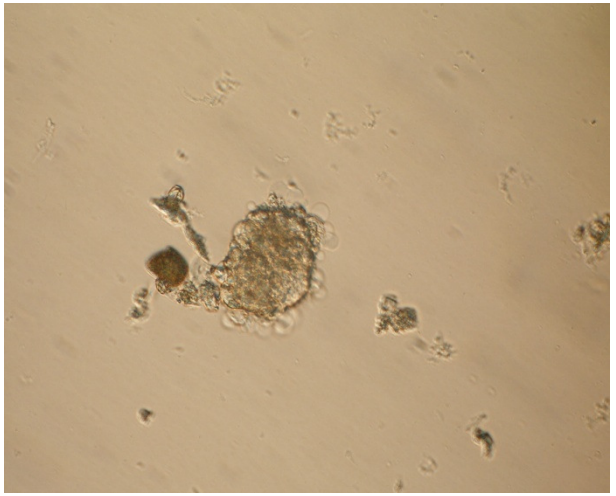
Methodology for hESC derivation

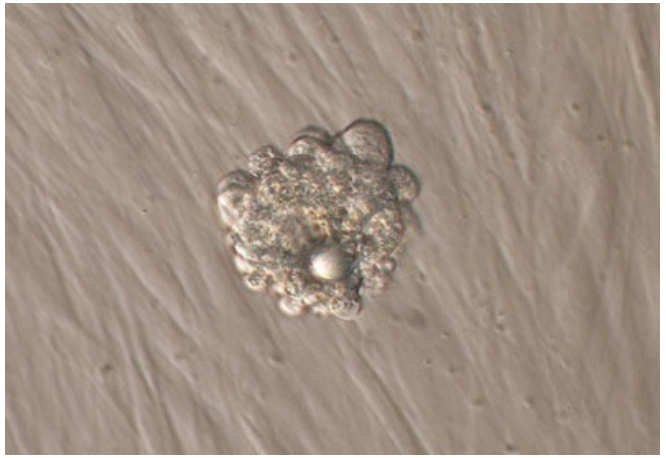
- ICM isolation

Methodology

ICM isolation (optional)

- Immunosurgery
- Laser dissection
- Mechanical dissection



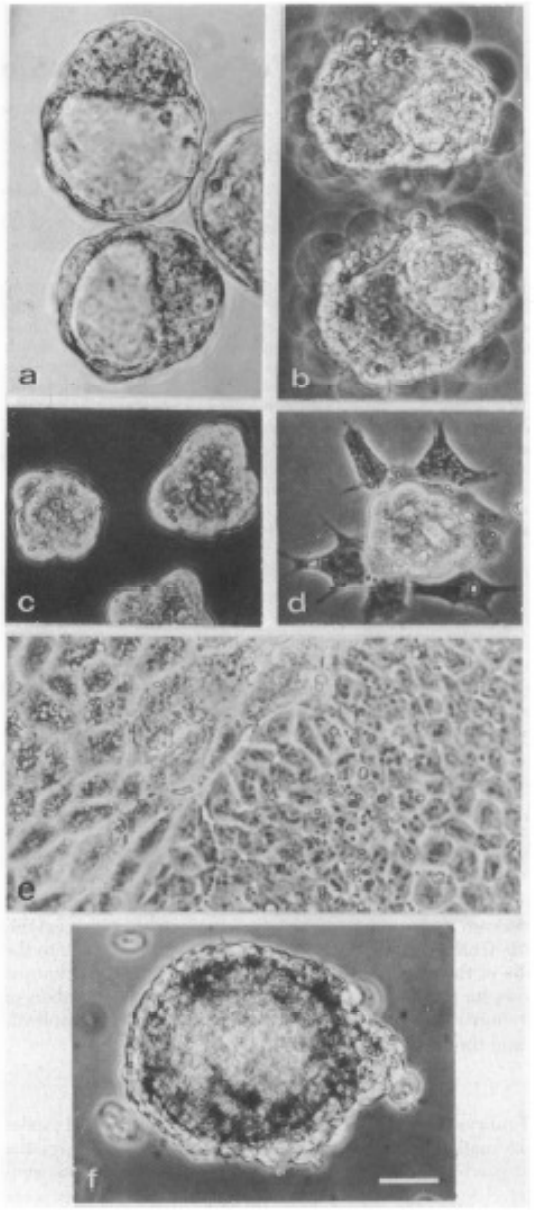


Methodology

Immunosurgery

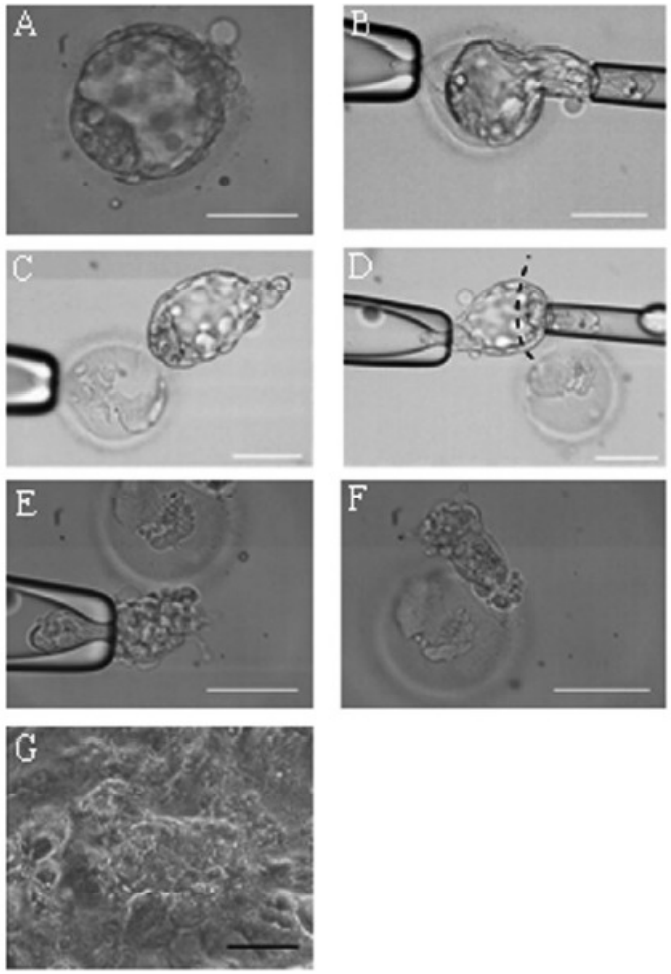
Use of antibodies (antihuman and complement) in order to lyse the cells of the trophectoderm

Solter and Knowles, 1975



Methodology

Laser dissection



Laser-assisted blastocyst dissection and subsequent cultivation of embryonic stem cells in a serum/cell free culture system: applications and preliminary results in a murine model

Noriko Tanaka[1], Takumi Takeuchi[1], Queenie V Neri[1], Eric Scott Sills[2] and Gianpiero D Palermo*[1]

2006

Evaluation of a laser technique to isolate the inner cell mass of murine blastocysts

José Luis Cortés*[|], Fernando Cobo*, Purificación Catalina*, Ana Nieto*, Carmen Cabrera*, Rosa Montes*, Ángel Concha† and Pablo Menendez*[|]

2007

Laser-assisted derivation of human embryonic stem cell lines from IVF embryos after preimplantation genetic diagnosis

T. Turetsky[1,†], E. Aizenman[2,†], Y. Gil[1], N. Weinberg[3], Y. Shufaro[1,2], A. Revel[2], N. Laufer[2], A. Simon[2], D. Abeliovich[3] and B.E. Reubinoff[1,2,4]

2008

Methodology

Mechanical dissection

Human Reproduction Vol.22, No.12 pp. 3051–3058, 2007
Advance Access publication on October 24, 2007
doi:10.1093/humrep/dem335

Mechanical isolation of the inner cell mass is effective in derivation of new human embryonic stem cell lines

Susanne Ström[1,†], José Inzunza[2,†], Karl-Henrik Grinnemo[3], Kerstin Holmberg[4], Eija Matilainen[1], Anne-Marie Strömberg[1], Elisabeth Blennow[4] and Outi Hovatta[1,5]

2007

Flexible metal needles (0.125 mm diameter) used to open the ZP and dissect the ICM

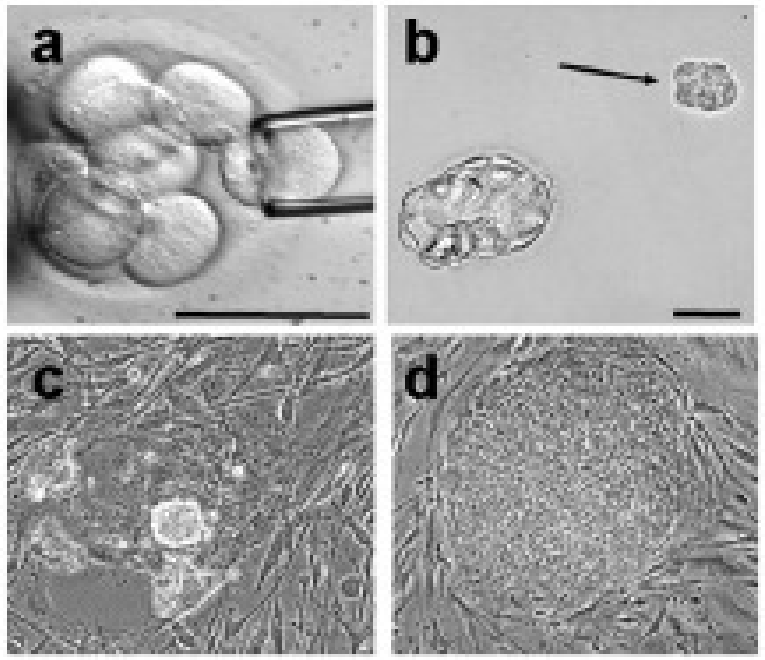
Methodology

Alternatives to the ICM isolation and seeding

- *Whole blastocysts*
  Heins, 2003

- Morula
  Strelchenko, 2004;
  Tesar, 2005

- Single blastomeres
  Klimanskaya, 2006
  Chung, 2007



Methodology

Embryo seeding – hESC culture

- ICMs or ZP free blastocysts are transferred to feeder dishes.
- hESC culture medium: Knockout DMEM with 2nmol/ml Glutamax, 0.05 mmol/l 2-mercaptoethanol, 8 ng/ml bFGF, 1% non essential aminoacids, 20% knockout SR and 0.5% penicillin-streptomycin.

Methodology

Feeder cells

- Unknown mechanisms that control the undifferentiated proliferation of hESC
- Feeder cells must produce factors associated with cell surface adherence and are responsible for the maintenance of the undiferentiated phenotype of the hESC

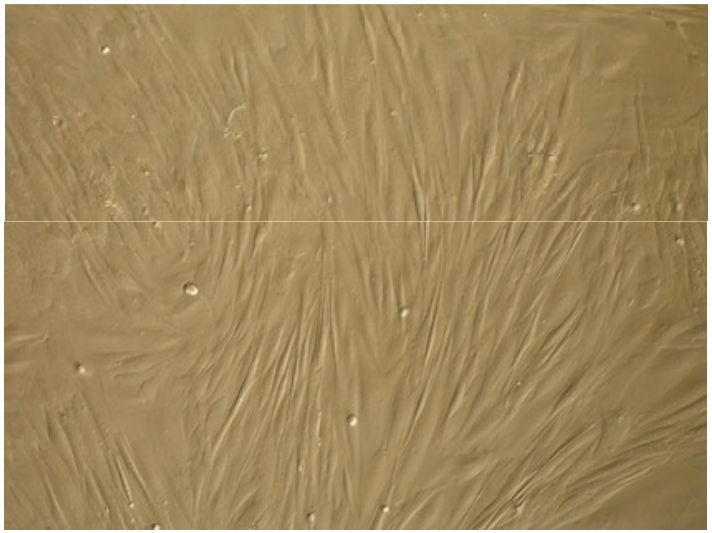
Methodology for hESC derivation

- hESC derivation and culture conditions
  - Feeder cells

Methodology

Feeder cells

- Mouse embryonic fibroblasts
  Thomson, 1998
- Human foreskin fibroblasts
  Hovatta, 2003
- Human placental fibroblasts
  Genbacev, 2005



Methodology for hESC derivation

- hESC derivation and culture conditions
  - Xeno-free, feeder free and GMP derivation

Methodology

Alternative matrices

Proteic matrix of complex composition

- Matrigel; *Xu, 2001*
- Mouse embryonic fibroblast extracellular matrix, *Klimanskaya, 2005*

Proteic matrix of defined composition

- Laminin; *Xu, 2001; Li 2005*
- Collagen IV, laminin, fibronectin and vitronectin; *Ludwig, 2006*

Human embryonic stem cells derived without feed ells

2005

Derivation of human embryonic stem cells in defined conditions

2006

Tenneille E Ludwig[1, 2, 3], Mark E Levenstein[1], Jeffrey M Jones[1, 4], W Travis Berggren[1], Erika R Mitchen[1], Jennifer L Frane[2, 3], Leann J Crandall[1], Christine A Daigh[2, 3], Kevin R Conard[1], Marian S Piekarczyk[1], Rachel A Llanas[1] & James A Thomson[1, 2, 3, 4, 5]

Report of derivation and culture of feeder free hESC with protein components derived from recombinant sources or purified human material

Derivation of a Xeno-Free Human Embryonic Stem Cell Line

2006

Catharina Ellerström,[a,b] Raimund Strehl,[a] Karina Moya,[a] Katarina Andersson,[a] Christina Bergh,[c] Kersti Lundin,[c] Johan Hyllner,[a] Henrik Semb[b]

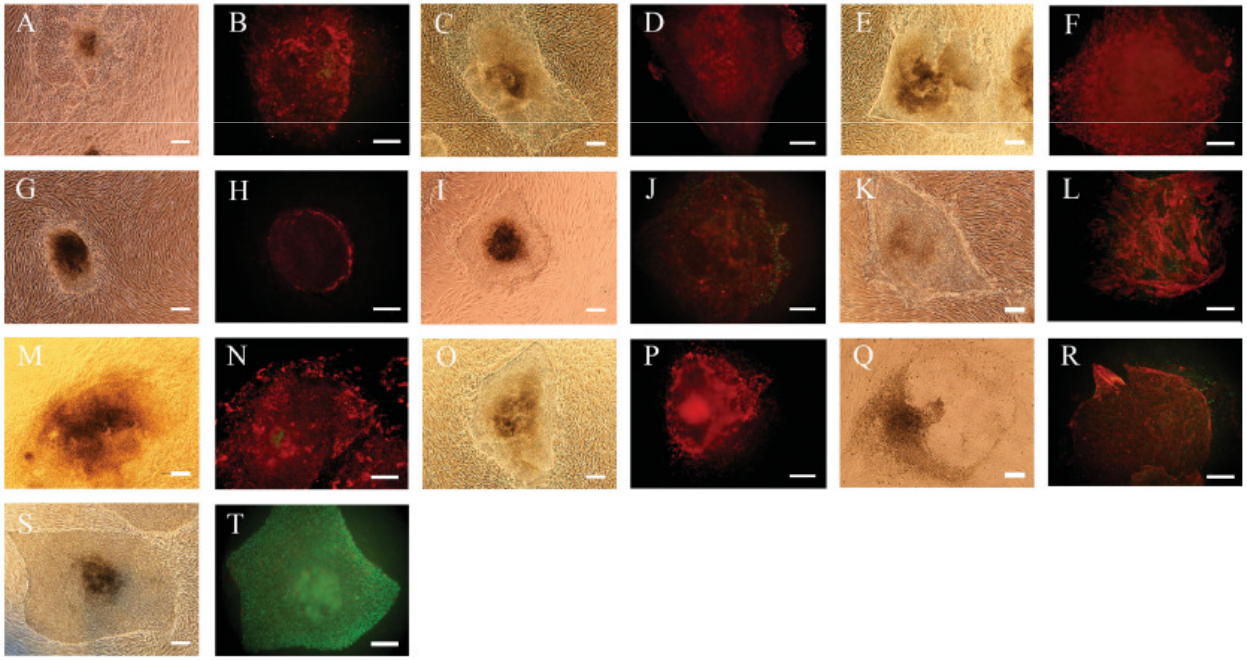
Report of the stablishment and characterisation of a hESC without animal origin components

Testing of nine different xeno-free culture media for human embryonic stem cell cultures

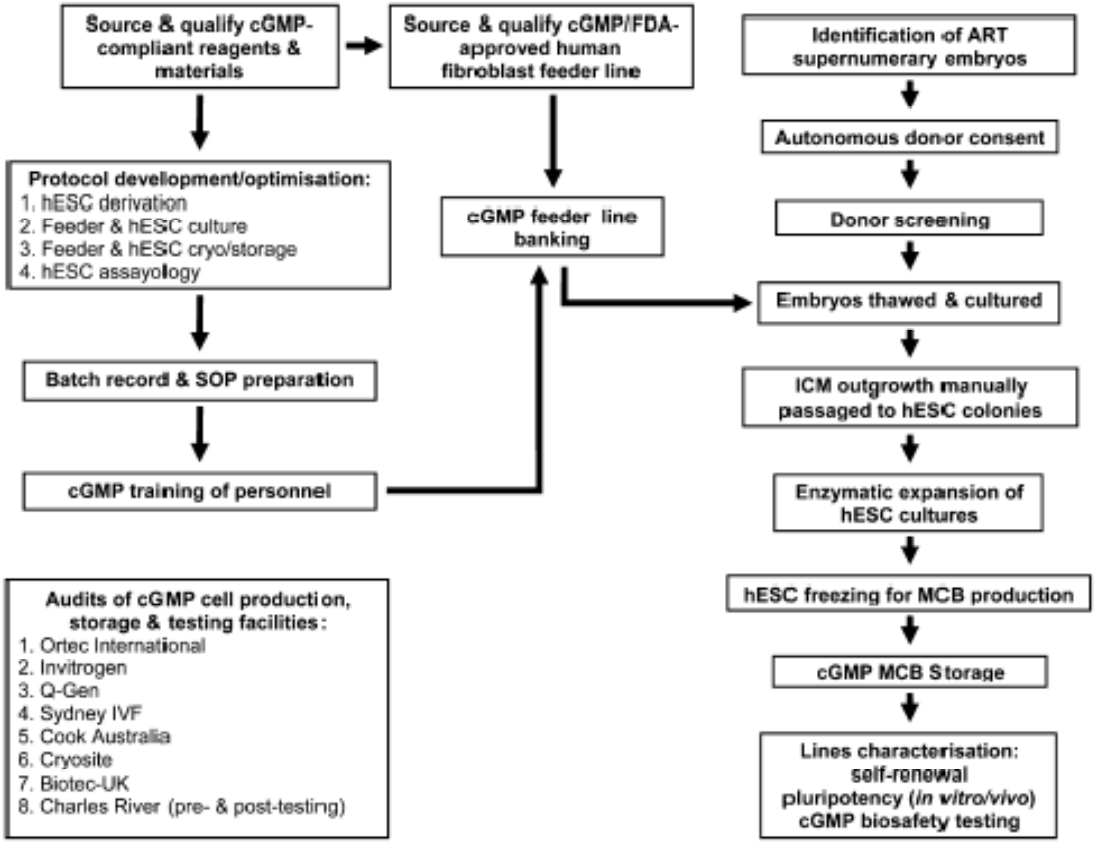
2007

Kristiina Rajala[1,4], Heidi Hakala[1], Sarita Panula[1], Suvi Aivio[1], Harri Pihlajamäki[2], Riitta Suuronen[1], Outi Hovatta[1,3] and Heli Skottman[1]

Test reagent
Control hESC medium
Human serum
Lipumin™ 10×
Plasmanate
SerEx 10×
Serum substitute supplement (SSS)
SR3
TeSR1
X-Vivo 10
X-Vivo 20



The Generation of Six Clinical-Grade Human Embryonic Stem Cell Lines



Crook et al, 2007

hESC culture

Isolation and culture conditions without animal origin products and defined conditions are needed to envisage therapeutic approaches (GMP conditions)

Results

Banc de Linies Cel.lulars de Barcelona Results

Results of embryo thawing and hESC derivation (1st series)

1st series	2PN zygotes	cleavage stage embryos	blastocysts	total
embryos thawed	20	35	6	61
embryos surviving (%)	10 (50%)	28 (80%)	4 (66.7%)	42 (68.8%)
blastocysts (%)	3 (30%)	10 (35.7%)		
blastocysts/ICM seeded	3	10	4	17
outgrowths	-	3	2	5
hESC lines	0	3 (30%)	2 (50%)	5 (29.4%)

Banc de Linies Cel.lulars de Barcelona Results

Results of embryo thawing and hESC derivation (2nd series)

2nd series	cleavage stage embryos
embryos thawed	66
embryos surviving (%)	58 (87.8%)
blastocysts (%)	13 (22.4%)
blastocysts/ICM seeded	11
outgrowths	1
hESC lines	1 (9.1%)

Banc de Linies Cel.lulars de Barcelona Results

Results of embryo thawing and hESC derivation (3rd series)

3rd series	cleavage stage embryos
embryos thawed	62
embryos surviving (%)	15 (24.2%)
blastocysts (%)	4 (26.7%)
blastocysts/ICM seeded	5
outgrowths	3
hESC lines	3 (60%)

Banc de Linies Cel.lulars de Barcelona Results

Results of embryo thawing and hESC derivation from abnormal embryos (PGD)

	Aneuploidies	Monogenic diseases	Total
Thawed blastocysts	67	19	86
Survival blastocysts	44 (65.7%)	14 (73.7%)	58 (68.1%)
Blastocysts/ICM seeded	33	9	42
Lines	-	1	1 (2.4%)

Autor	Year	Lab.	embryos	ICM (n)	Cell lines	Effic. (%)*
Thomson[4]	*1998*	WiCell, USA	36	14	H1,H7,H9, H13,H14	36
Reubinoff[31]	*2000*	Monash Inst. Austr./Singapore	N/A	2	HES-1, HES2	?
Lanzendorf[29]	*2001*	Jones Inst for Reprod. Med., USA	110	18	ES-76, ES-78-1, ES-78-2	17
Amit[40]	*2002*	Rambam Med. Center, Israel	5	5	I-3, I-4, I-6	60
Richards[50]	*2002*	Dept. Obs. Gyn. NU Singapore	1	1	1	?
Hovatta[32]	*2003*	Karolinska Inst. Sweden	N/A	2	HS181, HS207	?
Mitalipova[26]	*2003*	BresaGen, USA	19	8	BG01, BG02, BG03, BG04	50
Park [51]	*2003*	MizMedi Hosp Seoul, Korea	N/A	13	3	23
Pickering[38]	*2003*	Guy's, King's &St Thomas Med. UK	58	N/A	3	?
Baharvand[27]	*2004*	Royan Inst, Iran	N/A	1	Royan H11	?
Heins[52]	*2004*	Cellartis AB, Sweden	N/A	N/A	SA002,FC018 AS034,AS038 SA121 SA181(abn)	N/A
Cowan[33]	*2004*	Howard Hughes Medical Inst, USA	286 clev.+ 58 blasts	97	HUES1-17	18
Park[53]	*2004*	Maria Infertility Hospital, Korea	20blastos 20Pron.	11 3	MB01-07 MB08-09	64 67
Sjögren[20]	*2004*	Goteborg Univ., Sweden	748	114	22	19
Stojkovic[24]	*2004*	Univ. Newcastle, UK	11 day-2 embryos	7	HES-NCL1	14
Strelchenko[43]	*2004*	Repr. Genetic Inst Chicago, USA	46 morula, 39 blast. 32 ICM	**11** *12* 5	**8** *7* 5	**17** *18* 16
Suss-Toby[34]	*2004*	Rambam Medical Center, Israel	60	6	I9	17
Verlinsky[35]	*2005*	RGI Chicago, USA	72	N/A	18 abn	N/A
Chen[25]	*2005*	Tongi Hosp., China	130	10	2	20

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Author	Year	Lab.	embryos	ICM (n)	Cell lines	Effic. (%)*
Findikli[21]	*2005*	Istambul Mem. Hosp., Turkey	31	N/A	7	N/A
Genbacev[13]	*2005*	UCSF, USA	192 321	14 10	UCSF-2 UCSF-1	7 10
Inzunza[42]	*2005*	Karolinska Inst., Sweden	10	N/A	HS293, HS206	N/A
Kim HS[39]	*2005*	Seoul Nat. Univ., Korea	N/A	N/A	13	N/A
Kim SJ[54]	*2005*	MizMedi Hosp. Korea	N/A	16	Miz-Hes4-8, 10-13	56
Klimanskaya[12]	*2005*	ACT, USA	N/A	5	ACT-14	20
Lee[55]	*2005*	MRC MizMedi Hosp. Korea	8 PN-stage	7	Miz-endol,-2,-3	43
Li[56]	*2005*	Hosp. Sun Yat-sen Univ., China	N/A	4	CHES-1	25
Lysdahl[57]	*2006*	Aalborg Univ., Denmark	198	23	CLS1, CLS2, CLS3, CLS4	17
Mummery[37]	*2005*	Hubrecht Lab., The Nederlands	22	N/A	NL-HESC1	N/A
Oh[58]	*2005*	MRC, Seoul Nat Univ., Korea	73	10	SNUhES1-3	30
Pickering[46]	*2005*	Guy's, King's &St Thomas Med. UK	N/A	N/A	CF1 abn	N/A
Simon[36]	*2005*	Valecia Stem Cell Bank	40	16	VAL-1, VAL-2	13
Wang[59]	*2005*	Xiinhua Hospital, China	N/A	N/A	SH1, SH2,SH7	N/A
Baharvand[48]	*2006*	Royan Inst, Iran	N/A	N/A	Royan H2-3-4-5-6 (abn)	N/A
Hampl	*2006*	Acad. Sci. Czech Republic	98	14	CCTL8,9,6,10, 12,14	42
Mardal[60]	*2006*	Reliance Life Sciences, India	N/A	N/A	ReliCell®hES1	N/A
Mateizel[47]	*2006*	Res. C. Reprod. Genetics, Belgium	69	52	VUB1,2, 3,4,5 (abn)	10
Ludwig[15]	*2006*	WiCell	N/A	N/A	WA15, WA16	N/A

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Autor	Fresh/ Frozen embryos	Feeders	ICM Isolation	culture (d) before ICM isolation	initial split* (Mec. Coll. Disp)
Thomson[4]	Fresh/ frozen	Irrad MEF	Immuno	N/A	
Reubinoff[31]	Frozen	MitoC MEF	Immuno	6	MD
Lanzendorf[29]	Fresh	Irrad MEF	Immuno	6	M4-11
Amit[40]	Frozen	MEF	Immuno	N/A	
Richards[50]	Frozen	MitoC HF (FetMusl)	Immuno	N/A	MD 10
Hovatta[32]	Fresh	Irrad postnatHFF	Immuno	6	D9-19
Mitalipova[26]	Frozen	Irrad MEF	Immuno	6_7	M7-10
Park [51]	Frozen	MitoC MEF	Immuno		
Pickering[38]	N/A	N/A	N/A	N/A	N/A
Baharvand[27]	N/A	MitoC MEF	No Immuno	6	M1
Heins[52]	Fresh/ Frozen	MitoC MEF	Immuno	6_7	M7
Cowan[33]	Frozen	MitoC MEF	Immuno	N/A	M
Park[53]	Frozen	MitoC STO	Immuno	N/A	M5-8
Sjögren[20]	Fresh/ Frozen	MEF	Immuno	6_8	
Stojkovic[24]	Fresh	Irrad MEF	Immuno	8	M17
Strelchenko[43]	N/A	MitoC MEF or BRL	Immuno	N/A	
Suss-Toby[34]	Fresh	Inact MEF	No Immuno	N/A	M
Verlinsky[35]	Fresh PGD	MitoC MEF or BRL			ED8-14
Chen[25]	Fresh	MitoC MEF	Immuno	5_8	M5-8

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Author	Fresh/ Frozen embryos	Feeders	ICM Isolation	culture (d) before ICM isolation	Initial split* (Mec. Coll. Disp)
Findikli[21]	Fresh	MitoC MEF	15Immuno, 12 direct	5_7	7-10
Genbacev[13]	Frozen Fresh	Human Plancental fibroblasts	No Immuno	N/A	M
Inzunza[42]	3frozen, 7fresh	Irrad HFF	Immuno	6	M12
Kim HS [39]	Frozen	MitoC STO	Immuno/Mechanical	N/A	M7
Kim SJ [54]	Frozen	MitoC MEF	Immuno	N/A	M
Klimanskaya[12]	Frozen	MitoC Lysed MEFs	Immuno	N/A	
Lee[55]	Frozen	MitoC Human endometrial	Immuno	N/A	M6-8
Li [56]	Frozen	Irrad MEF	Immuno	N/A	
Lysdahl[57]	Fresh	Irrad HFF	Immuno	N/A	M10-15
Mummery[37]	Fresh	MitoC MEF	Immuno	N/A	MD8
Oh[58]	Frozen	MitoC STO	Immuno/ whole	5_7	M7-8
Pickering [46]	Fresh PGD	N/A	N/A	N/A	M17
Simon[36]	Frozen	Human Plancental	No Immuno	N/A	M15 M21
Wang [59]	N/A	Irrad MEF 55Gy EDF	Immuno	N/A	M/C 10-14
Baharvand [48]	N/A	MitoC MEF	Immuno	5,5	MD 10
Hampl	Frozen	MEF	Immuno	N/A	N/A
Mardal[60]	N/A	N/A	N/A	N/A	N/A
Mateizel [47]	Frozen/ Fresh	Irrad/MitoC MEF	Immuno	N/A	N/A
Ludwig [15]	Frozen	N/A	Immuno	N/A	N/A

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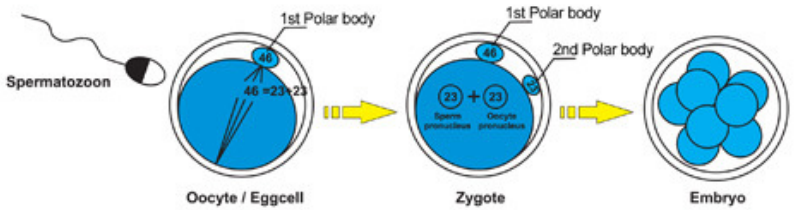
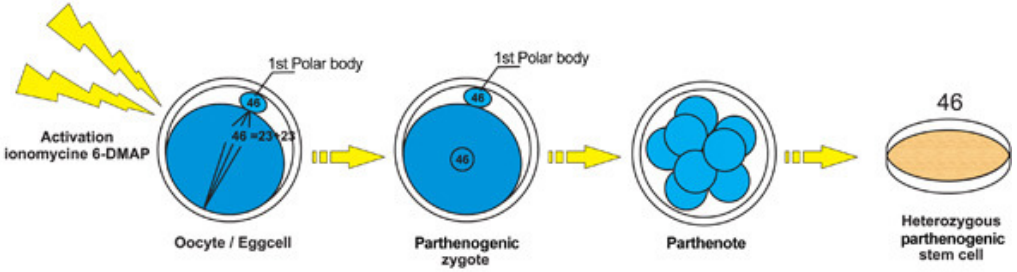
Parthenogenetic ES cells from human oocytes

Fertilization



Parthenogenesis



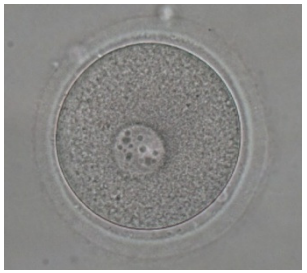
Activation:

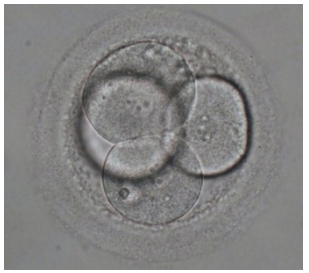
Ionomicin: flux of intracellular Ca++ → reactivation of meiosis

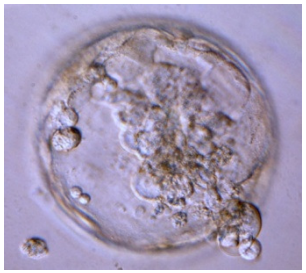
6-DMAP: block the 2nd PB extrusion → 2n embryo

Culture:

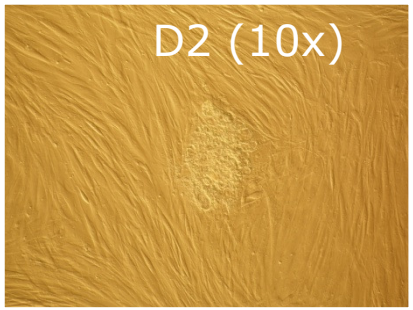
G1/G2 medium for human IVF embryos up to blast (D+5/7)

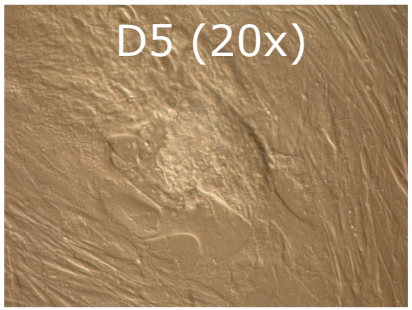


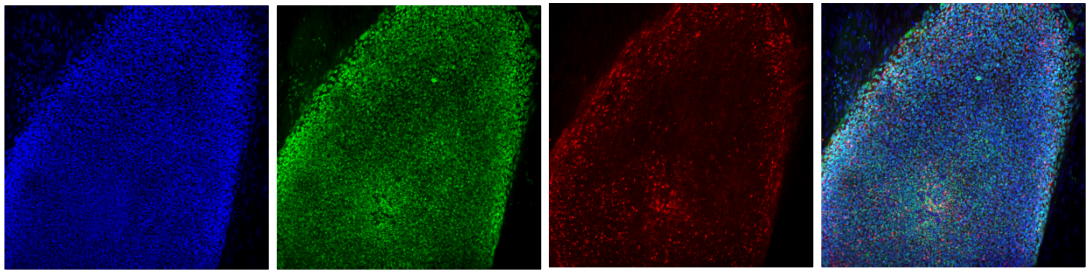




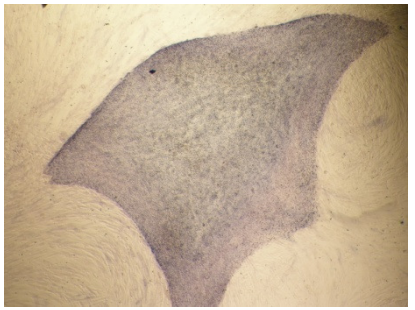
Oocytes	1PN1PB @ 18h	Cleaved @ D2	Blastocysts @ D5	Cell lines
17	12	16 (94%)	6 (37.5%)	1 (6.25%)

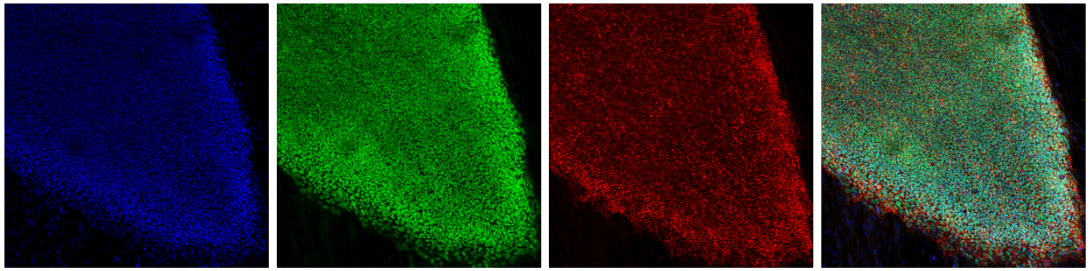




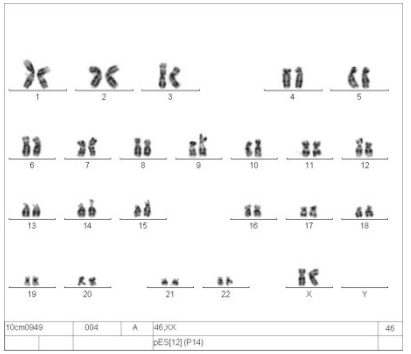


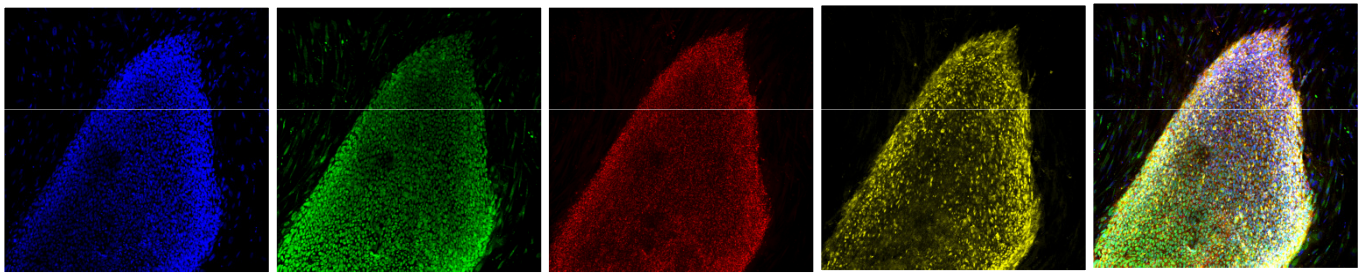
DNA
Nanog
TRA 1-81



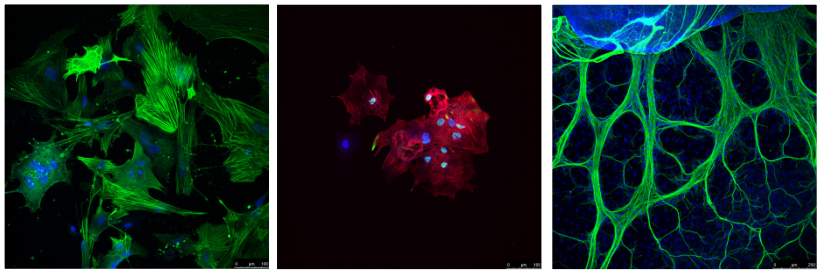


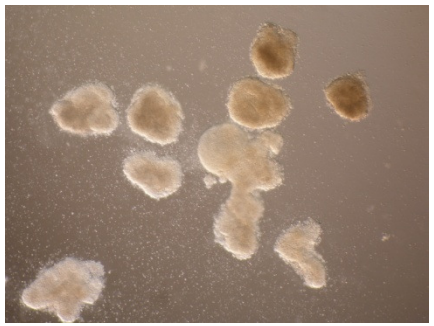
DNA
Oct4
SSEA3





DNA
Sox2
SSEA4
Tra 1-60





hESCreg

European Human Embryonic Stem Cell Registry

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  - Register
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- Legislation
- Ethics
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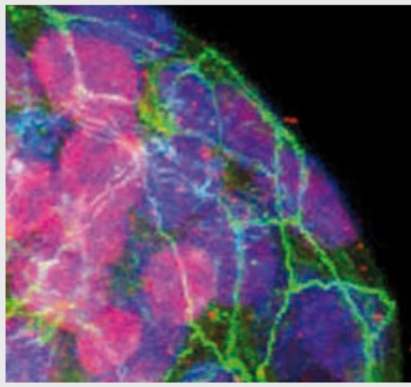
- Contact

Welcome



Register:

Cell Line
Research Project

Login to my hESCreg

Show:

Available Cell Lines
All Research Projects
All Research Provider

Welcome to the European Human Embryonic Stem Cell Registry – hESCreg

You have entered the work-in-progress site of the hESCreg database. Here you can browse the database or log in using the Username/Password combination issued to you. At the first login we advise you to change your personal Username/Password combination to one only known to yourself. If you are a provider of hES cell lines we are calling on you to complete the minimum registration information for your pre-registered cell lines. If there are any new lines please add them as well. We would greatly appreciate if you could complete this by the end of November 2007. Apart from that we are looking forward to receiving your comments or suggestions. Should you have any problems operating the database or would like to contribute to the further development of the database please direct all communication to helpdesk@hescreg.eu.

Until the official launch of hESCreg on January 18, 2008, in Berlin the interim web page at www.hescreg.eu will act as the official information resource of the initiative.

Thank you very much in advance for your contributions!

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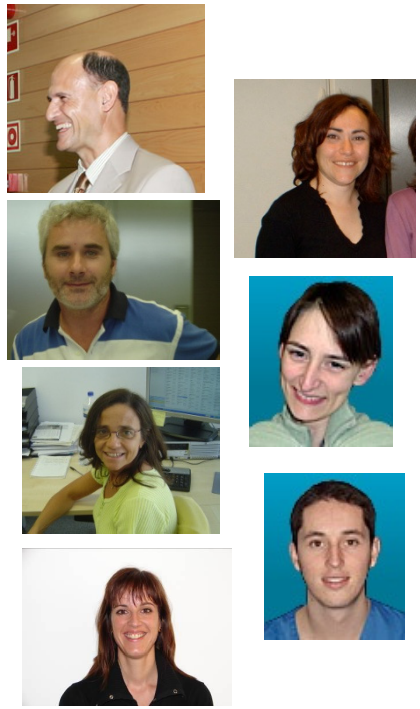
anavei@dexeus.com



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Centre de Medicina Regenerativa de Barcelona