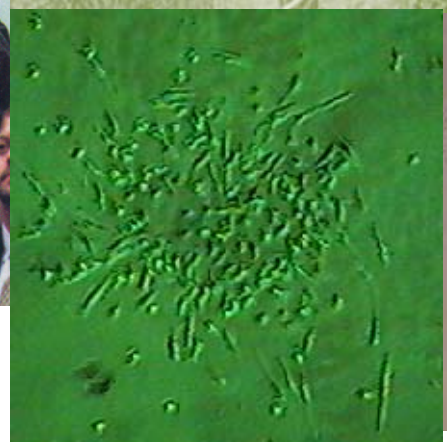
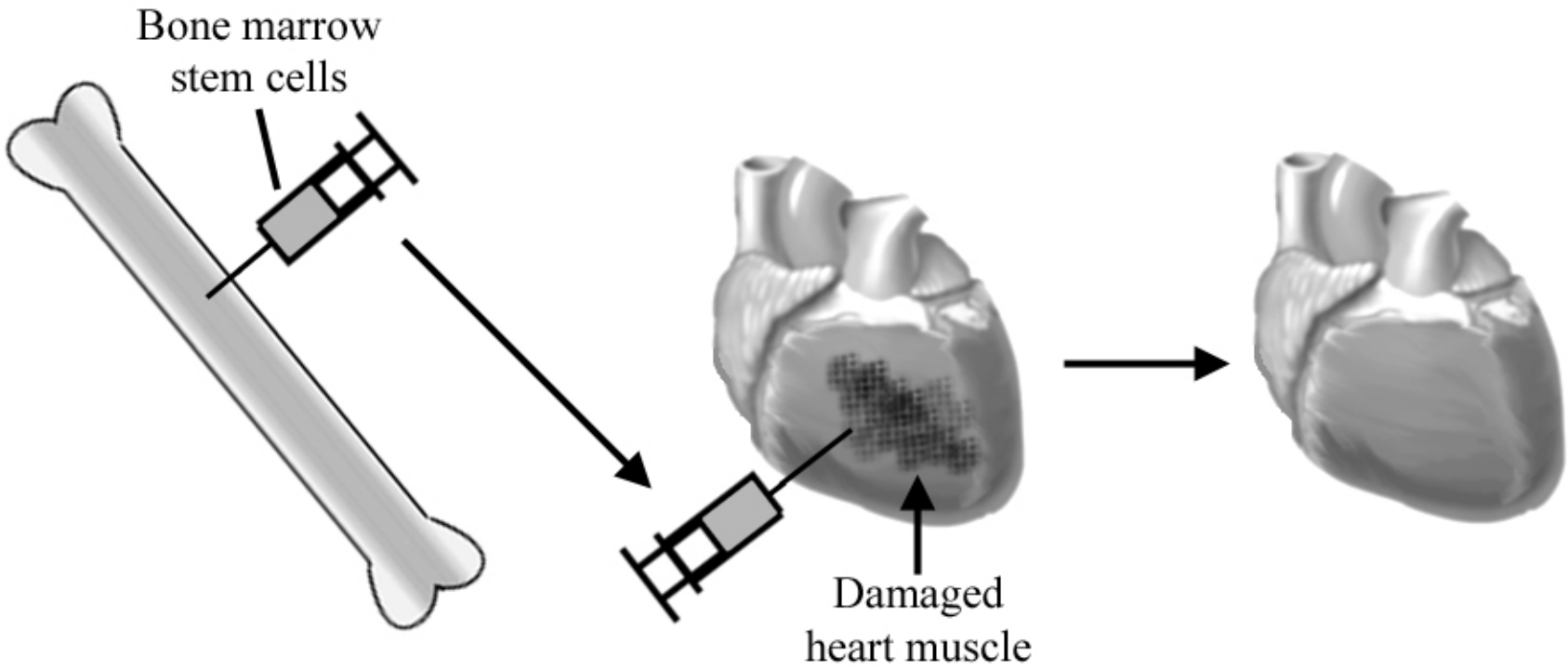
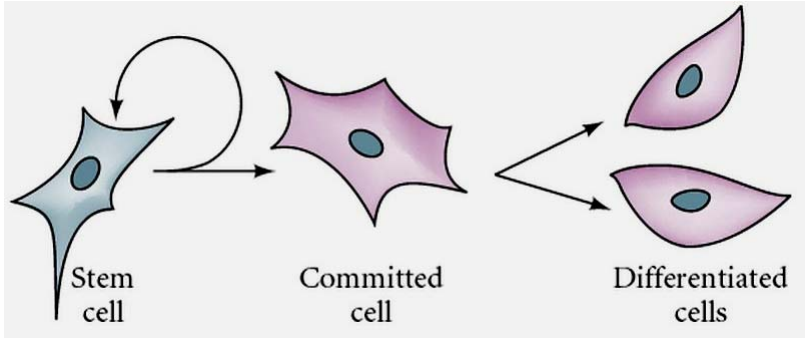
Stem Cells and Cloning



David A. Prentice, Ph.D.
Family Research Council and Georgetown University Medical School
Washington, D.C., USA

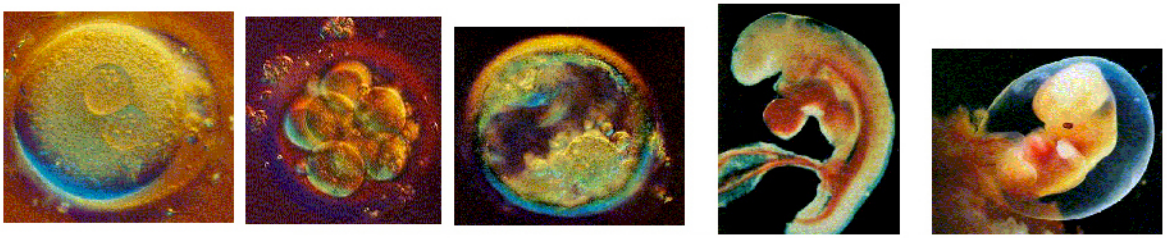
Regenerative Medicine with Stem Cells





Stem Cells

Human Developmental Continuum →

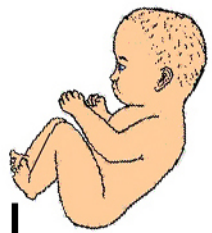


Single-cell Embryo | 3-day Embryo | 5-7 day Embryo | 4-week Embryo | 6-week Embryo

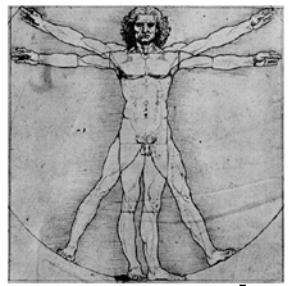
Embryonic Stem (ES) cells
Totipotent

Embryonic Germ (EG) cells
(primordial germ cells)
Pluripotent

Fetal Tissue Stem cells
Pluripotent or Multipotent



Infant



Adult

"Adult" Stem cells
Pluripotent or Multipotent

Teratocarcinoma (germ cell tumor)

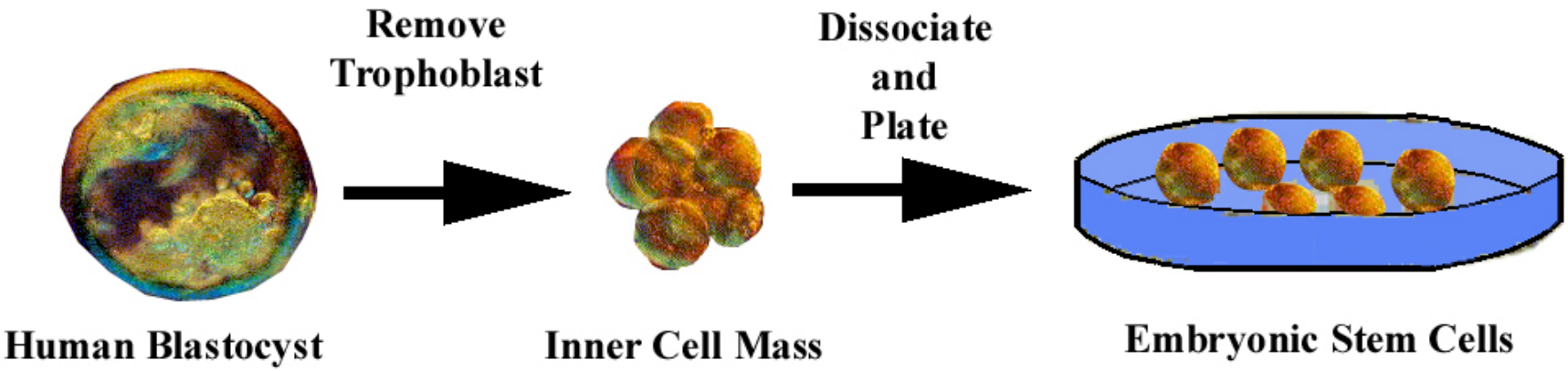
Embryonal Carcinoma (EC) cells
Pluripotent

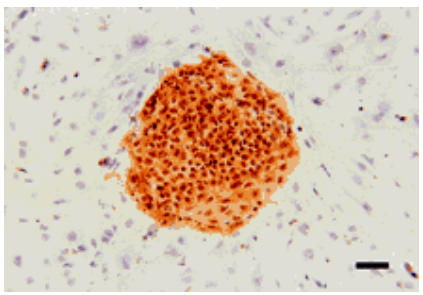
Cord Blood Stem cells
Placental Stem cells
Pluripotent or Multipotent

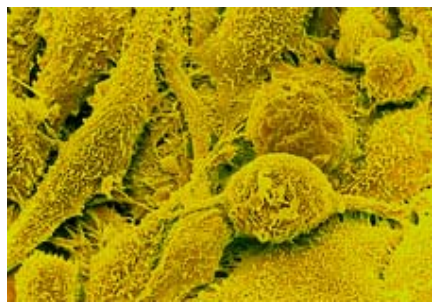
Isolation & Culture of Embryonic Stem Cells
(Human-1998; Mouse-1981)

Method patented
U.S. patent held by Univ. Wisconsin

Purported Advantages:
1) Proliferate indefinitely
2) Form any tissue







Claims unsubstantiated for embryonic stem cells

Current or potential embryonic stem cell problems:

- Difficult to establish and maintain
- Difficulty in obtaining pure cultures in the dish
- Potential for tumor formation and tissue destruction

  *Wakitani S *et al.*; "Embryonic stem cells injected into the mouse knee joint form teratomas and subsequently destroy the joint"; *Rheumatology* 42, 162-165; January 2003
- Questions regarding functional differentiation

  *Hansson M *et al.*, "Artifactual insulin release from differentiated embryonic stem cells", *Diabetes* 53, 2603-2609, October 2004

  *Sipione S *et al.*, "Insulin expressing cells from differentiated embryonic stem cells are not beta cells", *Diabetologia* 47, 499-508, 2004 (published online 14 Feb 2004)

  *Rajagopal J *et al.*; "Insulin staining of ES cell progeny from insulin uptake"; *Science* 299, 363; 17 Jan 2003

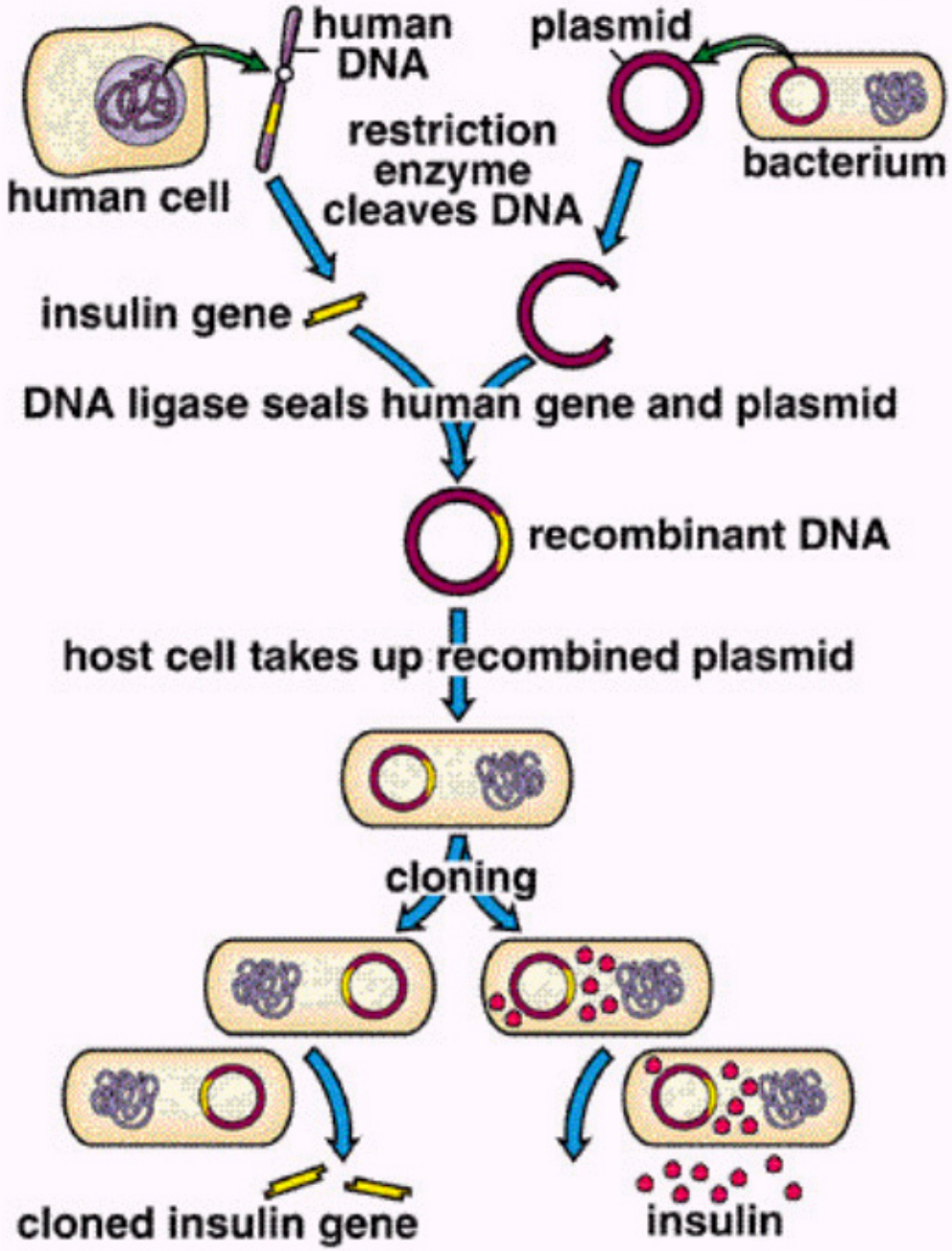
  *Zhang YM *et al.*; "Stem cell-derived cardiomyocytes demonstrate arrhythmic potential"; *Circulation* 106, 1294-1299; 3 September 2002
- Problem of immune rejection
- Genomic instability

  *Cowan CA *et al.*, "Derivation of embryonic stem-cell lines from human blastocysts", *New England Journal of Medicine* 350, 13; published online 3 March 2004

  *Draper JS *et al.*, "Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells", *Nature Biotechnology* 22, 53-54; January 2004

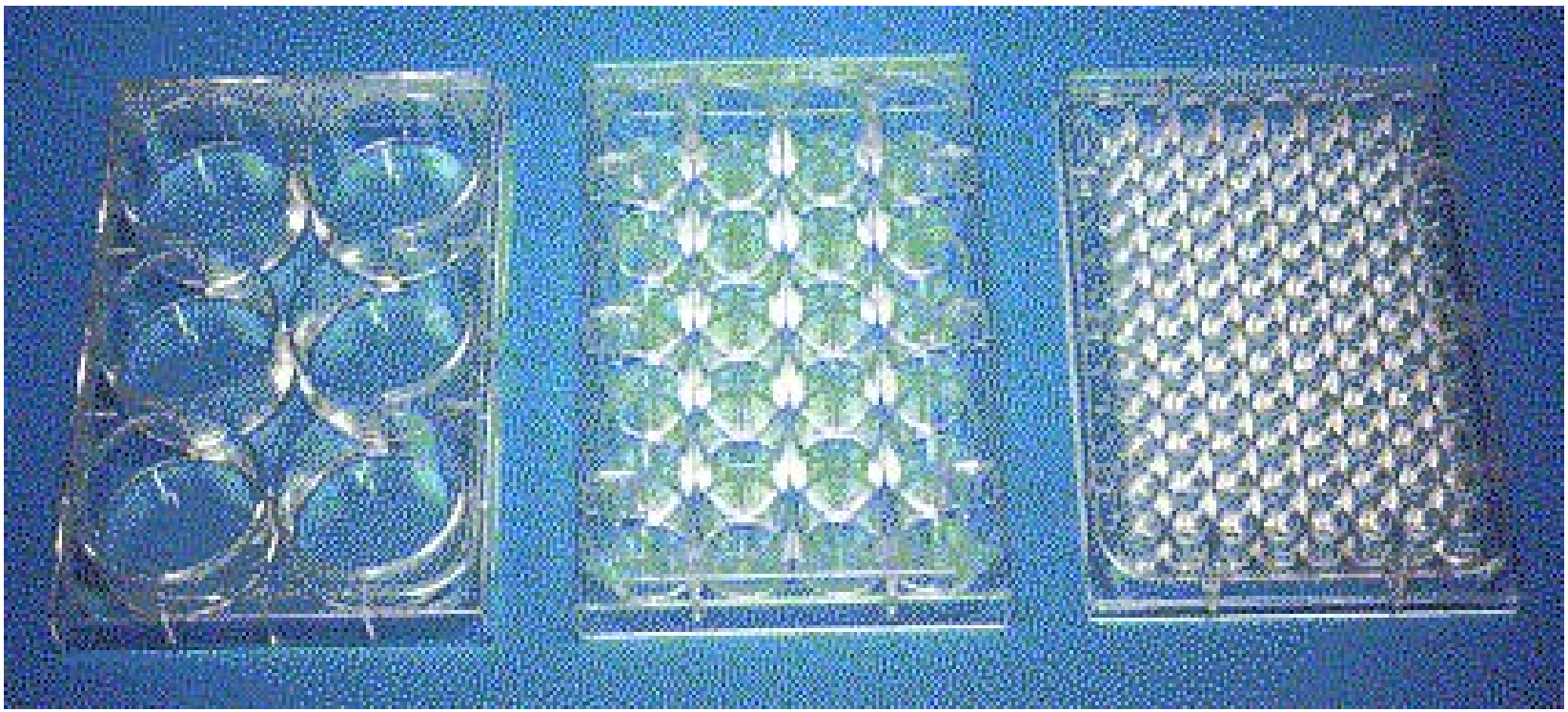
  *Humpherys D *et al.*; "Epigenetic instability in ES cells and cloned mice"; *Science* 293, 95-97; 6 July 2001
- Few and modest results in animals, no clinical treatments
- Ethically contentious

Human Gene Cloning

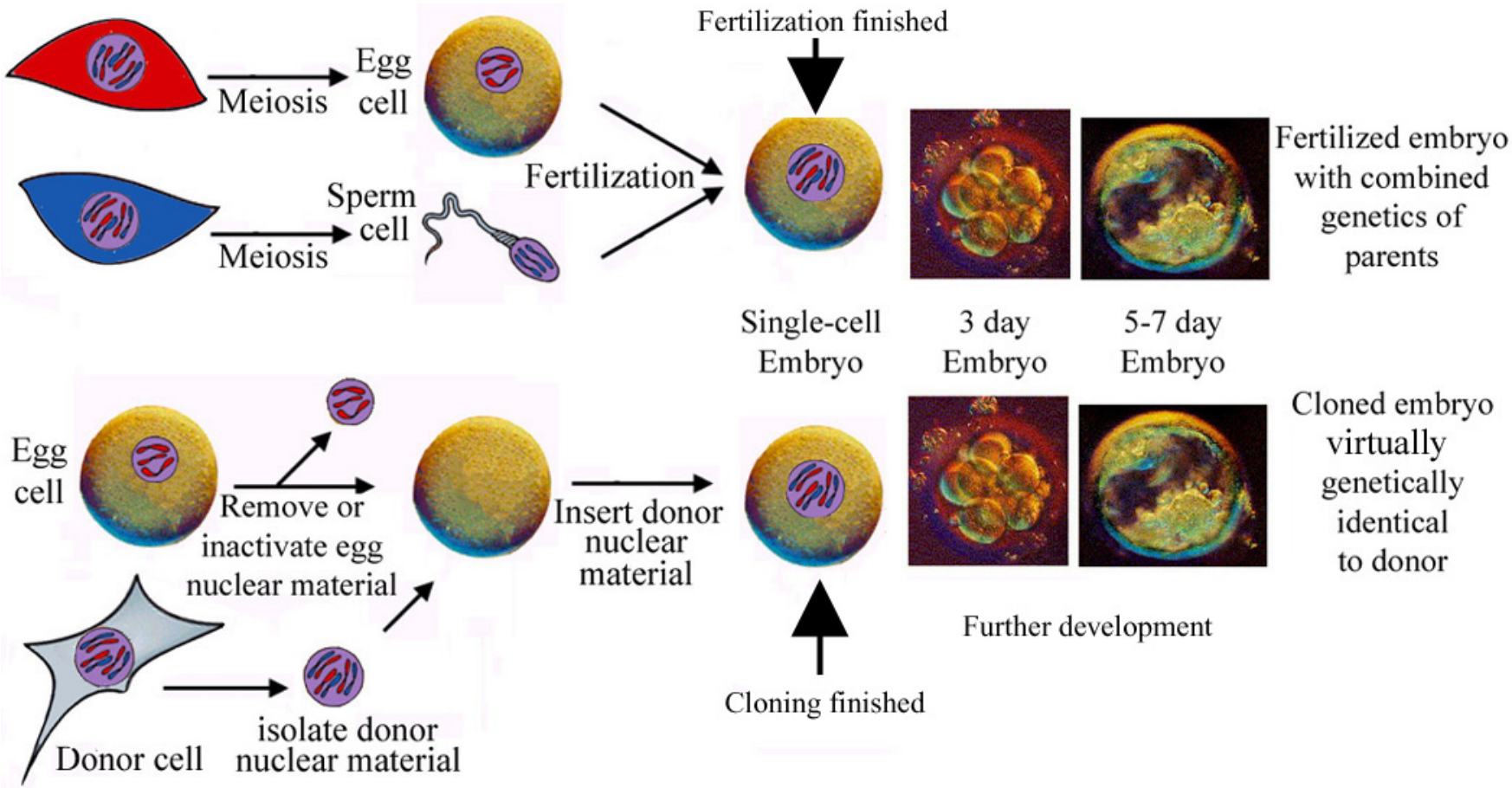


Cell Cloning

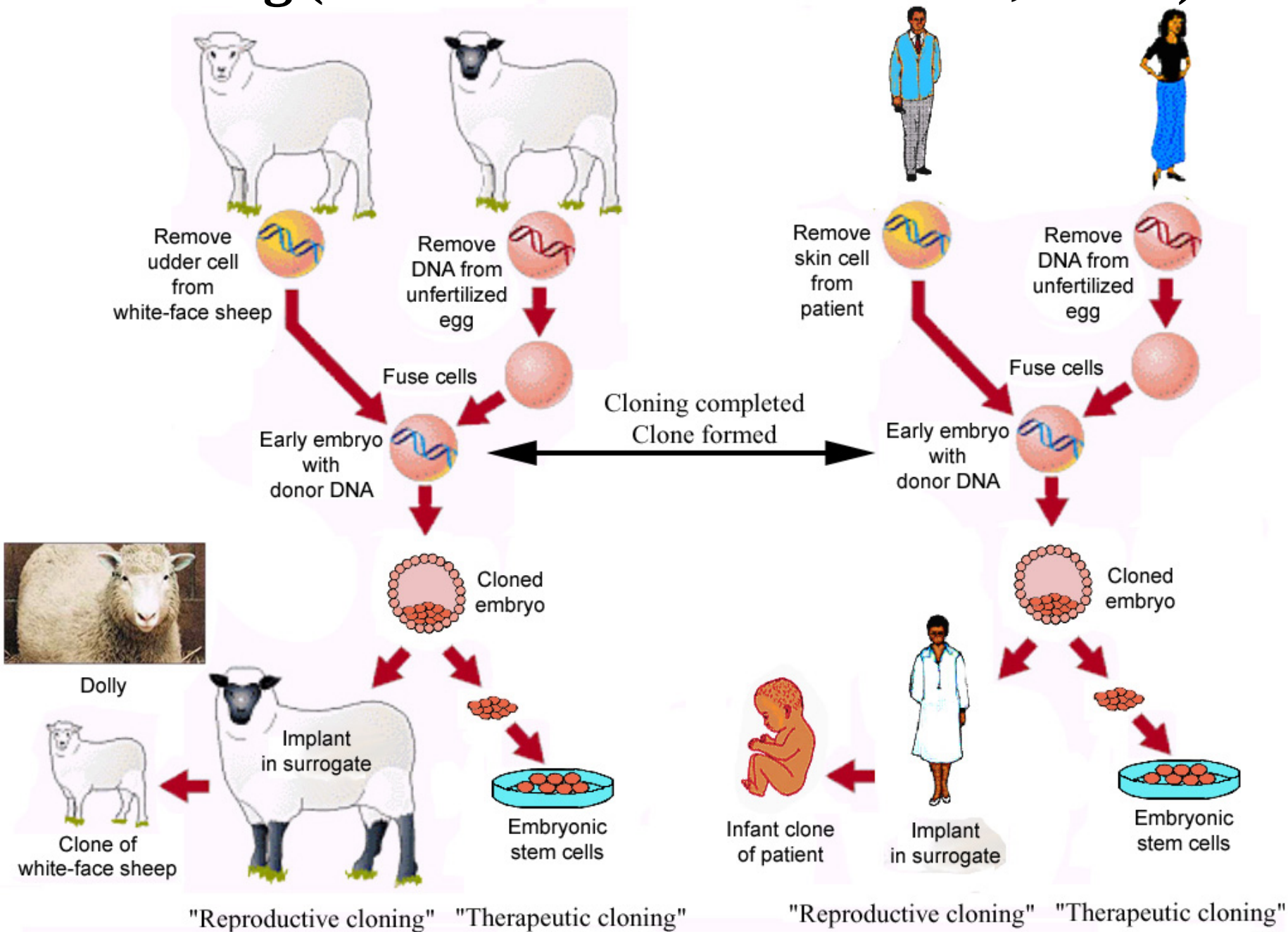
One cell is placed into the dish or well by itself. The cell divides and forms a population of identical cells (cell clones.)



Fertilization *vs*. Cloning (somatic cell nuclear transfer, SCNT)



Cloning (Somatic Cell Nuclear Transfer, SCNT)



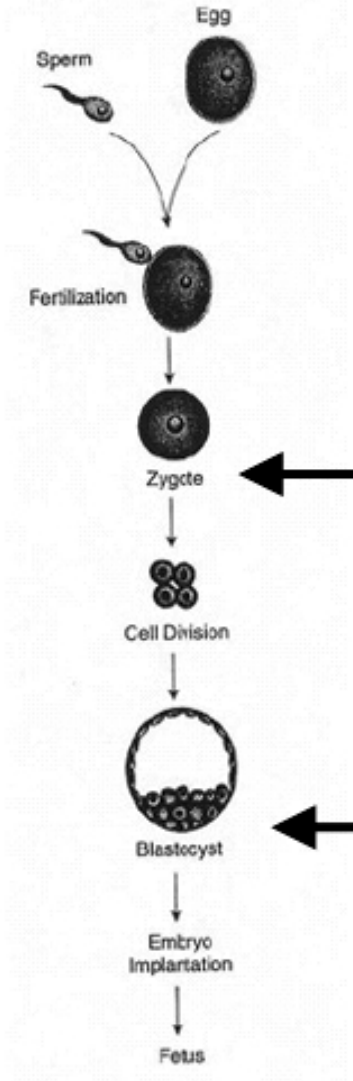


Figure 1 Stages of Development of the Human Embryo

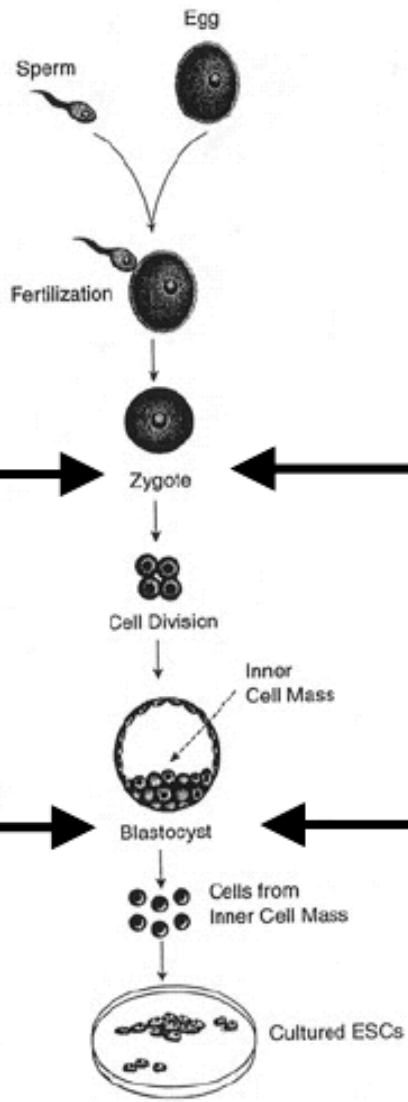


Figure 2 Isolation and Culture of Human ESCs from Blastocysts

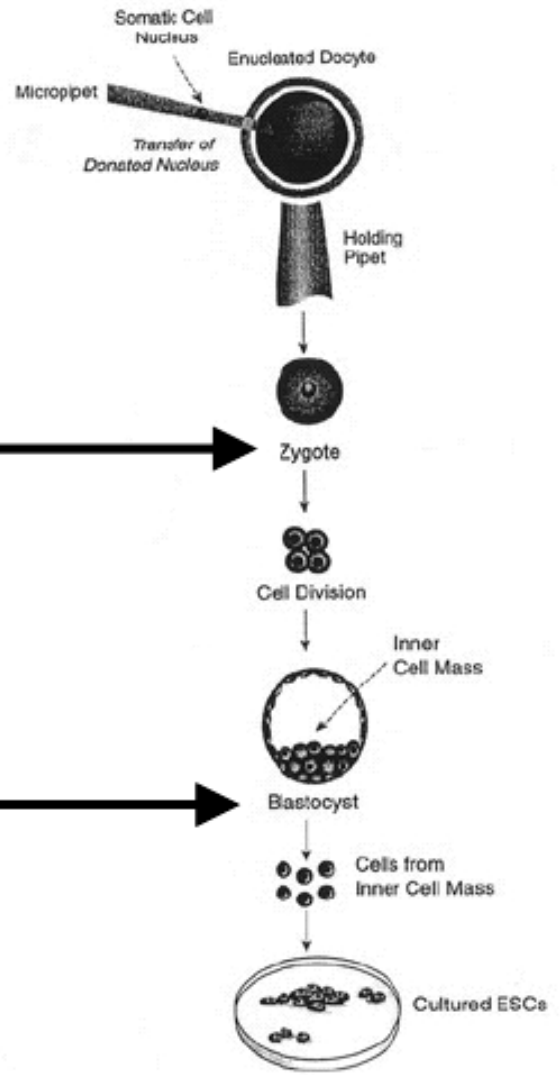


Figure 4 Somatic Cell Nuclear Transfer (SCNT).

[From: Stem Cells and the Future of Regenerative Medicine, Report of the National Academy of Sciences and the Institute of Medicine, National Academy Press, Washington, DC, Sept. 2001; Pg. 10, 11, 26]

Cloning (SCNT) produces a human embryo

"The method used to initiate the reproductive cloning procedure is called either nuclear transplantation or somatic cell nuclear transfer."
Scientific and Medical Aspects of Human Reproductive Cloning, Report of the National Academy of Sciences and the Institute of Medicine, National Academy Press, Washington, DC, Jan 2002

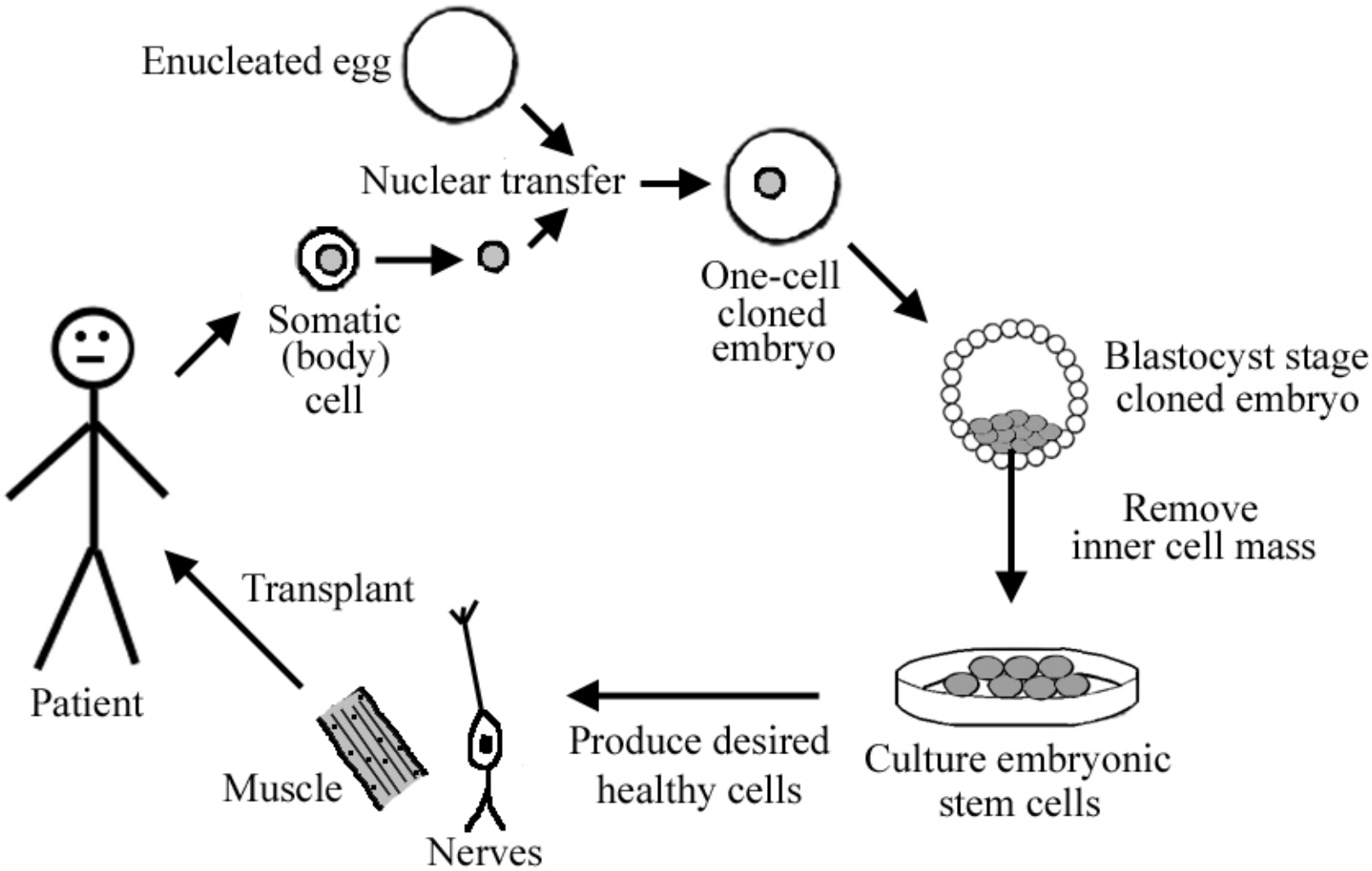
"The first product of SCNT is, on good biological grounds, quite properly regarded as the equivalent of a zygote, and its subsequent stages as embryonic stages in development."
"Human Cloning and Human Dignity: An Ethical Inquiry", Report of the President's Council on Bioethics, July 2002; p.50

"anything that you construct at this point in time that has the properties of those structures to me is an embryo, and we should not be changing vocabulary at this point in time. It doesn't change some of the ethical issues involved."
Dr. John Gearhart, Johns Hopkins University, 25 April 2002; before the U.S. President's Council on Bioethics.

"Therapeutic" Cloning creates an embryo for destruction

"Moreover, because therapeutic cloning requires the creation and disaggregation *ex utero* of blastocyst stage embryos, this technique raises complex ethical questions."
"Unlike much stem cell research, which can use spare embryos remaining from infertility procedures, CRNT [cell replacement through nuclear transfer, a.k.a. therapeutic cloning] requires the deliberate creation and disaggregation of a human embryo." **Robert P. Lanza, Arthur L. Caplan, Lee M. Silver, Jose B. Cibelli, Michael D. West, Ronald M. Green**; "The ethical validity of using nuclear transfer in human transplantation"; *The Journal of the American Medical Association* 284, 3175-3179; 27 Dec2000.

Theoretical Concept of “Therapeutic Cloning”



Therapeutic Cloning is a Failure

- **Transplanted cells from cloned mouse embryo rejected**

*WM Rideout *et al.*, "Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy," *Cell* 109, 17-27; 5 April 2002 (published online 8 March 2002)

"Our results raise the provocative possibility that even genetically matched cells derived by therapeutic cloning may still face barriers to effective transplantation for some disorders."

- *RYL. Tsai, R Kittappa, and RDG McKay; "Plasticity, niches, and the use of stem cells"; *Developmental Cell* 2, 707-712; June 2002

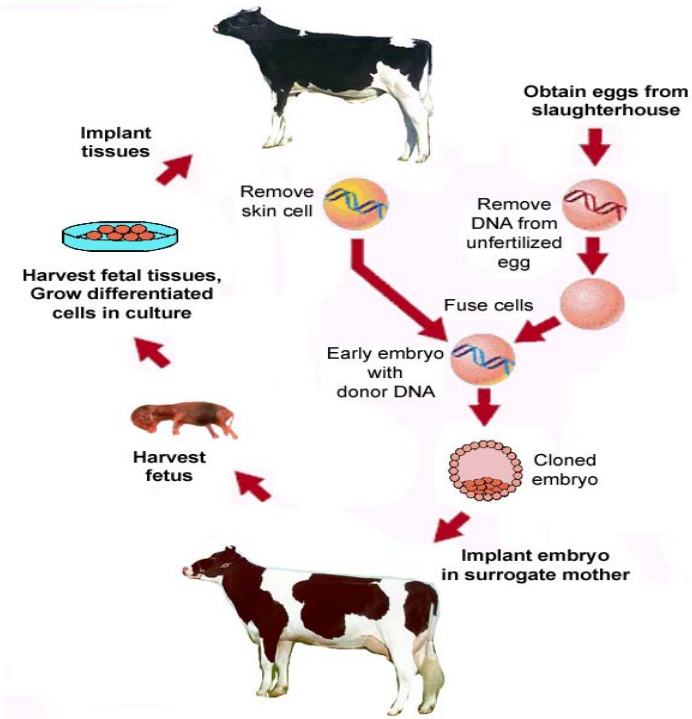
"Jaenisch addressed the possibility that ES clones derived by nuclear transfer technique could be used to correct genetic defects… However, the donor cells, although derived from the animals with the same genetic background, are rejected by the hosts."

- **Clones may need to be gestated to "harvest" already-differentiated tissues**

*R Lanza *et al.*; "Generation of histocompatible tissue using nuclear transplantation," *Nature Biotechnology* 20, 689-696; July 2002

*R Lanza *et al.*, "Regeneration of the infarcted heart with stem cells derived by nuclear transplantation," *Circulation Research* 94, 820-827, April 2004

*R Lanza *et al.*, "Long-term bovine hematopoietic engraftment with clone-derived stem cells", *Cloning and Stem Cells* 7, 95-106, July 2005



Transplant rejection still likely using cells from cloned embryos

- "**Robert Lanza**, chief scientist at Advanced Cell Technology in Worcester, Mass., an ardent advocate for both embryonic stem cell studies and therapeutic cloning, agreed that in the course of the political debate, the need for cloning to overcome immune system rejection has been overstated. 'It's not all or nothing. You can move ahead.'"
  San Francisco Chronicle, Monday, March 18, 2002 Page E – 1
- "There is no question in my mind that the possibility exists that if you are doing an egg donor, and nuclear transfer into an egg, that there possibly exists that that cell -- that the embryonic stem cells derived from that could be rejected. Absolutely."
  Dr. John Gearhart, Johns Hopkins, 25 April 2002 meeting of the President's Council on Bioethics
- "I should say that when you put the nucleus in from a somatic cell, the mitochondria still come from the host." He concluded, "And in mouse studies it is clear that those genetic differences can lead to a mild but certainly effective transplant rejection and so immunosuppression, mild though it is, will be required for that."
  Dr. Irving Weissman, Stanford, 13 February 2002 meeting of the President's Council on Bioethics

"Therapeutic cloning"—unlikely chance of clinical success

- "[T]he poor availability of human oocytes, the low efficiency of the nuclear transfer procedure, and the long population-doubling time of human ES cells make it difficult to envision this [therapeutic cloning] becoming a routine clinical procedure…"
  Odorico JS, Kaufman DS, **Thomson JA**, "Multilineage differentiation from human embryonic stem cell lines," *Stem Cells* 19, 193-204; 2001
- "However, it is unlikely that large numbers of mature human oocytes would be available for the production of ES cells, particularly if hundreds are required to produce each ES line. The technical capability for nuclear transfer would also need to be widely available and this is unlikely. In addition, epigenetic remnants of the somatic cell used as the nuclear donor can cause major functional problems in development, which must remain a concern for ES cells derived by nuclear transfer. …it would appear unlikely that these strategies will be used extensively for producing ES cells compatible for transplantation."
  Alan O.Trounson, "The derivation and potential use of human embryonic stem cells", *Reproduction, Fertility, and Development* 13, 523-532; 2001
- **Thomas Okarma**, CEO, Geron Corporation says: "The odds favoring success are vanishingly small, and the costs are daunting." "It would take thousands of [human] eggs on an assembly line to produce a custom therapy for a single person. The process is a nonstarter, commercially."
  Denise Gellene, "Clone Profit? Unlikely", Los Angeles Times, 10 May 2002

Development of "therapeutic" cloning techniques can lead to "reproductive" cloning:

"It is true that the techniques developed in CRNT [cell replacement through nuclear transfer, a.k.a. therapeutic cloning] research can prepare the way scientifically and technically for efforts at reproductive cloning."

Robert P. Lanza, Arthur L. Caplan, Lee M. Silver, Jose B. Cibelli, Michael D. West, Ronald M. Green; "The ethical validity of using nuclear transfer in human transplantation"; *The Journal of the American Medical Association* 284, 3175-3179; 27 Dec 27 2000

"If undertaken, the development of SCNT for such therapeutic purposes, in which embryos are not transferred for pregnancy, is likely to produce knowledge that could be used to achieve reproductive SCNT."

American Society for Reproductive Medicine Ethics Committee; "Human somatic cell nuclear transfer (cloning)"; *Fertility and Sterility* 74, 873-876; November 2000

“Cloning Unnecessary and Obsolete”
--leading embryonic stem cell expert

- **Alan Trounson**, Australian embryonic stem cell expert and a leader in the field worldwide, says that stem cell research has advanced so rapidly in the past few months that therapeutic cloning is now unnecessary. “My view is there are at least three or four other alternatives that are more attractive already,” he said.
  Trounson abandoned his call for therapeutic cloning, saying scientific breakthroughs mean there is now no need for the controversial technique.
  Professor Trounson said therapeutic cloning faced logistical problems, and that other techniques were showing great promise and offered better options. “I can't see why, then, you would argue for therapeutic cloning in the long term because it is so difficult to get eggs and you've got this issue of (destroying) embryos as well.”

“Stem-cell cloning not needed, says scientist”, The Age (Melbourne), pg. 2, July 29, 2002;
“Stem-cell research outpaces cloning”, The Australian, pg. 3, July 29, 2002;
“Therapeutic cloning no longer necessary: expert”, AAP Newsfeed, July 29, 2002

“Therapeutic” cloning places women at risk

Because both cloning and embryonic stem cell production are extremely inefficient, a tremendous number of eggs will be required.

For example, to treat only the 17 million Diabetes patients in the U.S.:

Will require 170 million-1.7 billion human eggs
(Optimistically 10-100 human eggs/patient, estimated cost US$100,000-200,000)
Mombaerts P, “Therapeutic cloning in the mouse”, *Proceedings of the National Academy of Sciences USA* 100, 11924-11925; 30 Sept 2003; Prentice DA, Stem Cells and Cloning, 1st edition, San Francisco: Pearson Education/Benjamin-Cummings, July 2002
[South Korean 1st human cloning -- one cell line, 242 eggs—would be >4 billion eggs for diabetes]
[2005 report, average 17 eggs/cell line = 289 million eggs for diabetes]

--Collecting 10 eggs/donor:
Will require 29-170 million women - childbearing age donors

Health risks—High-dose hormone therapy and surgery to obtain eggs risks the donor’s health and future reproductive success
Commercial exploitation—of women globally

Adult stem cell capabilities

"There is no evidence of an adult stem cell that is pluripotent. It has not been demonstrated that one adult stem cell can be directed to develop into any cell type of the body. That is, *no adult stem cell has been shown to be capable of developing into cells from all three embryonic germ layers*."

Stem Cells: Scientific Progress and Future Research Directions, National Institutes of Health, June 2001; Pg. ES-6 *(emphasis added)*

"Thus, at this stage, any therapies based on the use of human ES cells are still hypothetical and highly experimental."
"Whether embryonic stem cells will provide advantages over stem cells derived from cord blood or adult bone marrow hematopoietic stem cells remains to be determined."

Stem Cells: Scientific Progress and Future Research Directions, National Institutes of Health, June 2001; Pg. 17, 63

"Some adult stem cells appear to have the capability to differentiate into tissues other than the ones from which they originated; this is referred to as plasticity. Reports of human or mouse adult stem cells that demonstrate plasticity and the cells they differentiate or specialize into include: 1) *blood and bone marrow (unpurified hematopoietic) stem cells differentiate into the 3 major types of brain cells (neurons, oligodendrocytes, and astrocytes)* ***[ectoderm]****, skeletal muscle cells, cardiac muscle cells* ***[mesoderm]****, and liver cells* ***[endoderm]***; 2) bone marrow (stromal) cells differentiate into cardiac muscle cells, skeletal muscle cells, fat, bone, and cartilage; and 3) brain stem cells differentiate into blood cells and skeletal muscle cells."

Ibid, Pg. ES-7 ***[emphasis added]***

Adult Stem Cells

Bone Marrow

Marrow
Bone
Cartilage
Tendon
Muscle
Fat
Liver
Brain/Nerve
Blood cells
Heart
All Tissues

Stem Cells from Fat

Bone
Cartilage
Muscle
Nerves

Peripheral Blood

Bone Marrow
Blood cells
Nerves

Hair Follicle

Skin Brain
Smooth Muscle Fat

Gastrointestinal

Esophagus Small Intestine
Stomach Large Intestine/Colon

Placenta

Bone Nerve
Cartilage Muscle Tendon
Bone Marrow Blood vessel

Skeletal Muscle

Skeletal muscle
Smooth muscle
Bone
Cartilage
Fat
Heart

Brain

Brain
Nerves
Blood cells
Muscle
All Tissues
Cornea
Retina
Pancreas
Liver
Heart
Lung
Spermatogonia
Amniotic Fluid
Umbilical Cord Matrix

CORD BLOOD

Various Tissues

David A. Prentice

Evidence that Some Adult Stem Cells show Pluripotent Capacity

Nasal Stem Cells form multiple tissues.

Murrell W *et al.*, "Multipotent stem cells from adult olfactory mucosa, *Developmental Dynamics* 233, 496-515, June 2005

Common Pluripotent Adult Stem Cell isolated from multiple mouse tissues

Case J *et al.*, Clonal multilineage differentiation of murine common pluripotent stem cells isolated from skeletal muscle and adipose stromal cells, Annals NY Acad Sci 1044, 183-200, June 2005

Bone Marrow Stem Cells can form all 3 germ layers, and regenerate damaged heart.

Yoon Y-s *et al.*, "Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction", *Journal of Clinical Investigation* 115, 326-338, February 2005

Human Cord Blood stem cells show pluripotent potential and extensive proliferation

Kögler G *et al.*, "A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential", *J. Experimental Medicine* 200, 123-135, 19 July 2004

Human Bone Marrow Adult Stem Cells with pluripotent potential, Oct-4 expression

D'Ippolito G *et al.*, "Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential", *J. Cell Science* 117, 2971-2981, 15 July 2004

Peripheral blood stem cells can form cells from all 3 germ layers

Zhao Y *et al.*; "A human peripheral blood monocyte-derived subset acts as pluripotent stem cells"; *Proceedings of the National Academy of Sciences USA* 100, 2426-2431; 4 March 2003

Adult stem cells from bone marrow can form new neurons in the human brain.

Mezey E *et al.*; "Transplanted bone marrow generates new neurons in human brains"; *Proceedings of the National Academy of Sciences USA* 100, 1364-1369; 4 Feb 2003

Adult stem cells from bone marrow can form all body tissues

Jiang Y *et al.*; "Pluripotency of mesenchymal stem cells derived from adult marrow"; *Nature* 418, 41-49; 4 July 2002

A <u>single</u> adult mouse bone marrow stem cell can form functional marrow, blood cells, liver, lung, gastrointestinal tract, skin, heart and skeletal muscle.

Krause DS *et al.*; "Multi-Organ, Multi-Lineage Engraftment by a Single Bone Marrow-Derived Stem Cell"; *Cell* 105, 369-377; 4 May 2001

Adult stem cells effective in tissue repair

Stroke—Adult stem cells from brain, bone marrow, and umbilical cord blood provide therapeutic benefit after stroke. First clinical trials under way.

*Shyu W-C *et al.*, "Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells", *Circulation* 110, 1847-1854, 2004

*Willing AE *et al.*, "Mobilized peripheral blood stem cells administered intravenously produce functional recovery in stroke", *Cell Transplantation* 12, 449-454; 2003

*Arvidsson A *et al.*; "Neuronal replacement from endogenous precursors in the adult brain after stroke"; *Nature Medicine* 8, 963-970; Sept 2002

*Riess P *et al.*; "Transplanted neural stem cells survive, differentiate, and improve neurological motor function after experimental traumatic brain injury"; *Neurosurgery* 51, 1043-1052; Oct 2002

*Li Y *et al.*; "Human marrow stromal cell therapy for stroke in rat"; *Neurology* 59, 514-523; August 2002

*Chen J *et al.*; "Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats"; *Stroke* 32, 2682-2688; November 2001

Adult stem cells effective in tissue repair

Spinal Cord Injury—Adult stem cells capable of re-growth and reconnection in spinal cord. Clinical trials started in Portugal and Australia.

*Sigurjonsson OE *et al.*, Adult human hematopoietic stem cells produce neurons efficiently in the regenerating chicken embryo spinal cord, PNAS 102, 5227-5232, 5 April 2005

*Lu J *et al.*, Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord, *Brain* 125, 14-21, 2002

*Ohta M *et al.*, Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation, Experimental Neurology 187, 266-278, 2004

*Hofstetter CP *et al.*, "Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery", *Proc Natl Acad Sci USA* 99, 2199-2204; Feb 19, 2002

*M. Sasaki *et al.*, "Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons," *Glia* 35, 26-34; July 2001

*A. Ramon-Cueto *et al.*, "Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia," *Neuron* 25, 425-435; Feb 2000.

*M.S. Ramer *et al.*; "Functional regeneration of sensory axons into the adult spinal cord," *Nature* 403, 312-316; Jan 20, 2000.

*Shihabuddin *et al.*; "Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus," *J Neurosci* 20, 8727-8735; Dec 2000.

*Barnett *et al.*; "Identification of a human olfactory ensheathing cell that can effect transplant-mediated remyelination of demyelinated CNS axons," *Brain* 123, 1581-1588, Aug 2000

*A. Ramon-Cueto *et al.*, "Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glial transplants," *J Neurosci* 18, 3803-3815; May 15, 1998

Adult stem cells effective in tissue repair

Diabetes—Pancreatic, liver, intestinal, spleen or bone marrow cells can form insulin-secreting islets. FDA approval of first clinical trial, Denise Faustman, Harvard.

*Sapir *et al.*, Cell-replacement therapy for diabetes: generating functional insulin-producing tissue from adult human liver cells, *Proceedings of the National Academy of Sciences USA* 102, 7964-7969, 17 May 2005

*Seaberg BM *et al.*, "Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages", *Nature Biotechnology* 22, 1115-1124, Sept 2004

*Oh S-H *et al.*, "Adult bone marrow-derived cells transdifferentiating into insulin-producing cells for the treatment of type I diabetes," *Laboratory Investigation* 84, 607-617, 1 May 2004

*Kodama S *et al.*, "Islet regeneration during the reversal of autoimmune diabetes in NOD mice", *Science* 302, 1223-1227; 14 Nov 2003

*Hess D *et al.*, "Bone marrow-derived stem cells initiate pancreatic regeneration", *Nature Biotechnology* 21, 763-770; July 2003

*Steptoe RJ *et al.*; "Transfer of hematopoietic stem cells encoding autoantigen prevents autoimmune diabetes"; *Journal of Clinical Investigation* 111, 1357-1363; May 2003

*Suzuki A *et al.*; "Glucagon-like peptide 1 (1-37) converts intestinal epithelial cells into insulin-producing cells"; *Proc Natl Acad Sci USA* 100, 5034-5039; 29 April 2003

*Ianus A *et al.*; *In vivo* derivation of glucose competent pancreatic endocrine cells from bone marrow without evidence of cell fusion; *Journal of Clinical Investigation* 111, 843-850; March 2003

*Yang L *et al.*; "*In vitro* trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells"; *Proceedings of the National Academy of Sciences USA*, 99, 8078-8083; 11 June 2002

*S. Ryu *et al.*; "Reversal of established autoimmune diabetes by restoration of endogenous ß cell function," *J. Clin. Invest.* 108, 63-72; July 2001

*Ramiya VK *et al.*; "Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells," *Nature Medicine* 6, 278-282, March 2000.

Adult stem cells effective in tissue repair

Heart Damage—Bone marrow, muscle, and heart stem cells repair heart.

*****Dawn B *et al.*, "Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function", *Proceedings of the National Academy of Sciences USA* 102, 3766-3771, 8 March 2005

*****Yoon Y-s *et al.*, "Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction", *Journal of Clinical Investigation* 115, 326-338, February 2005

******Wollert KC *et al.*, "Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial", *Lancet* 364, 141-148, 10 July 2004

******Britten MB *et al.*, "Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction"; *Circulation* 108, 2212-2218; Nov 2003

******Perin EC *et al.*; "Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure"; *Circulation* 107, r75-r83; published online May 2003

******Stamm C *et al.*; "Autologous bone-marrow stem-cell transplantation for myocardial regeneration"; *The Lancet* 361, 45-46; 4 January 2003

******Tse H-F *et al.*; "Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation"; *The Lancet* 361, 47-49; 4 January 2003

******Strauer BE *et al.*; "Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans"; *Circulation* 106, 1913-1918; 8 October 2002

******Menasché P *et al.* "Myoblast transplantation for heart failure." *Lancet* 357, 279-280; 27 January 2001

*Orlic D *et al.*, "Mobilized bone marrow cells repair the infarcted heart, improving function and survival"; *Proceedings of the National Academy of Sciences USA* 98, 10344-10349, 28 August 2001.

Adult stem cells effective in tissue repair

Parkinson's Disease—Neural stem cells can form all neuronal types, migrate throughout brain to repair damage, and prevent loss of neurons associated with Parkinson's disease.

*Liker MA *et al.*; "Human neural stem cell transplantation in the MPTP-lesioned mouse"; *Brain Research* 971, 168-177; May 2003

*Åkerud P *et al.*; "Persephin-overexpressing neural stem cells regulate the function of nigral dopaminergic neurons and prevent their degeneration in a model of Parkinson's disease"; *Molecular and Cellular Neuroscience* 21, 205-222; Nov 2002

*Ourednik J *et al.*; "Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons"; *Nature Biotechnology* 20, 1103-1110; Nov 2002

Using the patient's own adult neural stem cells, a group at Los Angeles Cedars-Sinai Medical Center report a reversal of symptoms in the first Parkinson's patient treated.

Lévesque M and Neuman T, "Autologous transplantation of adult human neural stem cells and differentiated dopaminergic neurons for Parkinson disease: 1-year postoperative clinical and functional metabolic result", American Association of Neurological Surgeons annual meeting, Abstract #702; 8 April 2002

Injecting growth signals into the brain stimulates the patients' own adult neural stem cells, provided a 61% improvement.

*Gill SS *et al.*; "Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease"; *Nature Medicine* 9, 589-595; May 2003 (published online 31 March 2003)

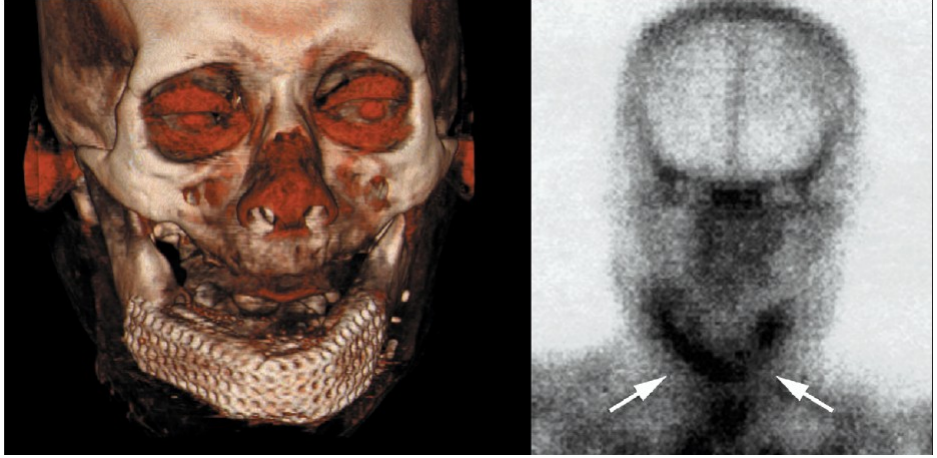
Current Clinical Uses of Adult Stem Cells

- **Cancers**—Lymphomas, multiple myeloma, leukemias, breast cancer, neuroblastoma, renal cell carcinoma, ovarian cancer
- **Autoimmune diseases—**multiple sclerosis, systemic lupus, rheumatoid arthritis, scleroderma, scleromyxedema, Crohn's disease
- **Anemias** (incl. sickle cell anemia)
- **Immunodeficiencies**—including human gene therapy
- **Bone/cartilage deformities**—children with osteogenesis imperfecta
- **Corneal scarring-**generation of new corneas to restore sight
- **Stroke**—neural cell implants in clinical trials
- **Repairing cardiac tissue after heart attack**—bone marrow or muscle stem cells from patient
- **Parkinson's**—retinal stem cells, patient's own neural stem cells, injected growth factors
- **Growth of new blood vessels**—*e.g.*, preventing gangrene
- **Gastrointestinal epithelia**—regenerate damaged ulcerous tissue
- **Skin**—grafts grown from hair follicle stem cells, after plucking a few hairs from patient
- **Wound healing**—bone marrow stem cells stimulated skin healing
- **Spinal cord injury**—clinical trials currently in Portugal, Italy, S. Korea

Diseases Treated in Human Patients

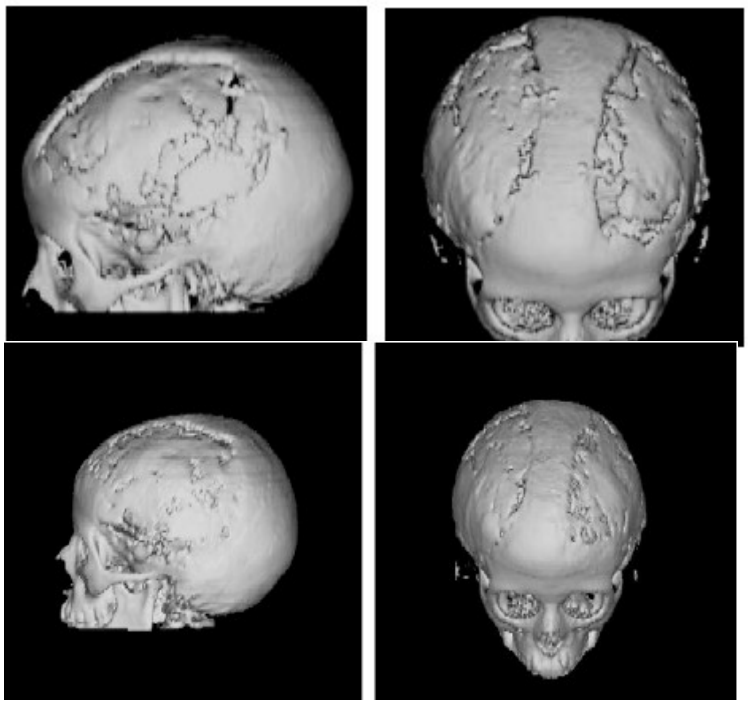
Laura Dominguez, Laura's father, and Susan Fajt.
Laura and Susan were treated for spinal cord injury with their own nasal adult stem cells.

Dennis Turner.
Treated for Parkinson's with his own brain adult stem cells.



Jaw regrown with adult bone marrow stem cells.

Skull bone grown for 7-year-old girl
using adult stem cells from fat.



Hwang Mi-Soon stands after 19 years with spinal cord injury, after treatment with umbilical cord blood stem cells.

Regeneration Mechanism?

(evidence for all of these)

Dedifferentiation-Redifferentiation

Cell fusion with already-differentiated cell

Transdifferentiation

Stimulate Differentiation of Tissue Cells

"[Robert] Lanza noted 'there is ample scientific evidence that adult stem cells can be used to repair damaged heart or brain tissue… if it works, it works, regardless of the mechanism,' he said."
Steve Mitchell, UPI; 12 October 2003

Adult Stem Cells

www.stemcellresearch.org

<u>Most promising</u> source for treatments

Able to generate virtually all adult tissues

Can multiply almost indefinitely, providing numbers sufficient for clinical treatments

Proven success in laboratory culture

Proven success in animal models of disease

Proven success in current clinical treatments

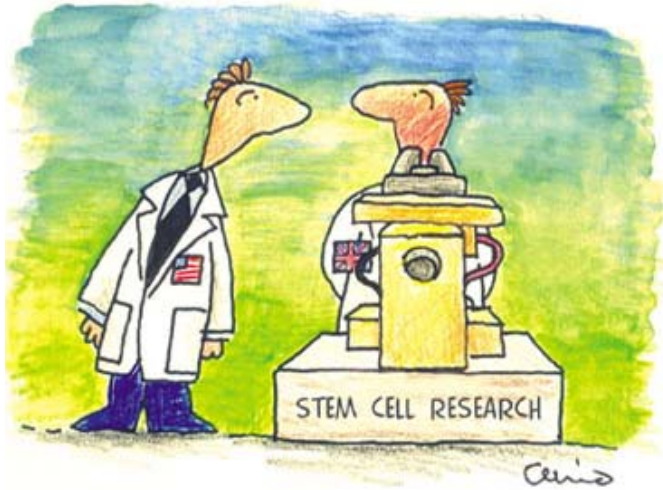
Ability to “home in” on damage

Avoid problems with tumor formation

Avoid problems with transplant rejection

Avoid ethical quandary

GLOBAL VIEW



- **All uses of human SCNT cloning banned: France (7 yrs jail), Canada (5 yrs jail), Australia, Germany, Norway, Switzerland, et al.**

- **United Nations**, 8 March 2005–
  Declaration to prohibit all forms of human cloning
- **European Parliament**, 10 March 2005–
  affirms UN declaration

U.S. FEDERAL LEGISLATION

- **HHS Appropriations language** (since 1996)

  SEC. 510.

  (a) None of the funds made available in this Act may be used for (1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.208(a)(2) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).

  (b) For purposes of this section, the term ‘‘human embryo or embryos’’ includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.