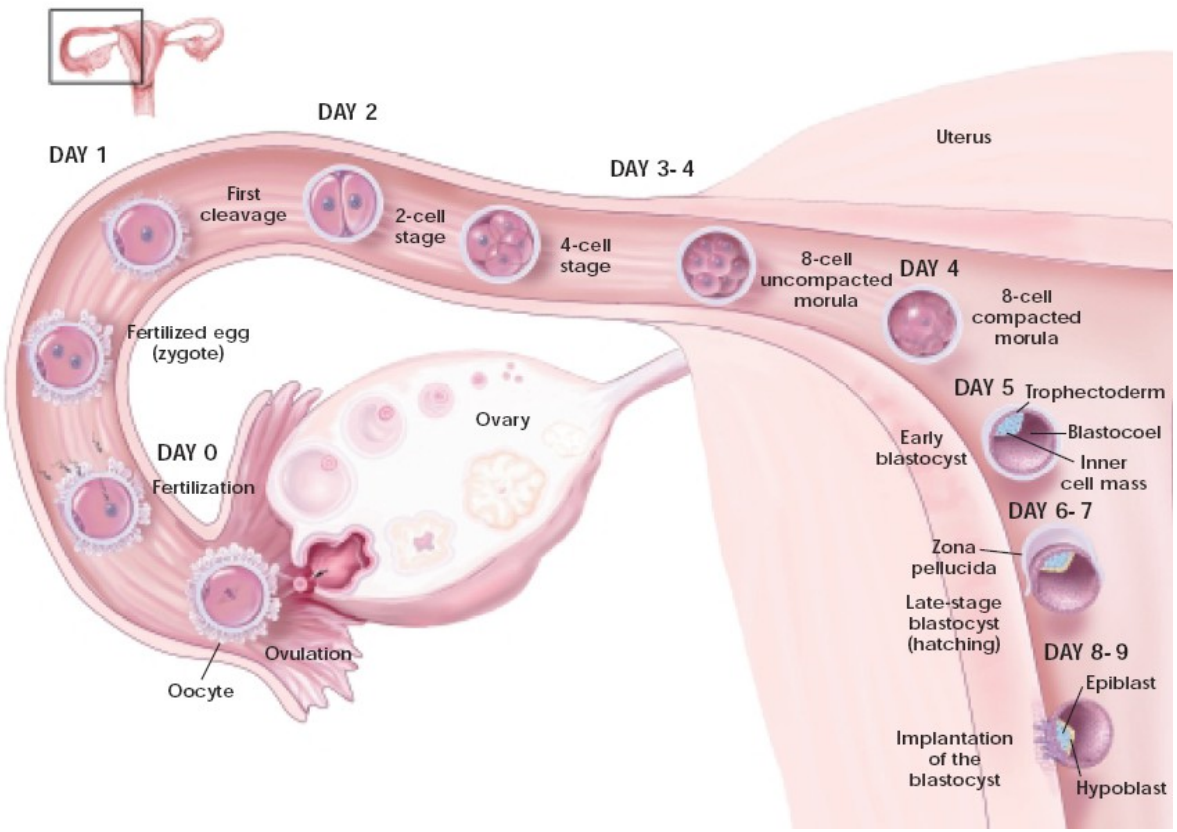
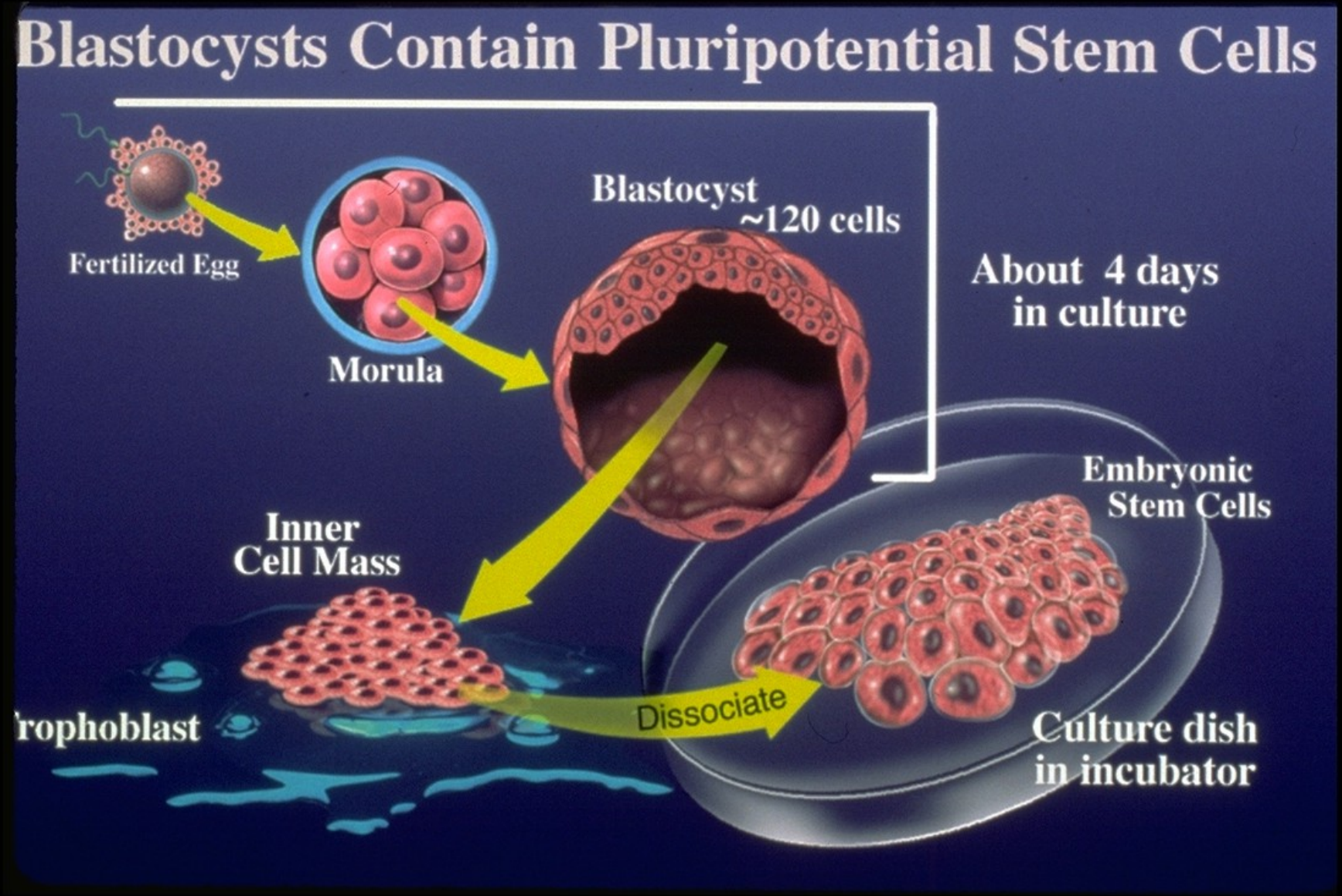
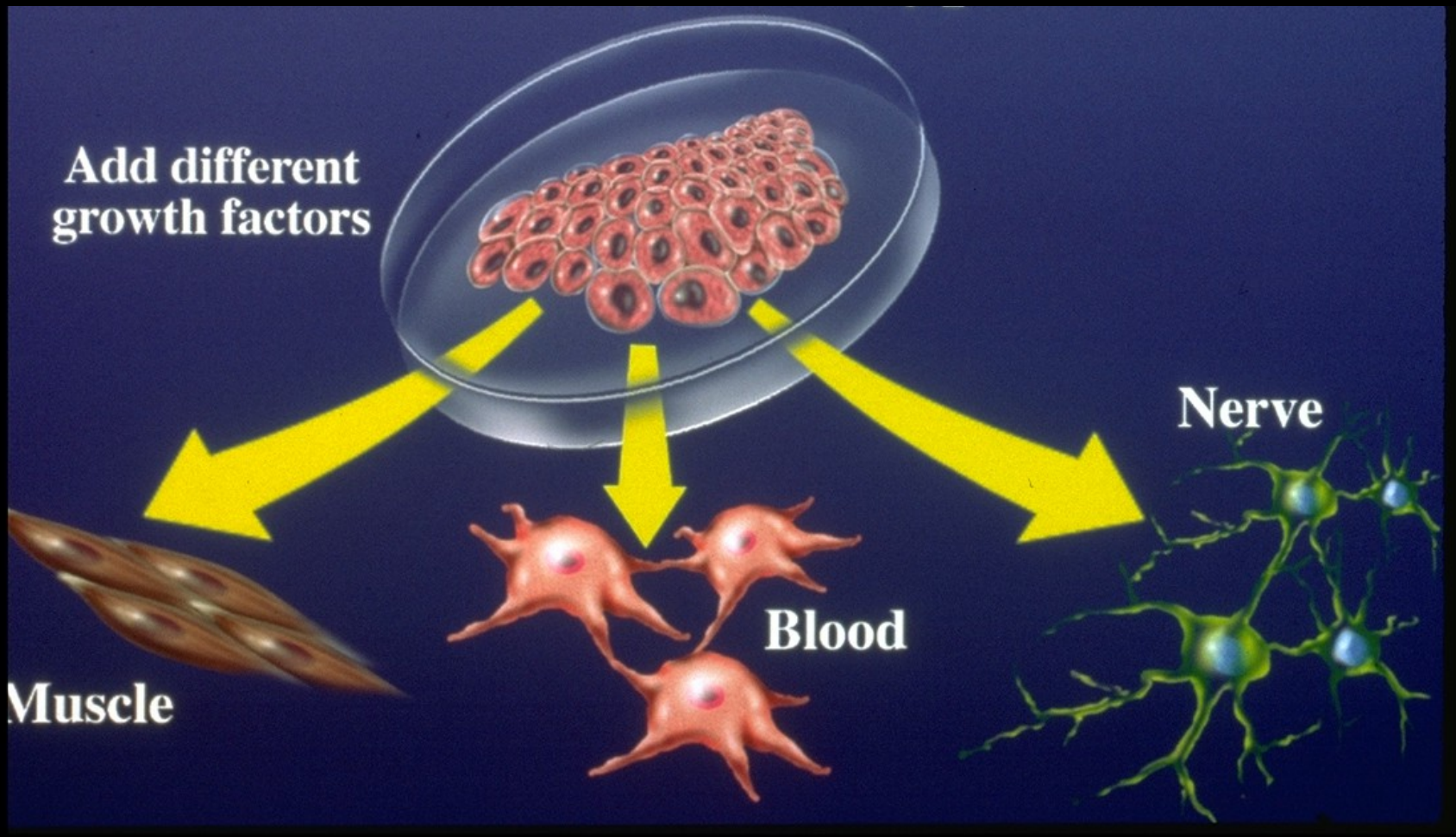
Izvorne celice

Kloniranje sesalcev


DAY 1
DAY 2
DAY 3- 4
Uterus
First cleavage
2-cell stage
4-cell stage
8-cell uncompacted morula
DAY 4
8-cell compacted morula
Fertilized egg (zygote)
Ovary
DAY 5
Trophectoderm
Blastocoel
Early blastocyst
Inner cell mass
DAY 0
Fertilization
DAY 6- 7
Zona pellucida
Late-stage blastocyst (hatching)
Ovulation
Oocyte
DAY 8- 9
Epiblast
Implantation of the blastocyst
Hypoblast

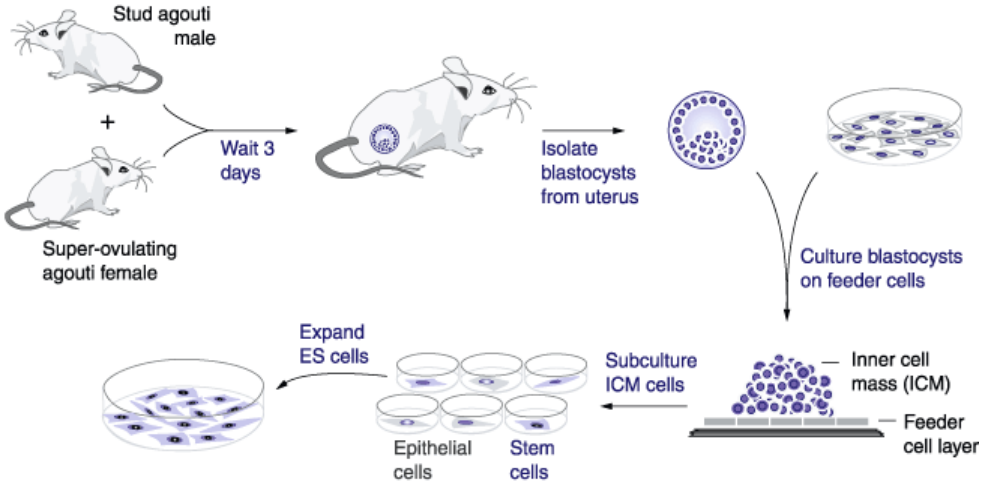

Blastocysts Contain Pluripotential Stem Cells
Fertilized Egg
Morula
Blastocyst
~120 cells
About 4 days
in culture
Embryonic
Stem Cells
Inner
Cell Mass
Dissociate
Culture dish
in incubator


Add different
growth factors
Nerve
Blood
Muscle

Izvorne celice

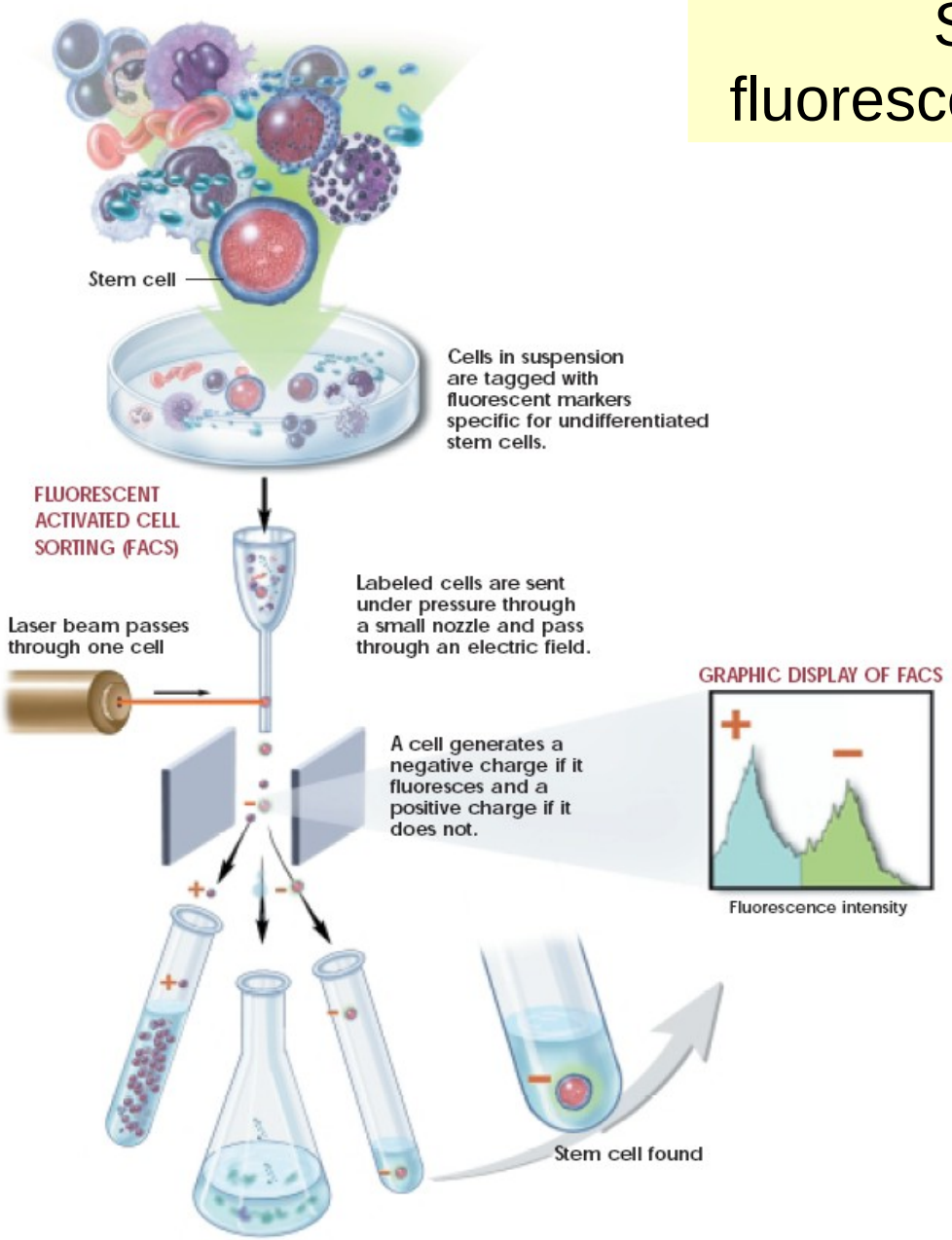
Blastocista (zarodek, star ~5 dni, ~100 celic): **embrionalne izvorne celice** (ES) predstavljajo notranjo maso blastociste in jih je ~30. ES-celice so pluripotentne in se lahko večkrat delijo, ne da bi se diferencirale. Pod določenimi pogoji jih je mogoče inducirati, da preidejo v funkcionalne celice s specializirano funkcijo. Pri človeku so jih uspeli izolirati in gojiti šele leta 1998.

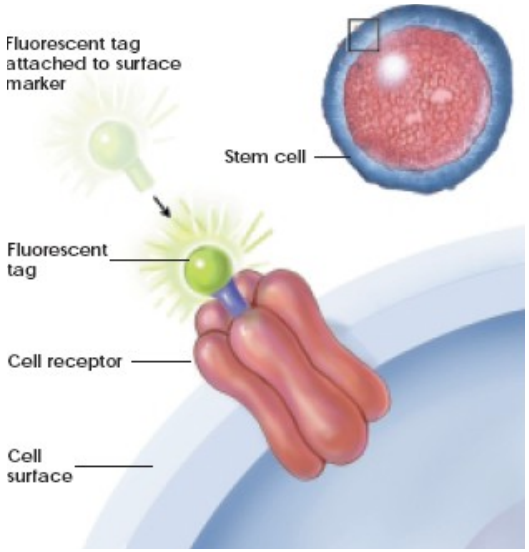
Raziskovalci preučujejo izvorne celice, da bi razumeli, kaj jim omogoča, da tako dolgo ostanejo nediferencirane in da bi odkrili, kateri signali lahko povzročijo njihovo specializacijo. Izvorne celice predstavljajo perspektiven način zdravljenja nekaterih hudih bolezni, ki jih trenutno še ne znamo pozdraviti.

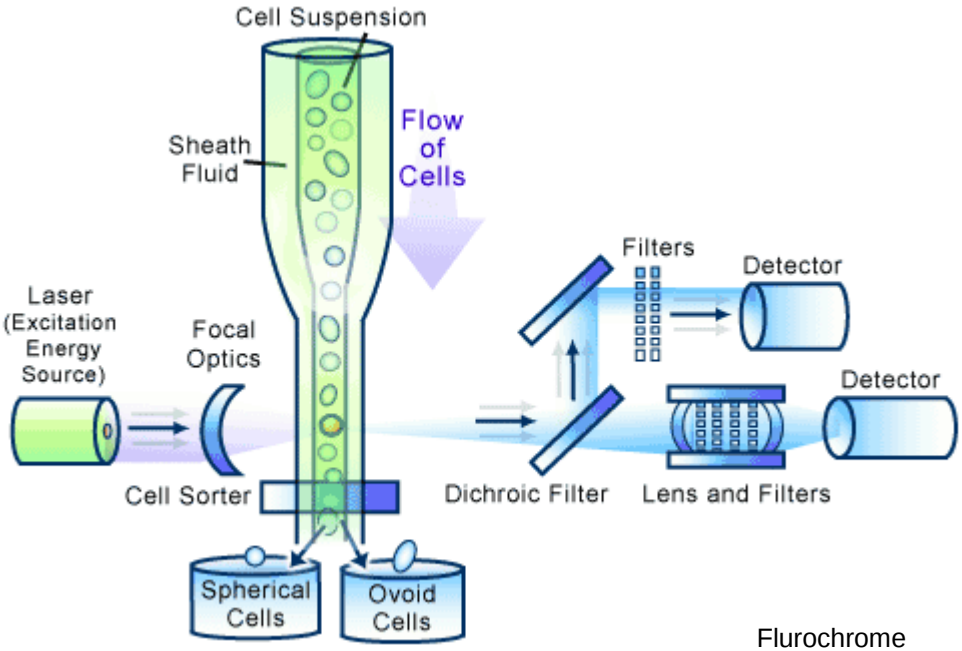


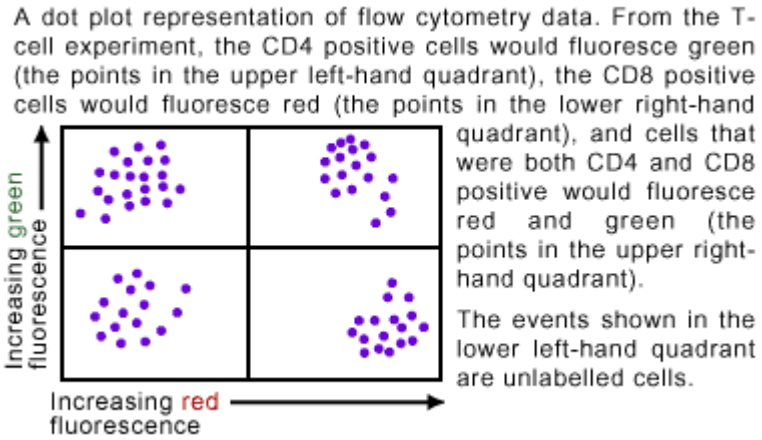
Embrionalne izvorne celice gojimo v posodah, ki so prekrite z embrionalnimi epitelnimi celicami, ki so spremenjene tako, da se ne morejo deliti *(‘feeder cells’)*. Te predstavljajo površino za pritrjevanje, hkrati pa izločajo rastne faktorje. Nedavno je uspelo gojiti ES-celice tudi v odsotnosti epitelnih celic.

Sortiranje celic s fluorescenčno aktivacijo (FACS)

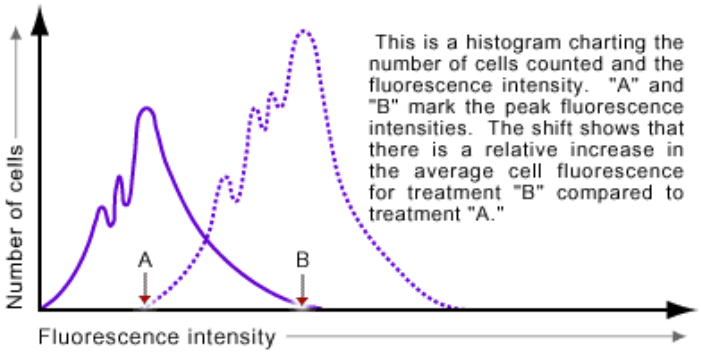








Flurochrome	Excitation Wavelength (nm)	Wavelength Emission (nm)	Emitted Color
Texas Red	488	615	Red
Phycoerythrin (PE)	488	575	Green
Fluorescein isothyocanate (FITC)	488	525	Green

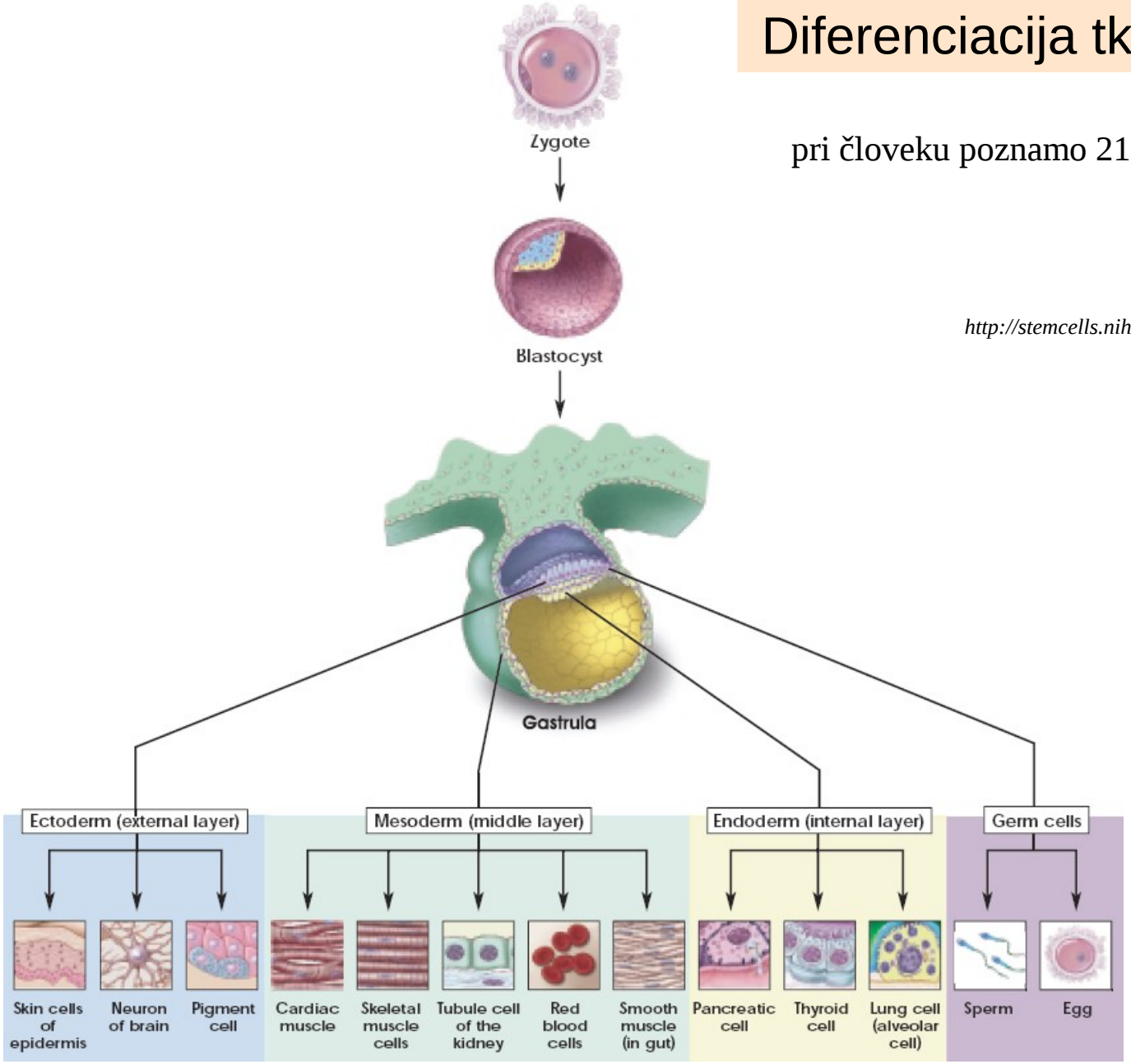


http://www.bioteach.ubc.ca/MolecularBiology/FlowCytometry/

Diferenciacija tkiv pri človeku

pri človeku poznamo 216 različnih tipov celic

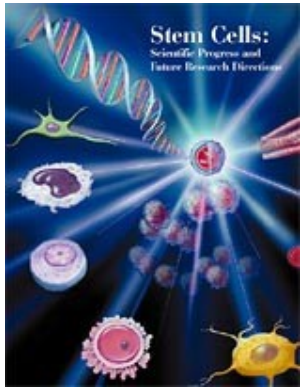
http://stemcells.nih.gov/stemcell/pdfs/fullrptstem.pdf



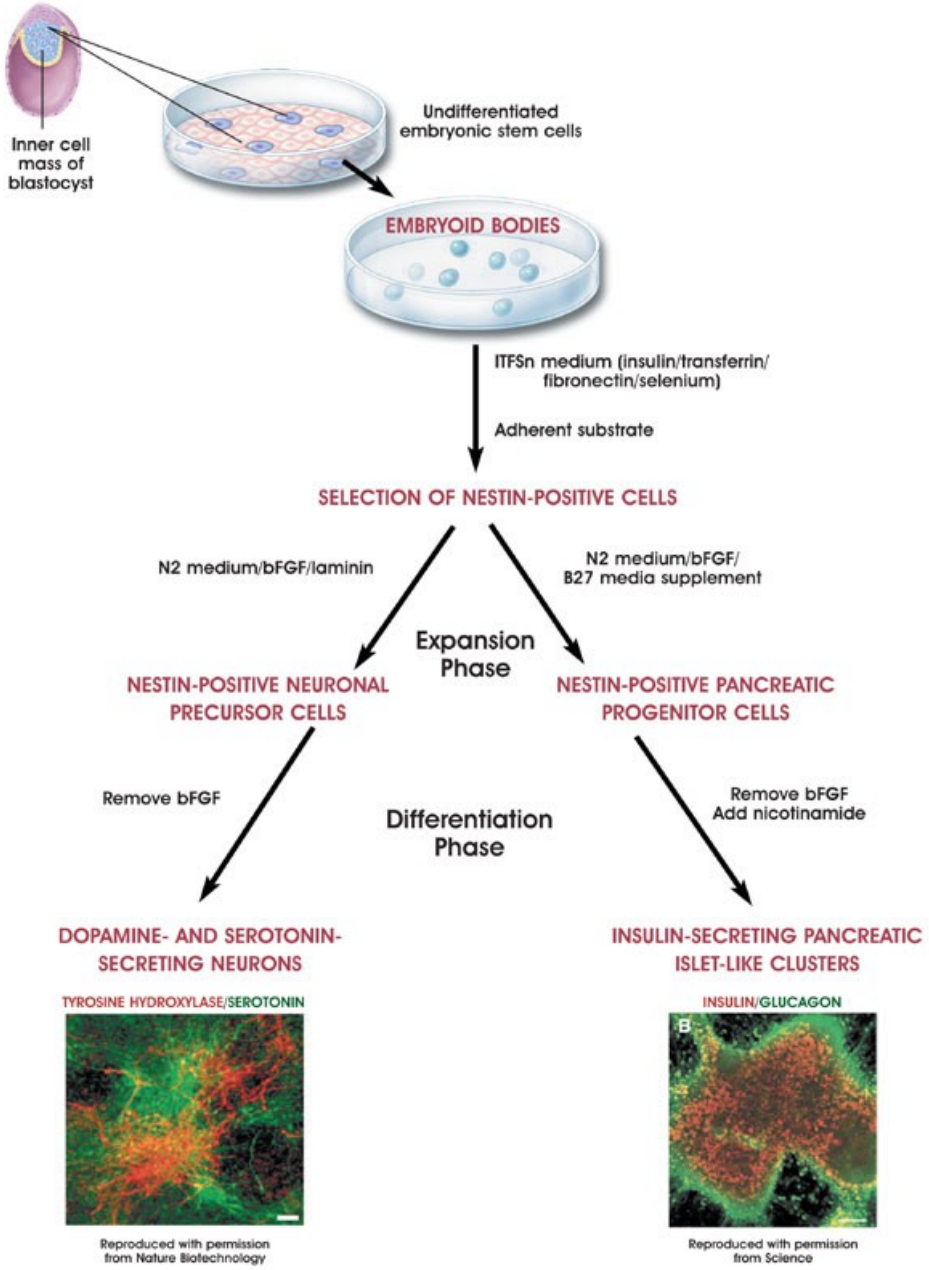
Izvorne celice /3

Diferenciacijo ES-celic lahko *in vitro* dosežemo na več načinov:

- celice pustimo, da tvorijo skupke (embrioidna telesca): razvijejo se celice različnih tipov
- spremenimo sestavo gojišča in površino posode za gojenje



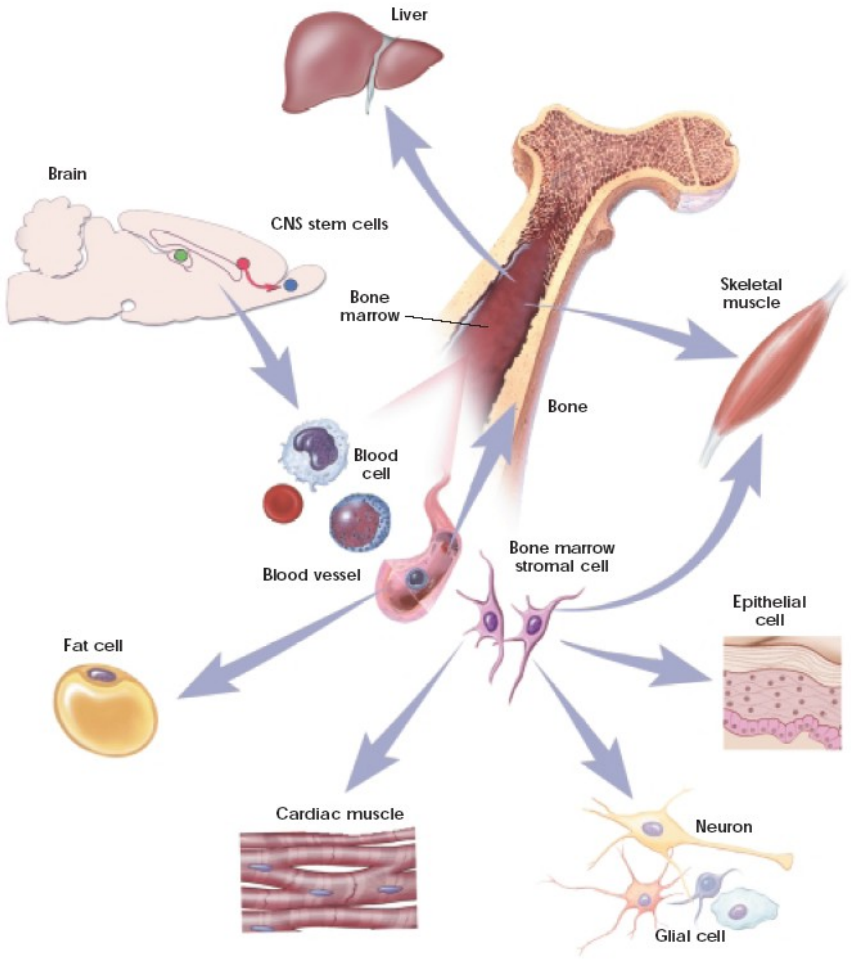
http://stemcells.nih.gov/index.asp
http://stemcells.nih.gov/stemcell/scireport.asp (222 strani)



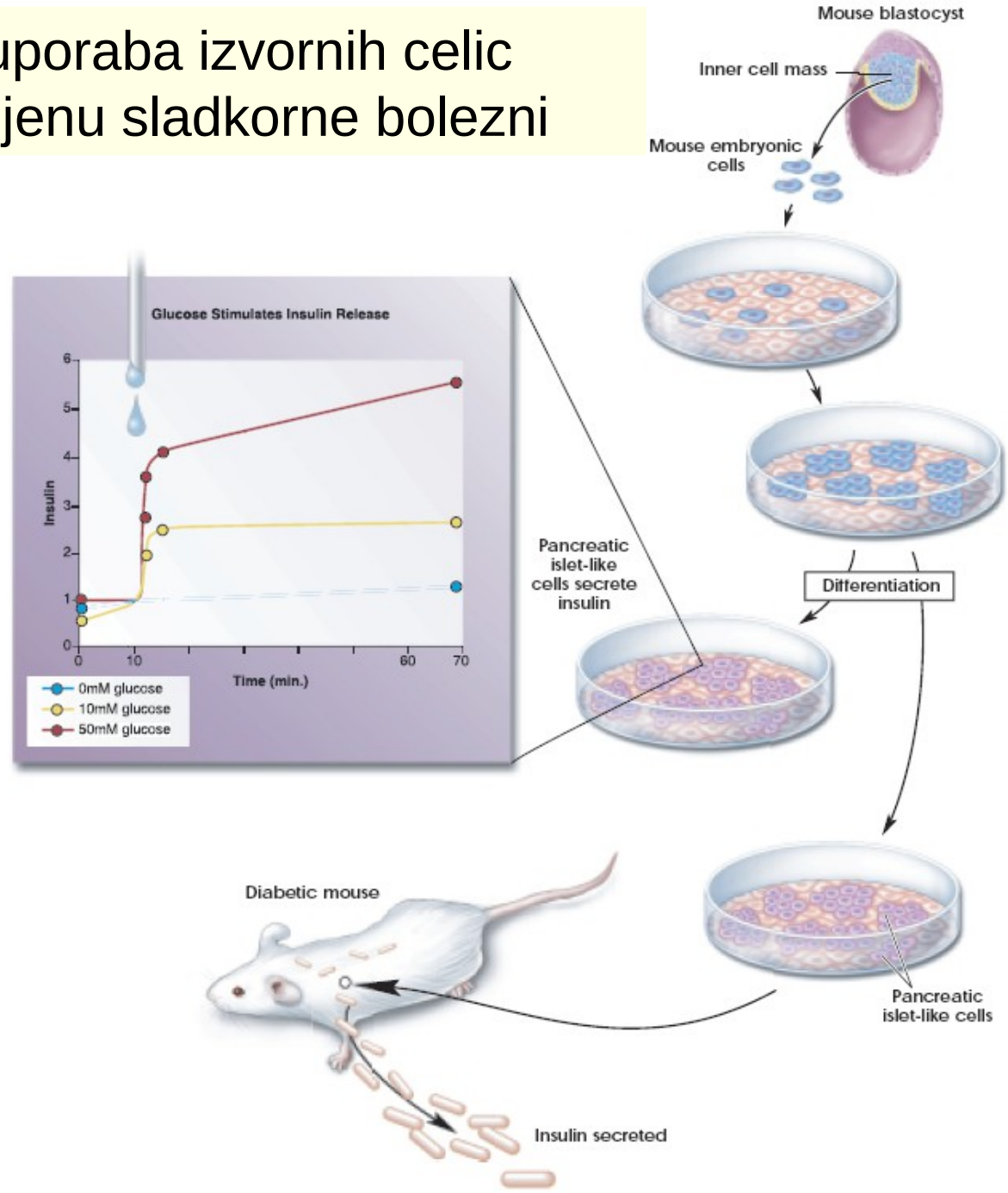
Izvorne celice /4

Izvorne celice pa obstajajo tudi pri odraslih (*adult stem cells*).
Našli so jih v kostnem mozgu, možganih, mišicah,koži,...
a jih je zelo malo.
Iz njih nastajajo celice, ki nadomestijo poškodovane ali okvarjene celice različnih tipov.
Odraslih izvornih celic ni mogoče dolgo gojiti, ne da bi se diferencirale.

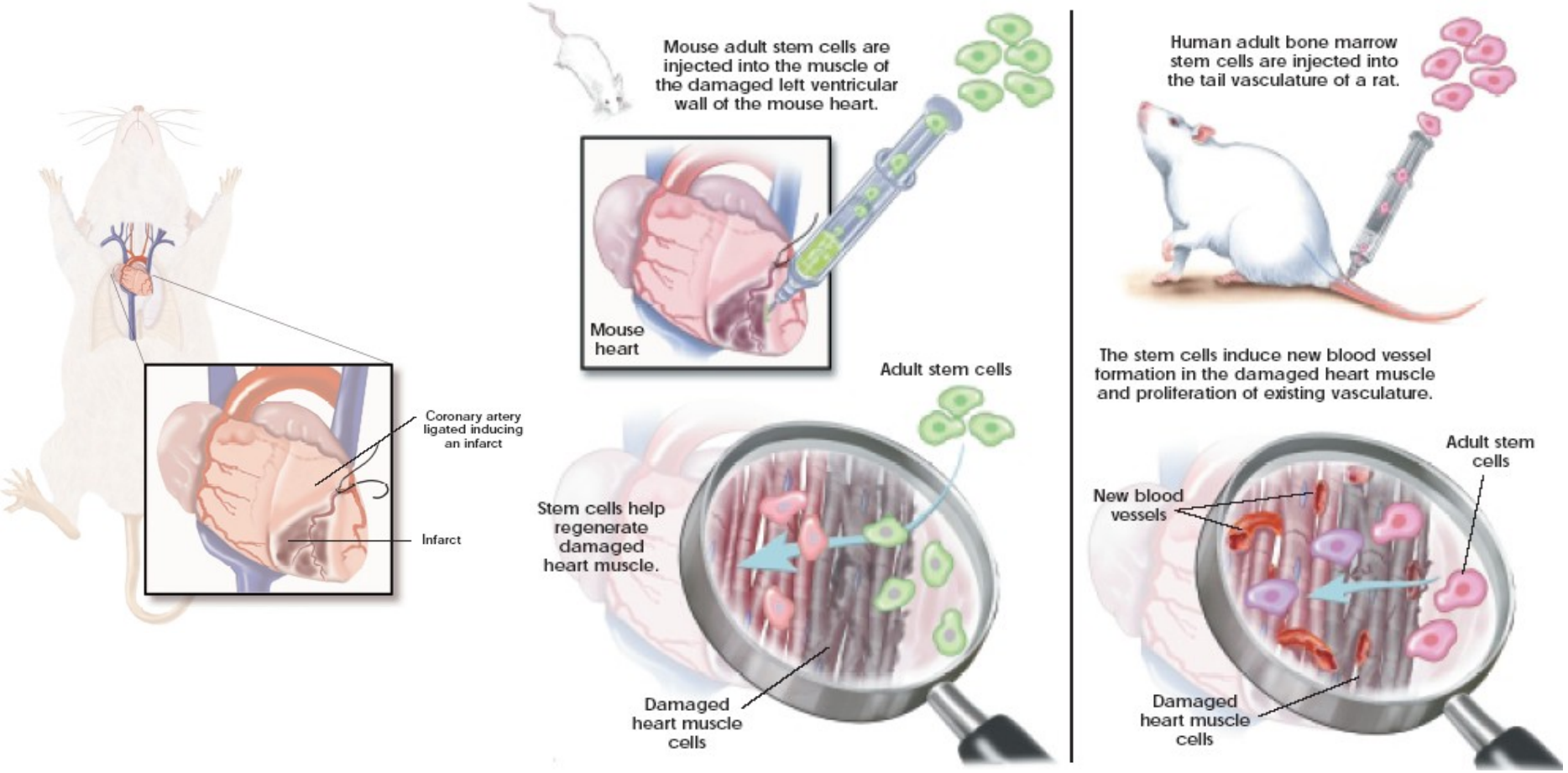
Odrasle (somatske) izvorne celice: dolgo je veljalo, da se lahko diferencirajo le v specializirano celico, ki ustreza tkivu, v katerem je (npr. izvorna celica v kostnem mozgu se razvije v eno od krvnih celic, ne more pa se razviti v možgansko živčno celico). Nekateri poskusi pa so pokazali, da je mogoča tudi diferenciacija v celico s povsem drugačno vlogo: ‘plastičnost’.



Možna uporaba izvornih celic pri zdravljenu sladkorne bolezni



Možna uporaba izvornih celic pri zdravljenu posledic srčnega infarkta

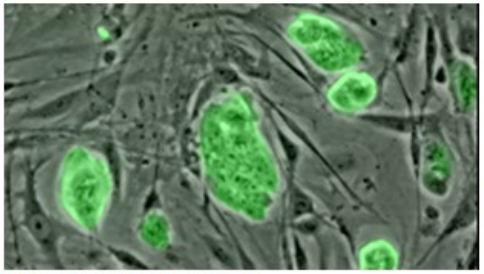


rezultati: Lanza, R. et al., Circulation Res., 2004

Chemical Reprogramming of Stem Cells

A new method for producing pluripotent stem cells using just small molecules, without the need for inserting genes, could yield safer therapies.

By Chris Palmer | July 22, 2013



Mouse embryonic stem cells
WIKIMEDIA COMMONS, CHONGDAE

Scientists first developed the ability to reprogram somatic cells into pluripotent stem cells—cells that could give rise to any of the body's cells types—in 2006. However, the technique has required the insertion of "master genes" that could increase the risk of mutations and cancer, limiting its potential clinical applications. A new study published Thursday (July 18) in *Science* demonstrates the creation of pluripotent stem cells in mice using a cocktail of small molecule compounds that can substitute for the potentially dangerous genes.

Though scientists have steadily reduced the need for additional genes to trigger pluripotency, one gene, *Oct4*, has been indispensable. Researchers led by Hongkui Deng at Peking University spent a year screening 10,000 compounds that would facilitate the reprogramming of mouse somatic cells into pluripotent cells without the need of *Oct4*. They identified a combination of seven small-molecule compounds that created pluripotent cells at a frequency of 0.2 percent, a rate comparable to the yield using gene insertion. The team dubbed its cellular creations chemically induced pluripotent stem cells (CiPSCs).

The researchers demonstrated that CiPSCs were indeed pluripotent by introducing the cells into developing mouse embryos. The resulting animals showed signs that CiPSCs gave rise to all major cell types, including brain, heart, liver, skin, and muscle.

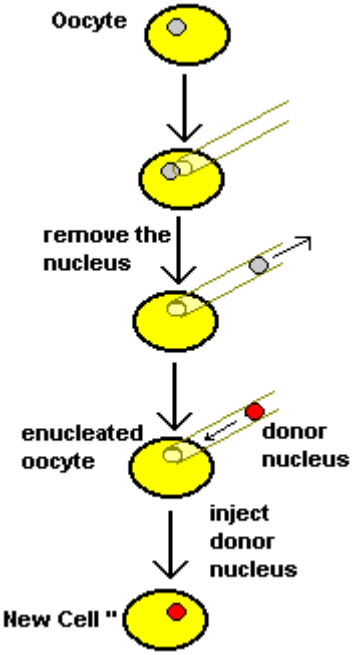
"People have always wondered whether all factors can be replaced by small molecules. The paper shows they can," Rudolf Jaenisch, a cell biologist at the Whitehead Institute at the Massachusetts Institute of Technology, told *Nature*.

The team described several advantages of using small molecules for reprogramming: they don't impact the immune system, they are cost effective; they are easily synthesized and preserved; and they pass through cell membranes easily, meaning they can be washed away after initiating reprogramming.

Sheng Ding, a reprogramming researcher at the Gladstone Institutes in San Francisco, told *Nature* that the study reflects "significant progress," but thinks that chemical reprogramming will not be widely used until the method is applied to human cells, not just mouse cells.

Kloniranje živali

O kloniranju vretenčarjev sta prva poročala Briggs in King že leta 1952. Poskus sta izvedla z žabo *Rana pipiens* in uspela dobiti klonirane paglavce (ne pa odraslih žab). Donorska jedra so bila iz blastul in sta jih mikroinjicirala v citoplazmo enukleiranih jajčnih celic. Uspešnost je bila 80 %.
"Jedra v stadiju blastule so torej totipotentna."



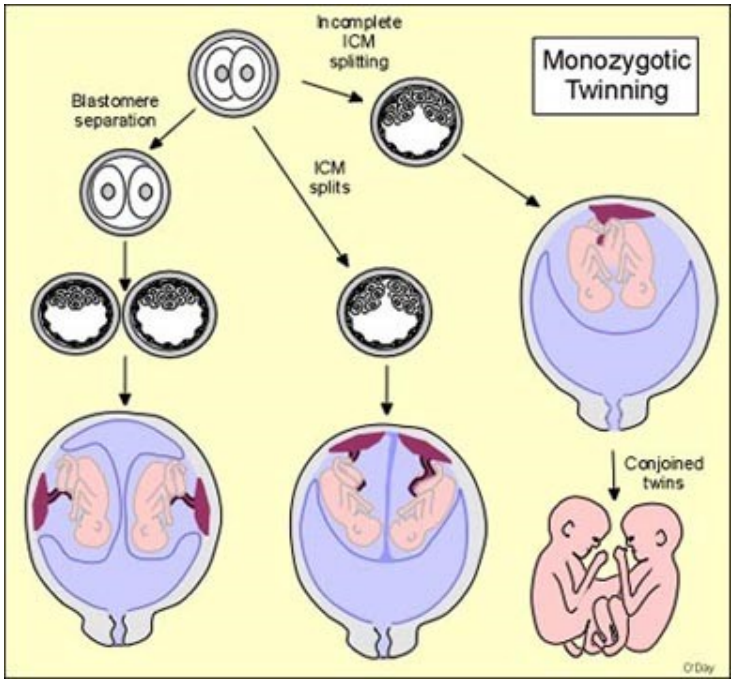
Gourdon (1962) je uporabil jedra diferenciranih črevesnih celic paglavcev dvoživke *Xenopus laevis*. Uspešnost je bila sedaj le še 2-5 %. Vprašanje pa je, ali je res uporabil končno diferencirane celice ali morda izvorne. *(Enako vprašanje se včasih pojavlja tudi v zvezi s kloniranjem ovce Dolly.)*

1966 so raziskovalci uspeli dobiti klonirano odraslo žabo iz črevesnih celic mladih paglavcev, 1975 pa so vzredili paglavce iz celic odraslih žab.

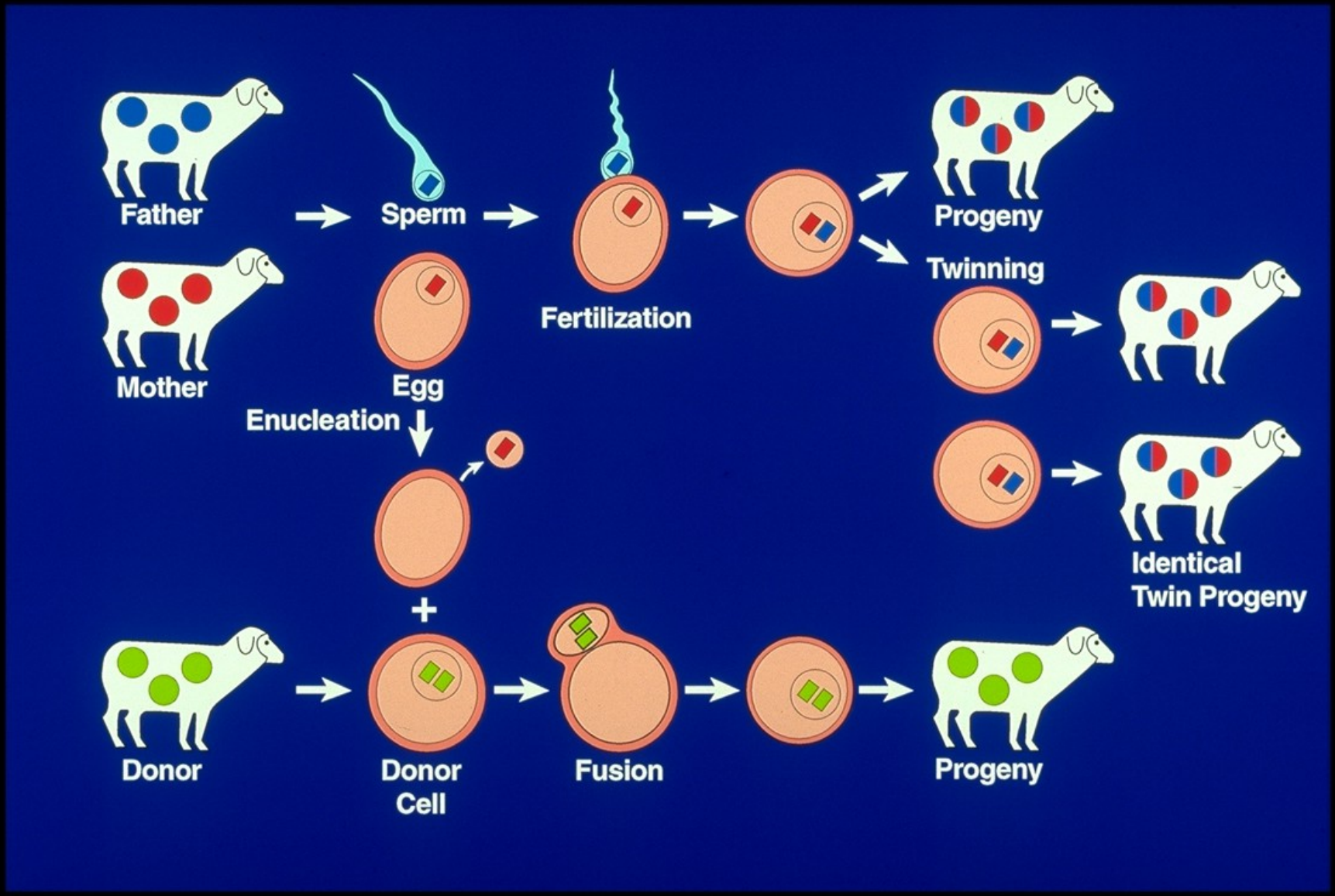
Kloniranje živali /2

1984 so vzgojili klone ovce tako, da so fizično ločili celice razvijajočih se zarodkov (‘twinning’). Enak postopek so izvedli tudi z zarodki drugih sesalcev.

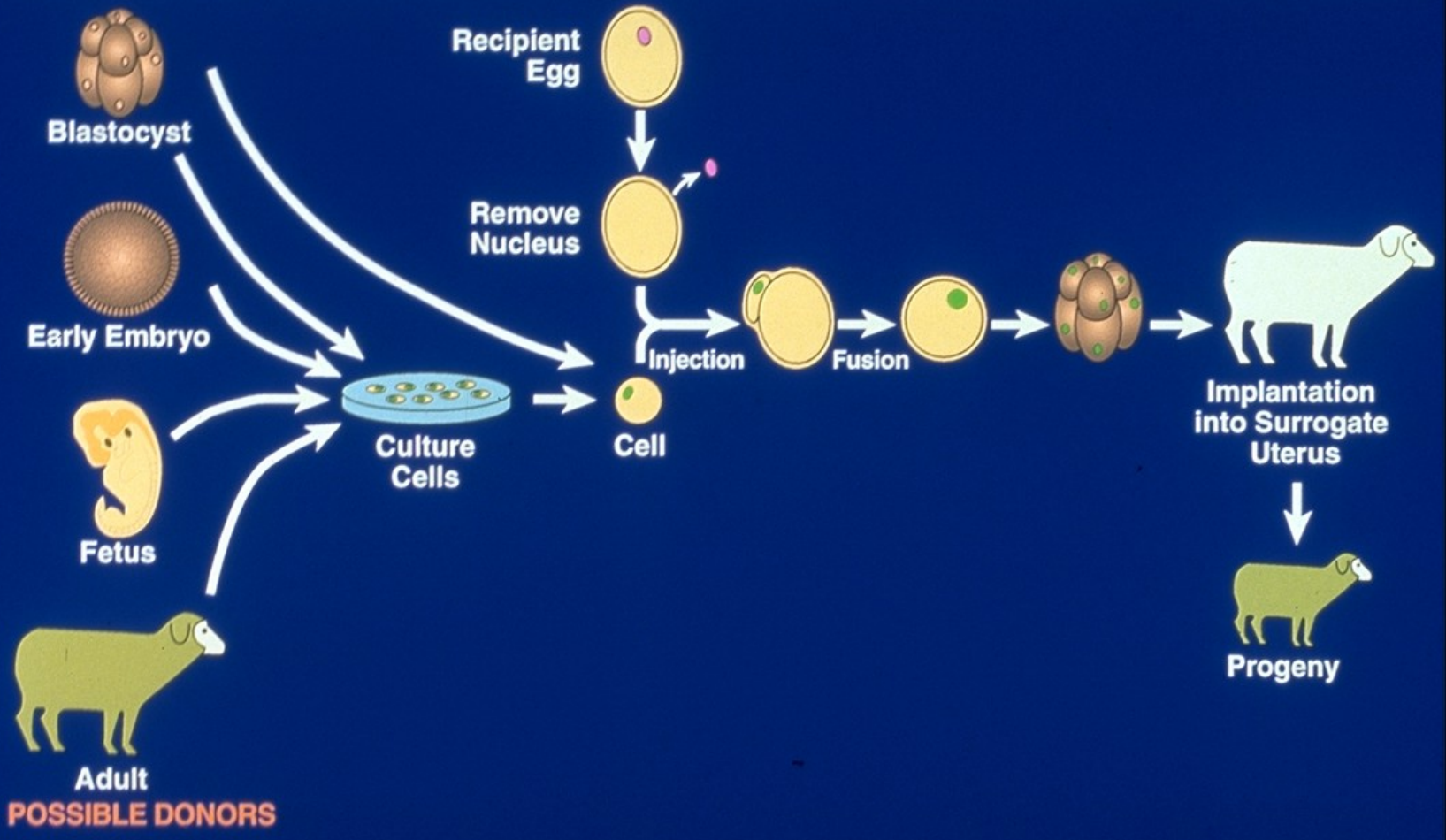
Predhodnici ovce Dolly sta bili ovci Megan in Morag. Na Roslinovem inštitutu v Edinburghu so ju klonirali 1995 iz embrionalnih izvornih celic, ki so jih pred tem več tednov gojili v laboratoriju.



http://www.utm.utoronto.ca/~w3bio380/picts/lectures/lecture11/Twinning1.jpg


Father
Sperm
Fertilization
Progeny
Twinning
Mother
Egg
Enucleation
Identical
Twin Progeny
Donor
Donor
Cell
Fusion
Progeny

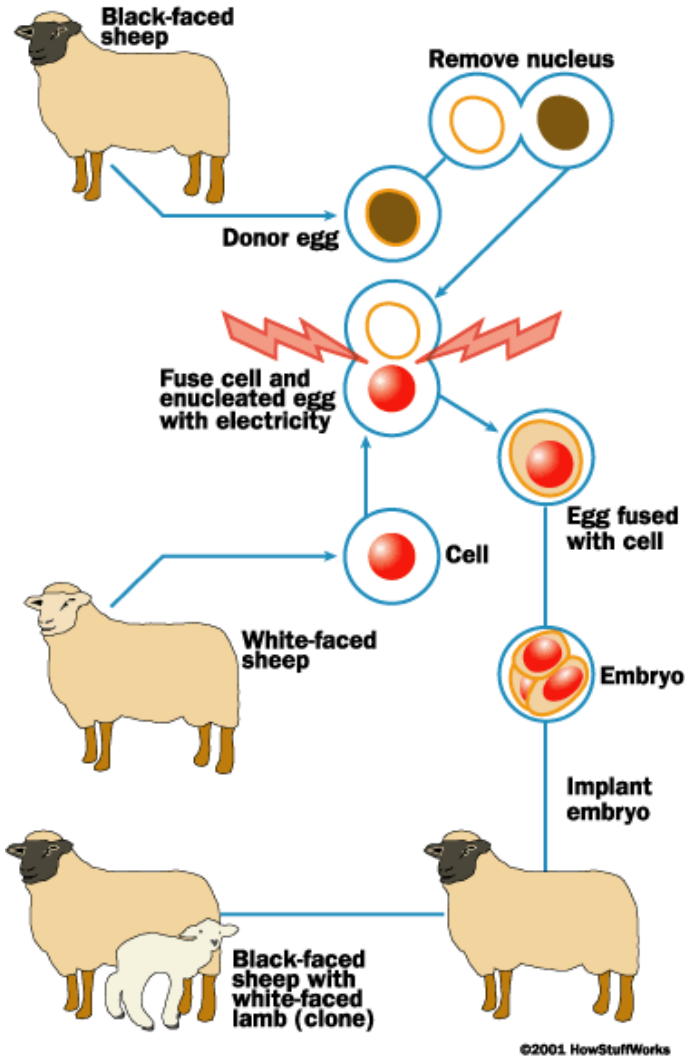
Cloning Procedures



Kloniranje z jedrnim prenosom

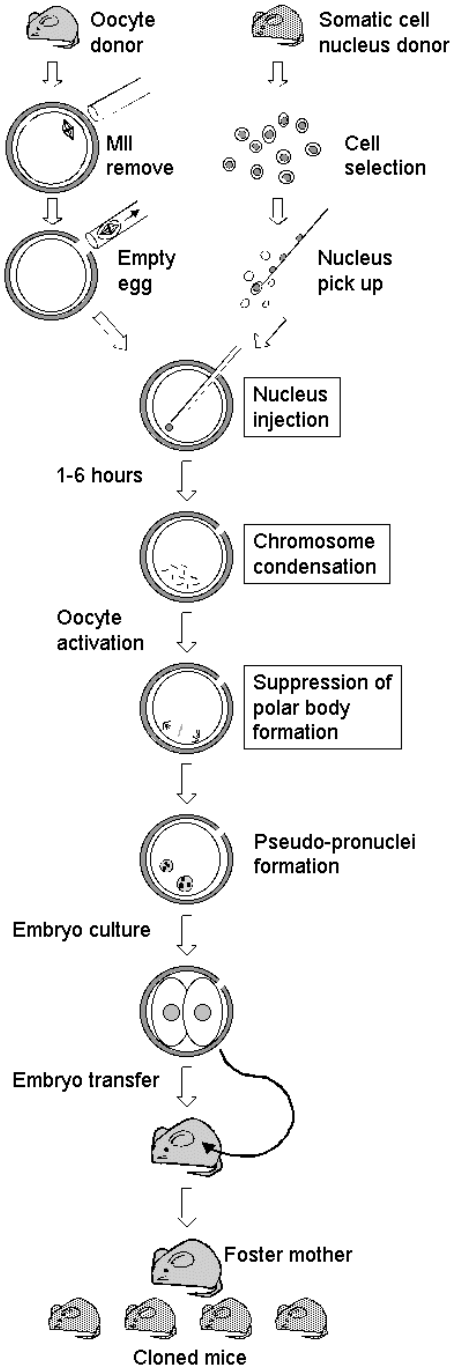
Za kloniranje višjih organizmov z želenimi lastnostmi najpogosteje uporabljajo metodo jedrnega prenosa.
Prvi uspešni poskusi so bili s prenosom embrionalnih jeder, pri ovci Dolly (5.7.1996) pa je šlo prvič za jedro iz celice odraslega organizma (epitel vimena 6 let stare ovce).
Epitelno celico so spravili v fazo mirovanja (G0), saj tudi jajčna celica pri prvih 3 delitvah samo pomnožuje DNA, do izražanja genov pa ne prihaja.

Dolly so uspavali 14.2.2003, ker je imela tumor na pljučih.
Pljučne bolezni so pri starih ovcah pogoste.
Ovce te pasme sicer živijo ~12 let. Leta 1999 so ugotovili, da so telomeri pri Dolly nekoliko krajši, kot bi pričakovali pri 3 leta stari ovci. Zadnja leta je imela tudi artritis v zadnjih nogah. Vseeno je skotila 4 zdrave mladiče (Bonnie 1998 in trojčke leta 1999).



Tehnika “Honolulu”

tehnika Honolulu → kloniranje miši (1998)



Ovca Dolly

Viable offspring derived from fetal and adult mammalian cells

I. Wilmut, A. E. Schnieke*, J. McWhir, A. J. Kind* & K. H. S. Campbell

Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, UK
** PPL Therapeutics, Roslin, Midlothian EH25 9PP, UK*

NATURE | VOL 385 | 27 FEBRUARY 1997

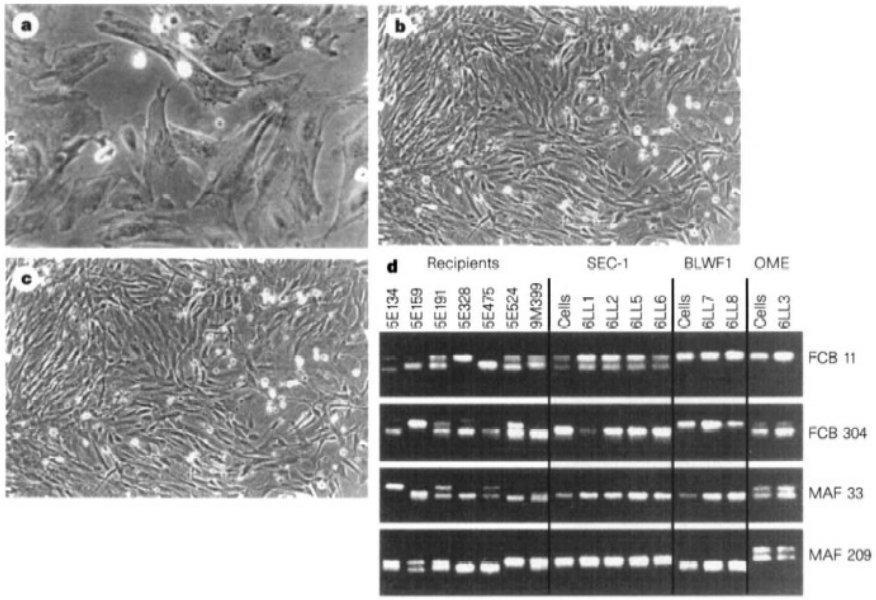


Figure 1 Phase-contrast photomicrograph of donor-cell populations: **a**, Embryo-derived cells (SEC1); **b**, fetal fibroblasts (BLWF1); **c**, mammary-derived cells (OME). **d**, Microsatellite analysis of recipient ewes, nuclear donor cells and lambs using four polymorphic ovine markers[22]. The ewes are arranged from left to right in the same order as the lambs. Cell populations are embryo-derived (SEC1), fetal-derived (BLW1), and mammary-derived (OME), respectively. Lambs have the same genotype as the donor cells and differ from their recipient mothers.

Table 2 Delivery of lambs developing from embryos derived by nuclear transfer from three different donor cells types, showing gestation length and birth weight

Cell type	Breed of lamb	Lamb identity	Duration of pregnancy (days)*	Birth weight (kg)
Mammary epithelium	Finn Dorset	6LL3	148	6.6
Fetal fibroblast	Black Welsh	6LL7	152	5.6
	Black Welsh	6LL8	149	2.8
	Black Welsh	6LL9†	156	3.1
Embryo-derived	Poll Dorset	6LL1	149	6.5
	Poll Dorset	6LL2‡	152	6.2
	Poll Dorset	6LL5	148	4.2
	Poll Dorset	6LL6‡	152	5.3

* Breed averages are 143, 147 and 145 days, respectively for the three genotypes Finn Dorset, Black Welsh Mountain and Poll Dorset.
† This lamb died within a few minutes of birth.
‡These lambs were delivered by caesarian section. Overall the nature of the assistance provided by the veterinary surgeon was similar to that expected in a commercial flock.

Figure 2 Lamb number 6LL3 derived from the mammary gland of a Finn Dorset ewe with the Scottish Blackface ewe which was the recipient.

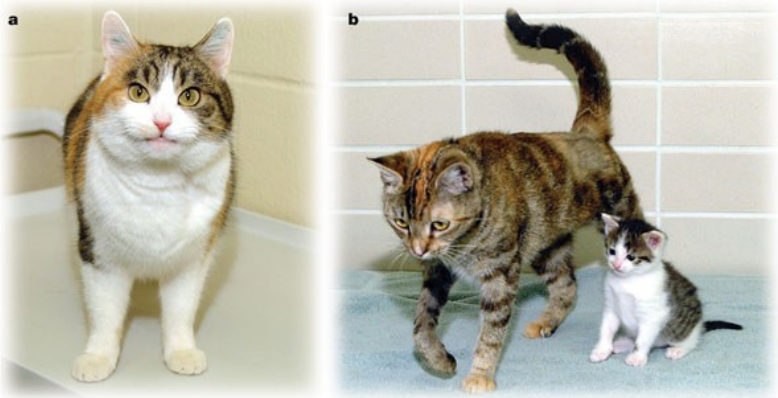
Table 1 Development of embryos reconstructed with three different cell types

Cell type	No. of fused couplets (%)*	No. recovered from oviduct (%)	No. cultured	No. of morula/ blastocyst (%)	No. of morula or blastocysts transferred†	No. of pregnancies/ no. of recipients (%)	No. of live lambs (%)‡
Mammary epithelium	277 (63.8)[a]	247 (89.2)	-	29 (11.7)[a]	29	1/13 (7.7)	1 (3.4%)
Fetal fibroblast	172 (84.7)[b]	124 (86.7)	-	34 (27.4)[b]	34	4/10 (40.0)	2 (5.9%)
			24	13 (54.2)[b]	6	1/6 (16.6)	1 (16.6%)§
Embryo-derived	385 (82.8)[a]	231 (85.3)	-	90 (39.0)[a]	72	14/27 (51.8)	4 (5.6%)
			92	36 (39.0)[b]	15	1/5 (20.0)	0

Kloniranje mačk

2000: Kitajci so klonirali kozo Yuanyuan.
2001: Francozi klonirali zajca na osnovi DNA iz odrasle somatske celice.

CC=CopyCat je bila prva klonirana mačka. Raziskovalce je presenetilo, ker je bil njen kožuh drugačen kot pri donorki kumulusnih celic (DNA). CC je bila edina rojena mačka od 87 ustvarjenih embrijev. (Nature 2/2002)

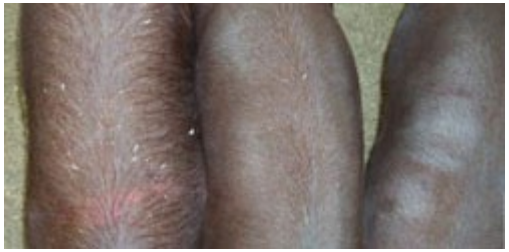


Leta 2004 so na osnovi DNA mačka Tahinija s t.im. “prenosom kromatina” klonirali mačka (Tabouli in Baba Ganoush). Kasneje so pri podjetju “Genetics Savings & Clone” klonirali še več drugih mačk: Mango → Peaches, Nicky → Little Nicky, Gizmo → Little Gizmo; zadnja dva po naročilu.

Kloniranje prašičev

Podjetje PPL Therapeutics je po Dolly kloniralo še prašiče (3/2000): Millie, Christa, Alexis, Carrel, Dotcom. Končni cilj je bil pripraviti živali z izbitimi geni in za vir organov za (kseno)transplantacijo. Klone prašičev z izbito eno kopijo gena za α-1,3-galaktoziltransferazo so pripravili konec leta 2002 (Noel, Angel, Star, Joy in Mary).
Na Univerzi Missouri so le malo zatem objavili podatek, da so pripravili 3000 gensko spremenjenih embrijev in jih vstavili v 28 nadomestnih svinj. Do skotitve se jih je razvilo le 7, od teh so 3 kmalu poginili.

S kloniranimi prašiči so izvedli več študij njihove morfološke, fiziološke in etiološke podobnosti. Ugotovili so, da so si genetsko identični kloni med seboj različni.



“Behavioral variation among cloned pigs”
Authors: Gregory S. Archer and T.H. Friend, Texas A&M University; J. Piedrahita, North Carolina State University; C.H. Nevill, S. Walker, Texas A&M University
Published: Feb. 19, 2003, in the early online edition of Applied Animal Behaviour Science

“Hierarchical Phenotype and Epigenetic Variation in Cloned Swine”
Authors: Greg S. Archer, Scott Dindor, Ted H. Friend, Shawn Walker, Gretchen Zaunbrecher, Bruce Lawhorn, Texas A&M University; Jorge A. Piedrahita, North Carolina State University
Published: Accepted by *Biology of Reproduction*

Kloniranje drugih sesalcev

Japonci so že 7/1998 klonirali govedo (Noto in Kaga, kasneje še Noto II in III). Leta 2000 je Kaga II povrgla tele, ki je bilo spočeto z umetno osemenitvijo.

Ameriškim znanstvenikom je (Science, 5/2003) uspelo klonirati mulo (križanca med oslom, ki ima 62 kromosomov in kobilo, ki jih ima 64). DNA izhaja iz gojenih fetusnih celic, ki so jih 1998 začeli gojiti na Univerzi Idaho. Po implantaciji 305 oocit se je nadomestni materi, kobili Syringi, skotila ena mula (s 63 kromosomi), ki je bila zdrava. Ime ji je Idaho Gem.

Kloniranje drugih sesalcev /3

Psa je prvič uspelo klonirati korejskim znanstvenikom (Nature, 4.8.2005).
Somatsko celico so odvzeli iz uhlja afganistanskega hrta Taija in jo spojili z izpraznjeno jajčno celico. Nadomestna mati je bila druge pasme.
Psa so tako pozno klonirali, ker oocite še ne dozorijo v jajčnikih in jih je tudi težko gojiti v laboratoriju. Jajčne celice so zato odvzeli šele 3 dni po ovulaciji in jim odvzeli DNA. Po fuziji in kemičnem šoku so se celice začele deliti, zgodnje zarodke pa so vstavili v nadomestne matere (123).
Implantirali so 1095 zgodnjih embriev, zaznali pa so le tri brejosti. V enem primeru je prišlo do splava, dva psa pa sta se rodila živa s carskim rezom. En je poginil zaradi pljučnice, preživelemu pa je ime Snuppy (Seoul National University). Ker psi zbolevajo za podobnimi boleznimi kot ljudje (visok krvni tlak, okvare srca, rak na dojki), bi bili psi lahko uporabni za raziskave teh bolezni.

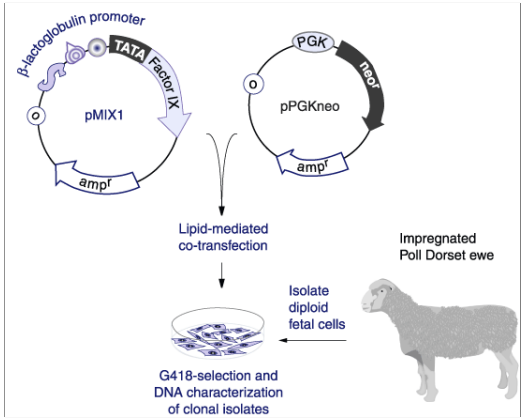
Figure 1 | Dog cloned by somatic-cell nuclear transfer. **a,** Snuppy, the first cloned dog, at 67 days after birth (right), with the three-year-old male Afghan hound (left) whose somatic skin cells were used to clone him. Snuppy is genetically identical to the donor Afghan hound. **b,** Snuppy (left) was implanted as an early embryo into a surrogate mother, the yellow Labrador retriever on the right, and raised by her.

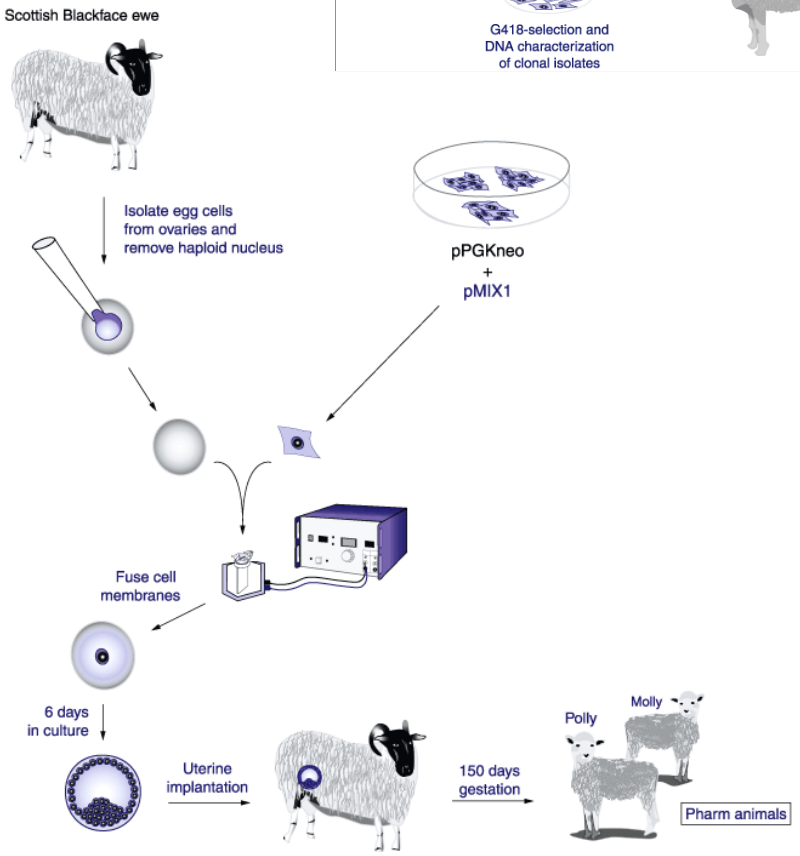
Kloniranje ogroženih vrst /2

Kloniranje gaura Noaha (ACT, januar 2001)
Epitelne celice samca, ki je poginil 8 let pred tem, so odmrznili in jih spojili z jajčnimi celicami navadne krave. Porabili so 692 jajčec, rodilo pa se je samo eno tele. 48 ur po rojstvu je klonirano ogroženo azijsko govedo gaur poginilo zaradi griže.

Prva klonirana ogrožena divja žival, ki so jo klonirali, je bil **muflon** (2000).
Kot nadomestna mati je služila običajna ovca, ki je bila tudi donorka (izpraznjene) jajčne celice. Somatske celice so odvzeli poginulemu divjemu muflonu in izvedli fuzijo. Analiza mtDNA je pokazala, da je klon imel mtDNA identično kot donorka oocit.
23 celičnih fuzij, 7 implantiranih embrijev, 1 mladič
(Nature Biotechnology, 10/2001)

Transgenske klonirane živali





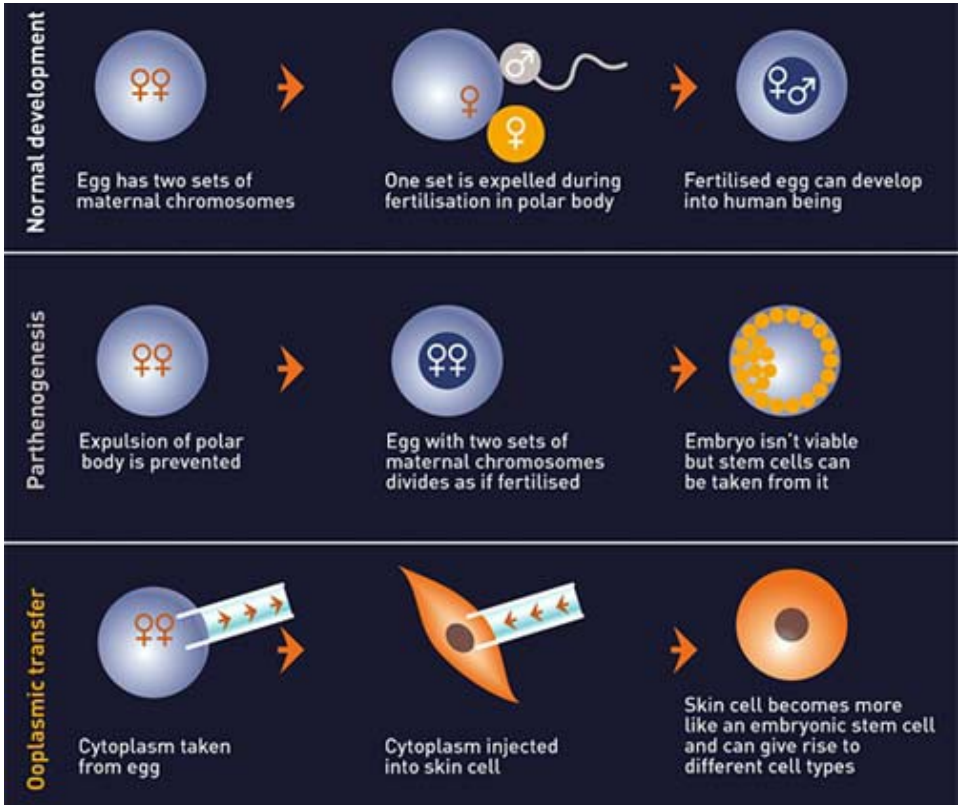
Za ovcami (1996) so z jedrnim prenosom klonirali še več drugih živali.
Transgenske živali, ki proizvajajo uporabne proteine (tabela) so pripravili s kombinacijo klasične transgenske tehnologije in jedrnega prenosa.

Human gene product	Pharmaceutical use	Mammary gland-specific promoter	Transgenic animal
Factor IX	blood clotting protein, treatment of hemophilia B	sheep β-lactoglobin	**sheep**
α-1-antitrypsin	protease inhibitor, treatment of emphysema and cystic fibrosis	sheep β-lactoglobin	**sheep**
antithrombin III	blood clotting protein, treatment of ATIII deficiency disease and use in open heart surgery	cow casein	**goat**
tissue plasminogen activator	dissolves blood clots, used as an acute treatment of heart attacks	mouse whey acidic protein	**goat**
lactoferrin	iron transport protein, infant formula additive	cow α-S-casein	**cow**
protein C	anticoagulant, treatment of hemophilia and used for surgery	mouse whey acid protein	**pig**

Kloniranje človeka

Pri kloniranju človeka ločimo **terapevtsko** in **reproduktivno kloniranje**. Reproduktivno kloniranje je po svetu zakonsko prepovedano, glede terapevtskega pa so mnenja manj odklonilna zaradi možne uporabe za zdravljenje težkih bolezni.

Za kloniranje človeka bi rabili primeren vir donorske DNA. Uporabne bi lahko bile embrijske celice, kar pa je povezano z etičnimi vprašanji.



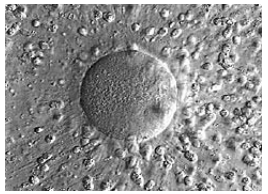
Za terapevtske namene bi lahko pridobili izvorne celice tudi brez klasičnega postopka kloniranja. V naravi včasih prihaja do spontane delitve neoplojenih jajčnih celic (partenogeneza), ki pa se običajno konča z odmrtjem ‘zarodka’ po nekaj dneh.

UK: Urad za človeško oplojevanje in embriologijo bi take poskuse dovolil.

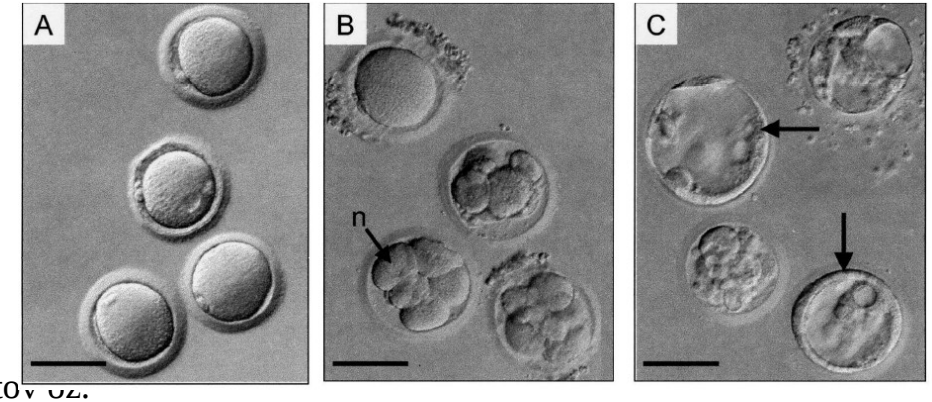
Pri ooplazemskem prenosu pa iz neoplojene jačne celice odvzamemo citoplazmo in jo prenesemo v diferencirano celico ter s tem dosežemo delno dediferenciacijo.

Kloniranje človeka: ACT 2001

J. Regenerative Med. 2, 25-31 (26.11.2001), Cibelli, J.B. et al.: Somatic Cell Nuclear Transfer in Humans: Pronuclear and Early Embryonic Development

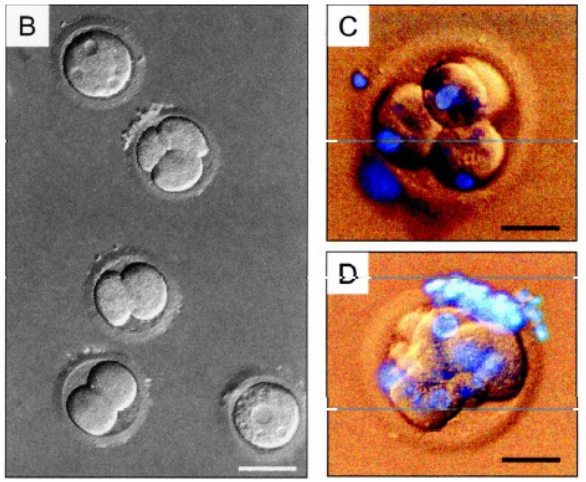


Zdrave ženske (7) so hormonsko stimulirali (superovulacija) in jim odvzeli jajčne celice (71). Somatske celice so bile kožni fibroblasti, ki so jih nekaj časa po biopsiji še gojili v kulturi. V ločenem poskusu so uporabili tudi kumulusne celice (obdajajo in prehranjujejo jajčno celico).



a) 22 jajčnih celic so kemično stimulirali (jonomicin, dimetilaminopurin), da je prišlo do partenogeneze. Po 4 dneh gojenja so nastale deleče se celice, ki so bile podobne embrijem (6) in so obstale v kulturi do 7. dne.

b) V 49 enukleiranih jajčnih celic so injicirali jedra fibroblastov oz. kumulusnih celic (8). Po 12 urah se je pronukleus pojavil pri 7 od 19 celic, ki so jih uspešno rekonstruirali z vnosom fibroblastnega jedra. Od 8 celic, ki so jim uspešno vbrizgali jedra kumulusnih celic, se je pronukleus pojavil pri 4, od teh so se 3 (vse iz iste donorke) delila do 4- oz. 6-celičnega stadija.



Kloniranje človeka: Hwang et al., 2004

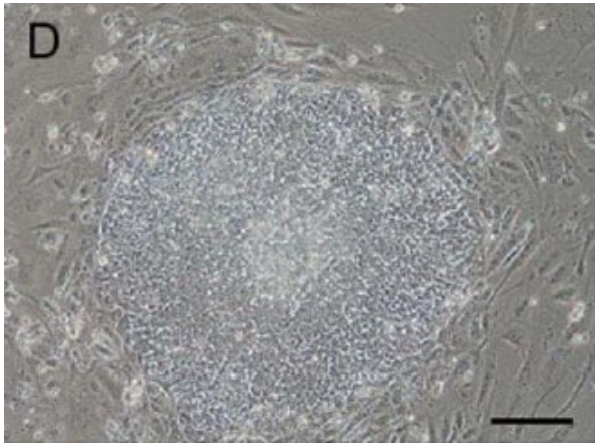
Hwang, W.S., et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. ScienceExpress 12. Feb. 2004

Zbrali so 242 jajčnih celic od 16 prostovoljk po ovarijski stimulaciji. Uporabili so jih 176 (v metafazi II), ki so jih gojili 1-2 uri preden so jih enukleirali. Celične fuzije so izvajali s kumulusnimi celicami istih donork (!). 2 h po fuziji so dodali reagenta, ki sta povzročila delitev celic: Ca-ionofor kalcimicin (10 µM, 5 min) in 6-dimetilaminopurin (2 mM,

4 h). Uspešnost kloniranja je bila pri optimiziranem postopku 25 %.

Experiment	Activation condition*		Reprogramming time (hrs)	1st step medium†	2nd step medium	No. of oocytes	No. (%) of cloned embryos developed to		
							2-cell	Compacted morula	Blastocyst
1st set	10 µM ionophore	6-DMAP	2	G 1.2	hmSOFaa	16	16 (100)	4 (25)	4 (25)
	10 µM ionophore	6-DMAP	4	G 1.2	hmSOFaa	16	15 (94)	1 (6)	0
	10 µM ionophore	6-DMAP	6	G 1.2	hmSOFaa	16	15 (94)	1 (6)	1 (6)
	10 µM ionophore	6-DMAP	20	G 1.2	hmSOFaa	16	9 (56)	1 (6)	0
2nd set	10 µM ionophore	6-DMAP	2	G 1.2	hmSOFaa	16	16 (100)	5 (31)	3 (19)
	5 µM ionophore	6-DMAP	2	G 1.2	hmSOFaa	16	11 (69)	0	0
	10 µM ionomycin	6-DMAP	2	G 1.2	hmSOFaa	16	12 (75)	0	0
	5 µM ionomycin	6-DMAP	2	G 1.2	hmSOFaa	16	9 (56)	0	0
3rd set	10 µM ionophore	6-DMAP	2	G 1.2	hmSOFaa	16	16 (100)	4 (25)	3 (19)
	10 µM ionophore	6-DMAP	2	G 1.2	G 2.2	16	16 (100)	0	0
	10 µM ionophore	6-DMAP	2	Continuous hmSOFaa		16	16 (100)	0	0
4th set	10 µM ionophore	6-DMAP	2	G 1.2	hmSOFaa	66	62 (93)	24 (36)	19 (29)

*Fused donor oocytes and somatic cells were activated in either calcium ionophore A23187 (5 or 10 µM) or ionomycin (5 or 10 µM) for 5 min, followed by 2 mM 6-dimethylaminopurine (DMAP) treatment for 4 hrs. †Oocytes were incubated in first medium for 48 hrs.



Kloniranje sesalcev: problemi

Table 1 | **Key pathological phenotypes reported in species that have been cloned**

Organ	Cattle	Sheep	Goats	Pigs	Mice	References
Cloning efficiency (%)	0–5	0.4–4.3	0.7–7.2	0.1–0.9	0.2–5.8	*
Placenta	Impaired development, oedematous cotyledons‡, enlarged umbilical vessels, hydrallantois§	Reduced vascularity	–	–	Placentomegaly‖	15–17,41,88,89
BW	Higher	–	–	Lower	–	41,90,91
Heart	RV enlargement	Hypertrophy	–	RV enlargement	–	42,92,93
Lungs	Hypertension¶	Hypertension, MPV	Pneumonia	–	Pneumonia	42,64,92,94,95
CNS	–	Pathology	–	–	–	96
Kidneys	Abnormalities (including size abnormalities)	Defects, hydronephrosis#	–	–	–	41,42,97,98
HLS	Lymphoid hypoplasia**, anaemia	–	–	–	Immune impairment	95,99,100
Endocrine	Diabetes	–	–	–	–	101
Liver	Fibrosis, fatty liver	Enlargement, BDP, fibrosis	–	–	Hepatic necrosis	16,41,42,88,95
MS	Limb deformities	Body-wall defects	–	–	–	42, 97
Other	–	–	–	–	Obesity	19,102

The cloning efficiency is the number of live offspring expressed as a percentage of the total number of nuclear transfer oocytes. *Data were obtained from the amalgamation of many studies (see Somatic cell nuclear transfer (cloning) efficiency in online links box) except where specifically referenced. Rabbits, horses, cats and rats have also been cloned, but no specific phenotypes were described in failed clones[6,9,10]. ‡Cotyledons are focal zones on the placenta of apposition of maternal and fetal tissue. §Hydrallantois is the excessive accumulation of fluid in the allantoic sac of the placenta. ‖Placentomegaly is enlargement of the placenta beyond its normal size. ¶Hypertension is high blood pressure, which causes the enlargement of vascular structures. #Hydronephrosis is dilation of the renal pelvis, which is caused by obstruction more distally in the urinary tract. **Lymphoid hypoplasia is an incomplete or underdeveloped lymphoid system. BDP, bile-duct paucity (reduction in the number of bile ducts in the liver); BW, body weight; CNS, central nervous system; HLS, haemolymphatic system (the organs involved in the generation and function of red and white blood cells); MPV, misaligned pulmonary vessels (a condition in which there is abnormal alignment of the veins and arteries in the lungs); MS, musculoskeletal; RV, right ventricle.

HUMAN CLONING: CAN IT BE MADE SAFE?

Susan M. Rhind, Jane E. Taylor‡, Paul A. De Sousa‡, Tim J. King‡, Michelle McGarry‡ and Ian Wilmut‡*

Kloniranje sesalcev: problemi /2

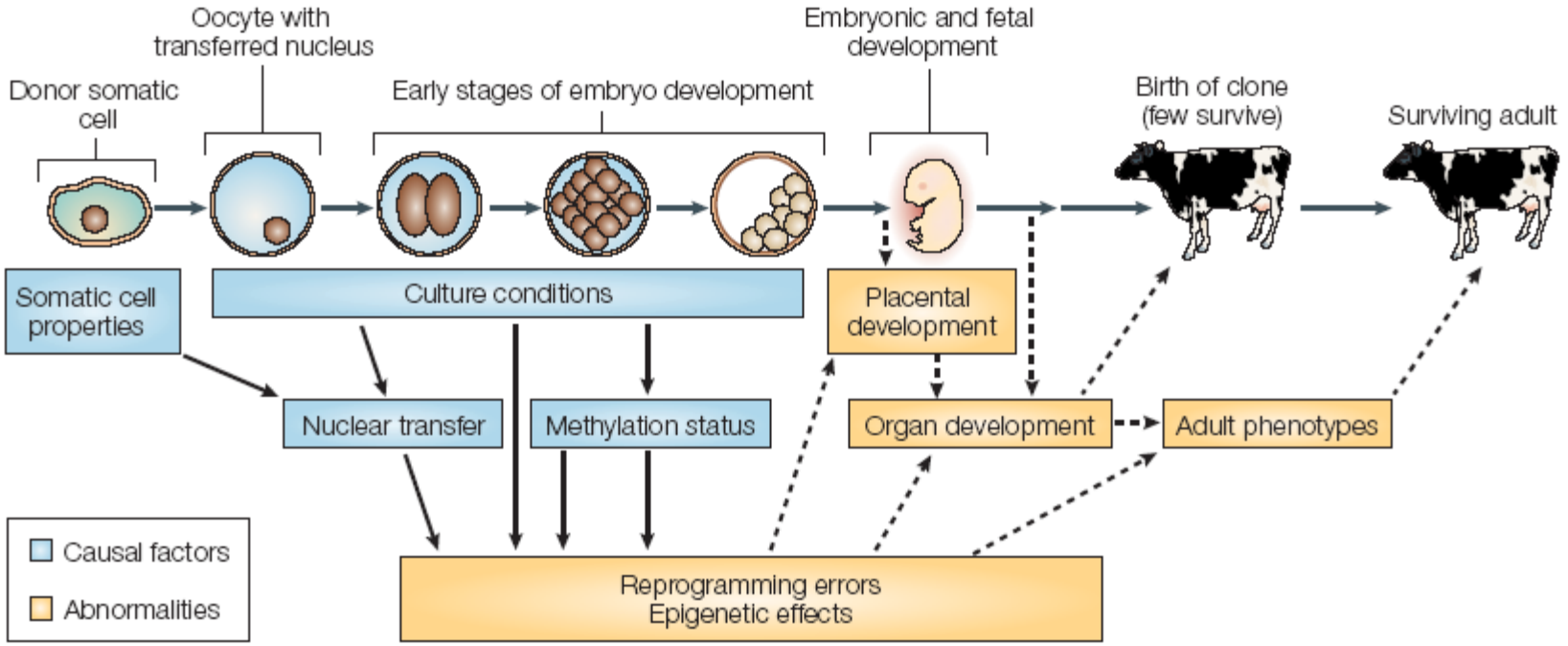


Figure 2 | **Overview of clone development showing the factors that contribute to clone failure.** Factors that affect clone development include the nature of the donor cell and features of the methodology that interfere with reprogramming and epigenetic mechanisms. Aberrant methylation also causes gene dysregulation. These errors have the potential to impact on placental and organ development, resulting in failure during embryogenesis and fetal development, or around birth/during the neonatal period. Animals that survive into adulthood might have more subtle abnormalities that are compatible with life, or might be phenotypically normal. Solid arrows represent recognized links; broken arrows representative speculative links.