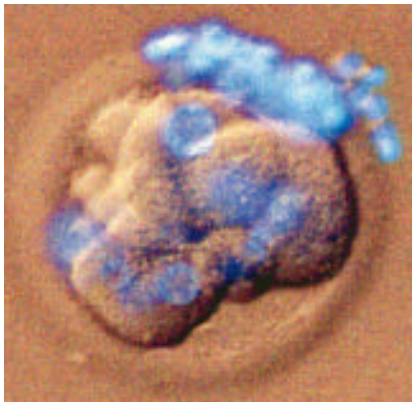
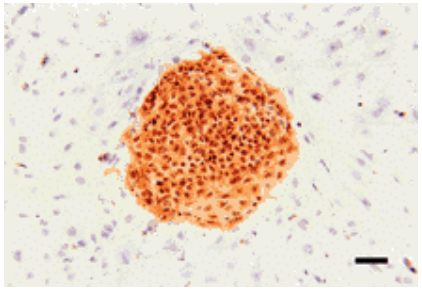
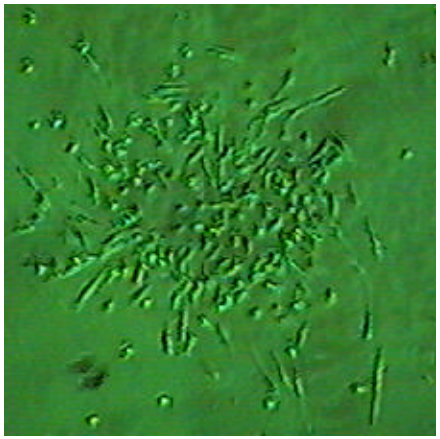
The Science of Human Cloning



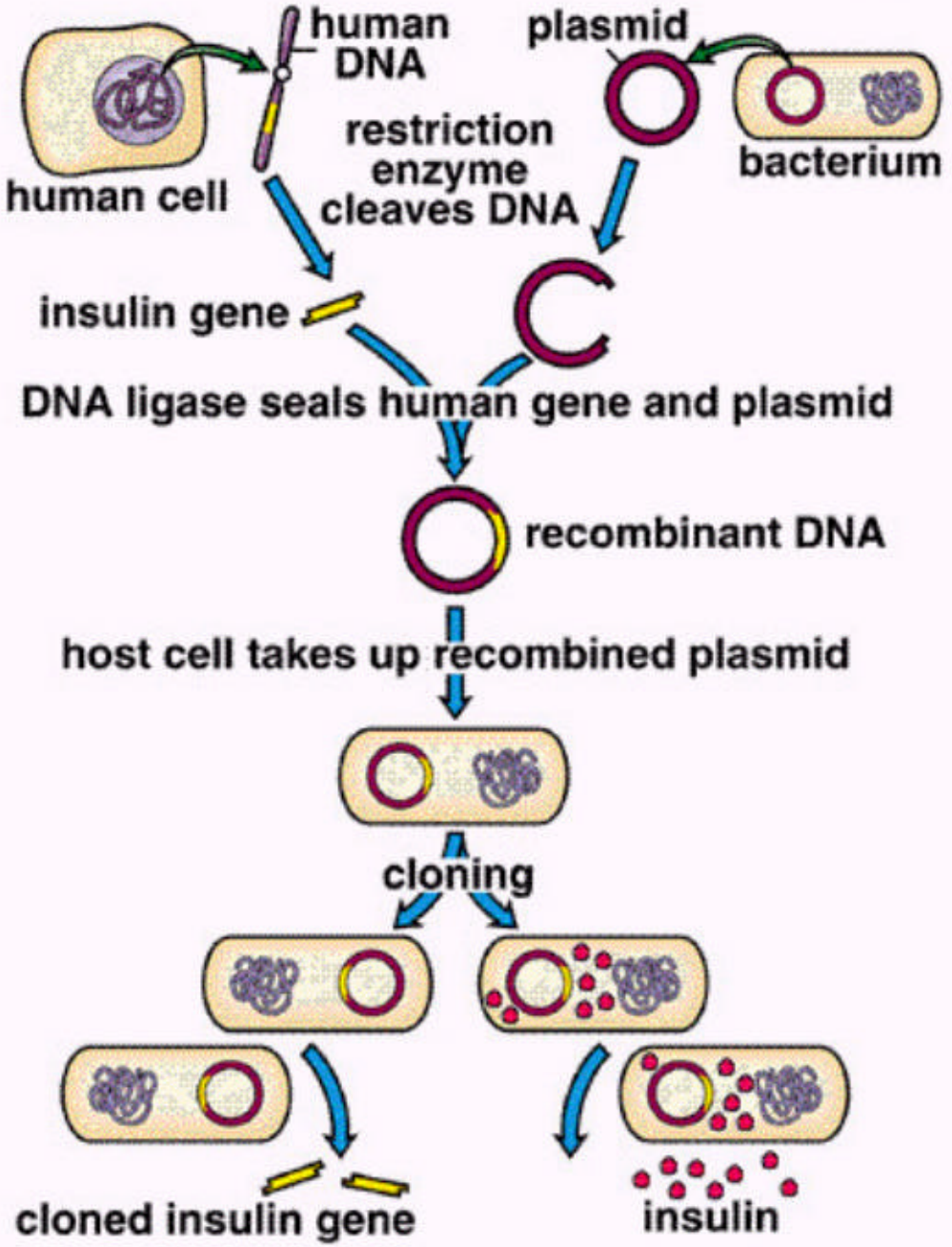




David A. Prentice, Ph.D.
Department of Life Sciences
Indiana State University, USA

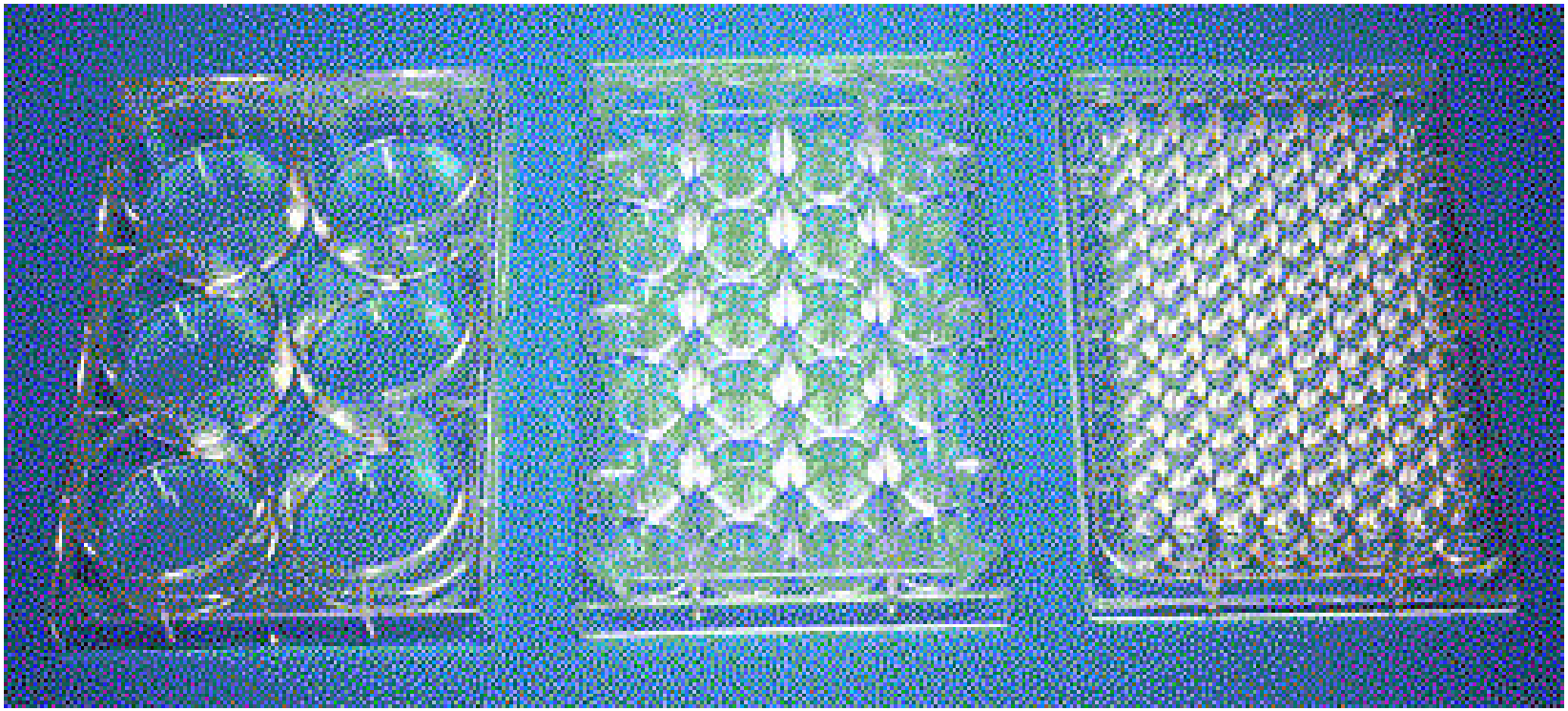
Human Cloning

Human Gene Cloning

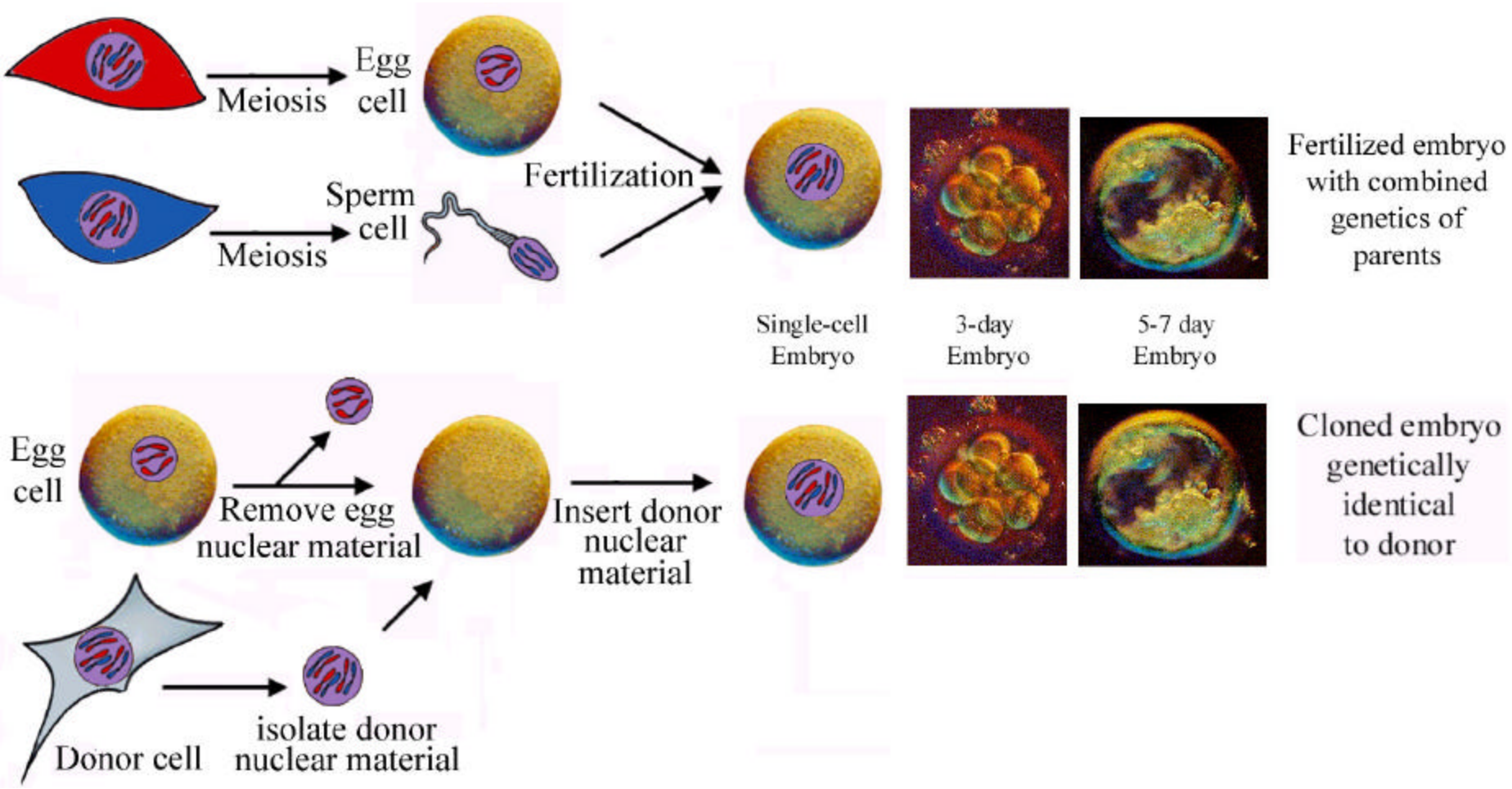


Cell Cloning

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**)



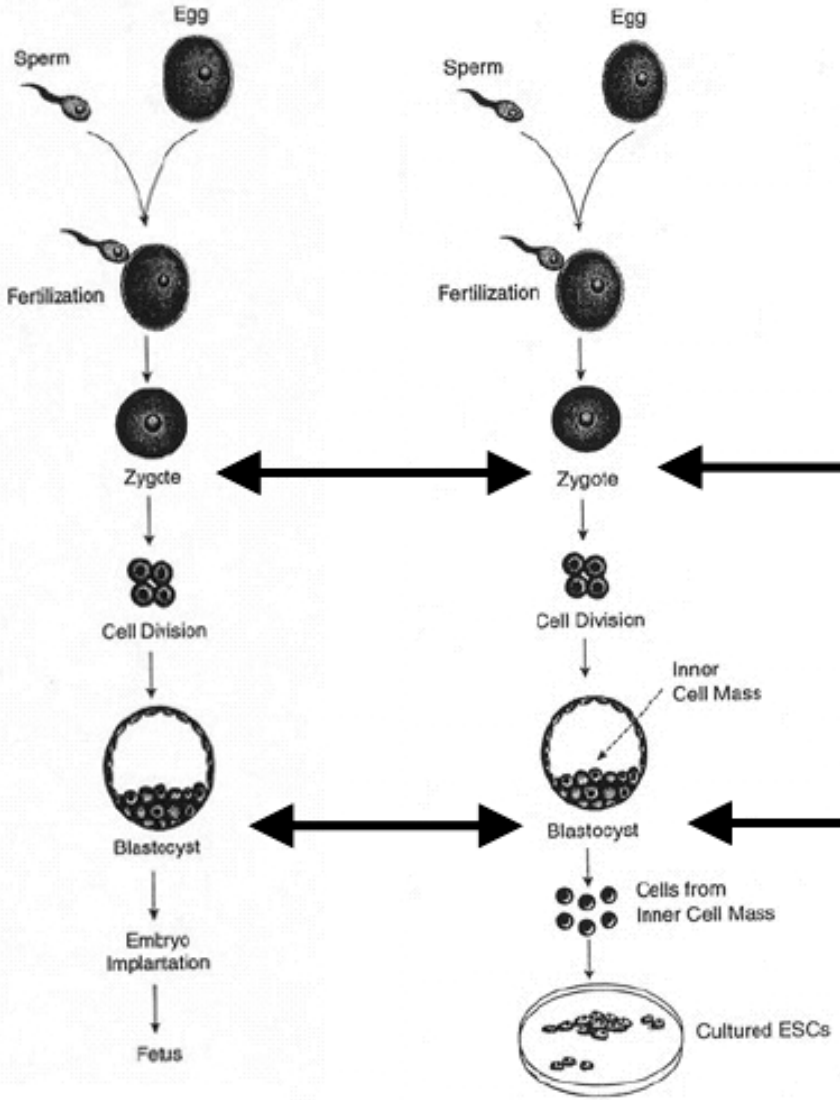


Figure 1 Stages of Development of the Human Embryo

Figure 2 Isolation and Culture of Human ESCs from Blastocysts

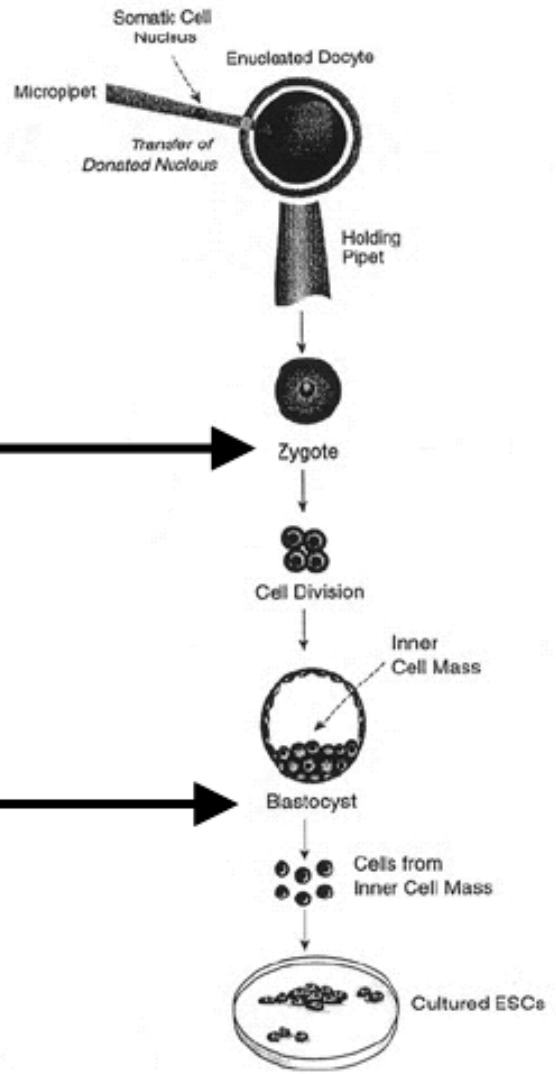
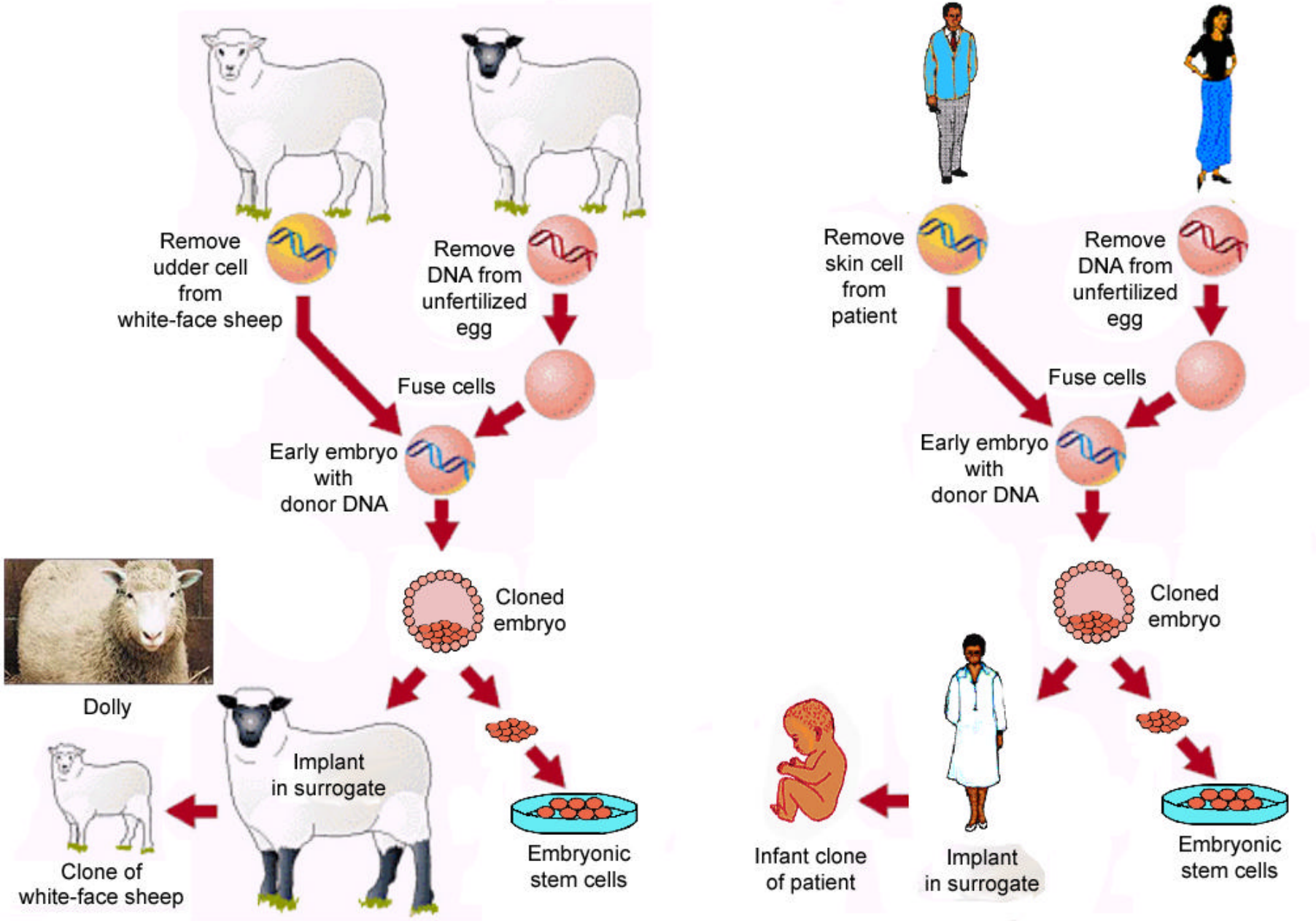


Figure 4 Somatic Cell Nuclear Transfer (SCNT).

[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]


Remove udder cell from white-face sheep
Remove DNA from unfertilized egg
Fuse cells
Early embryo with donor DNA
Cloned embryo
Dolly
Implant in surrogate
Clone of white-face sheep
Embryonic stem cells
Remove skin cell from patient
Remove DNA from unfertilized egg
Fuse cells
Early embryo with donor DNA
Cloned embryo
Infant clone of patient
Implant in surrogate
Embryonic stem cells

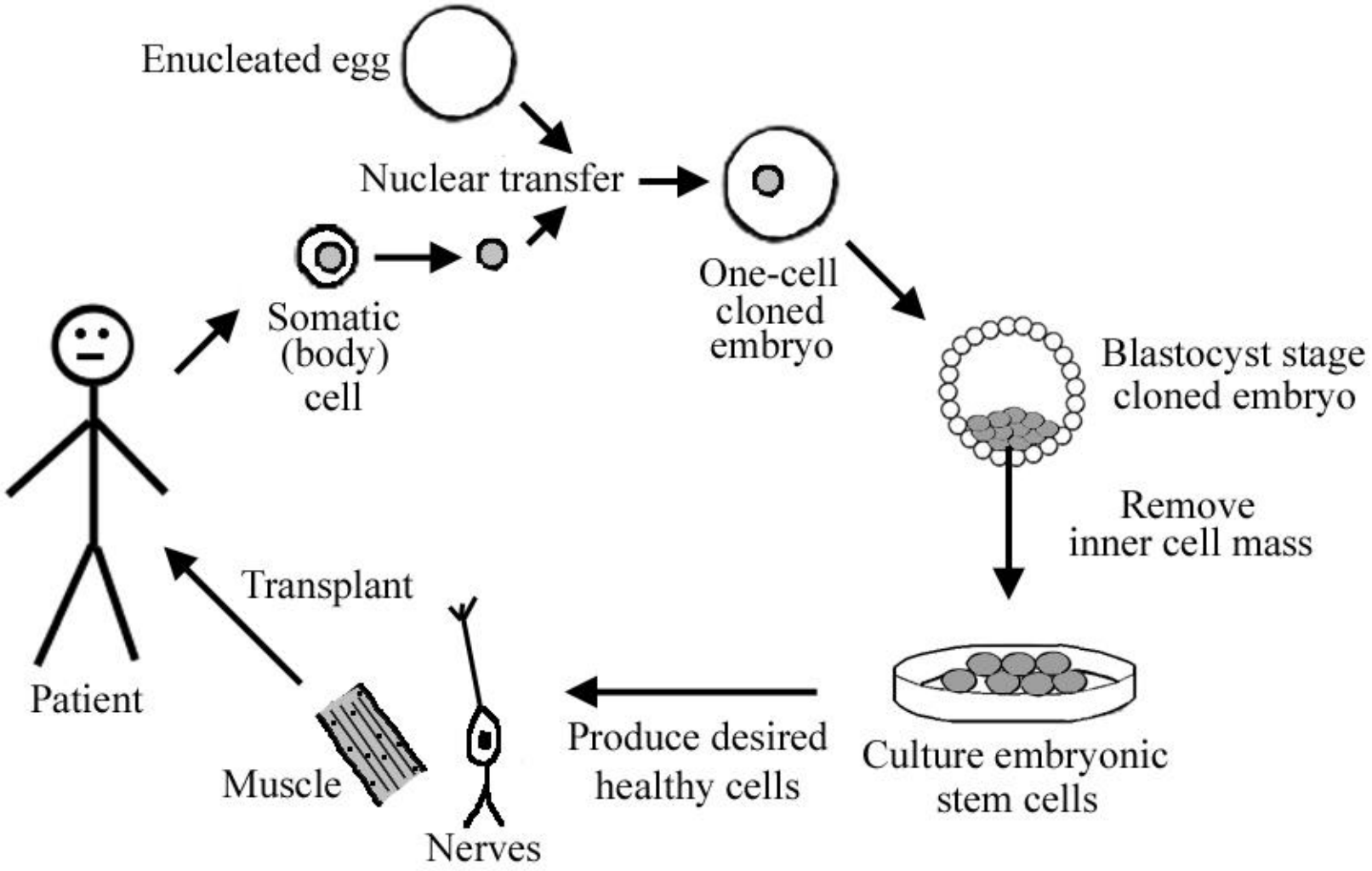
Cloning is unsafe for the clone *and* the surrogate mother

- Even apparently healthy clones have gene expression abnormalities.
  *Humpherys D *et al.*; "Epigenetic instability in ES cells and cloned mice"; *Science* 293, 95-97; July 6, 2001
  *Humpherys D *et al.*; "Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei"; *Proc. Natl. Acad. Sci. USA* 99, 12889-12894; October 1, 2002
- A review of all the world's cloned animals suggests that every one of them is genetically and physically defective. Ian Wilmut said. "There is abundant evidence that cloning can and does go wrong and no justification for believing that this will not happen with humans." "Gene defects emerge in all animal clones", Sunday Times of London, April 28, 2002

- Dolly the **sheep**, first cloned mammal: 1 live birth out of 277 cloned embryos (0.4%)
- Cloned **mice**: 5 live births out of 613 cloned embryos (0.8%)
  5 live births out of 314 cloned embryos implanted (1.6%) (0.8%; 1 survived)
  26 live births out of 312 cloned embryos implanted (8.3%) (4.2%; 13 survived)
- Cloned **pigs**: 5 live births out of 72 cloned embryos implanted (7%)
- Cloned **goats**: 3 live births out of 85 cloned embryos implanted (3.5%)
- Cloned **cattle**: 30 live births out of 496 cloned embryos implanted (6%) (4.8%; 24 survived)
- Cloned **cat**: 1 live birth out of 188 cloned embryos (0.5%); of 87 embryos implanted (1.1%)
- Cloned **gaur**: 1 live birth out of 692 cloned embryos (81 blastocysts) (0.1%) (0%; 0 survived)
- Cloned **rabbits**: 6 live births out of 1852 cloned embryos (0.3%) (0.2%; 4 survived)

- **Health risk for the surrogate mother—"large offspring syndrome"**

Conceptualization of “Therapeutic Cloning”



Human Embryo Cloning Places Women at Risk

Example—to treat the 17 million Diabetes patients in the United States:

Collecting 10 eggs/donor (ACT--71 eggs from 7 donors)

At *generous* 20% cloning efficiency (to achieve blastocyst stage)

At *generous* 10% efficiency at initiating ES cell culture

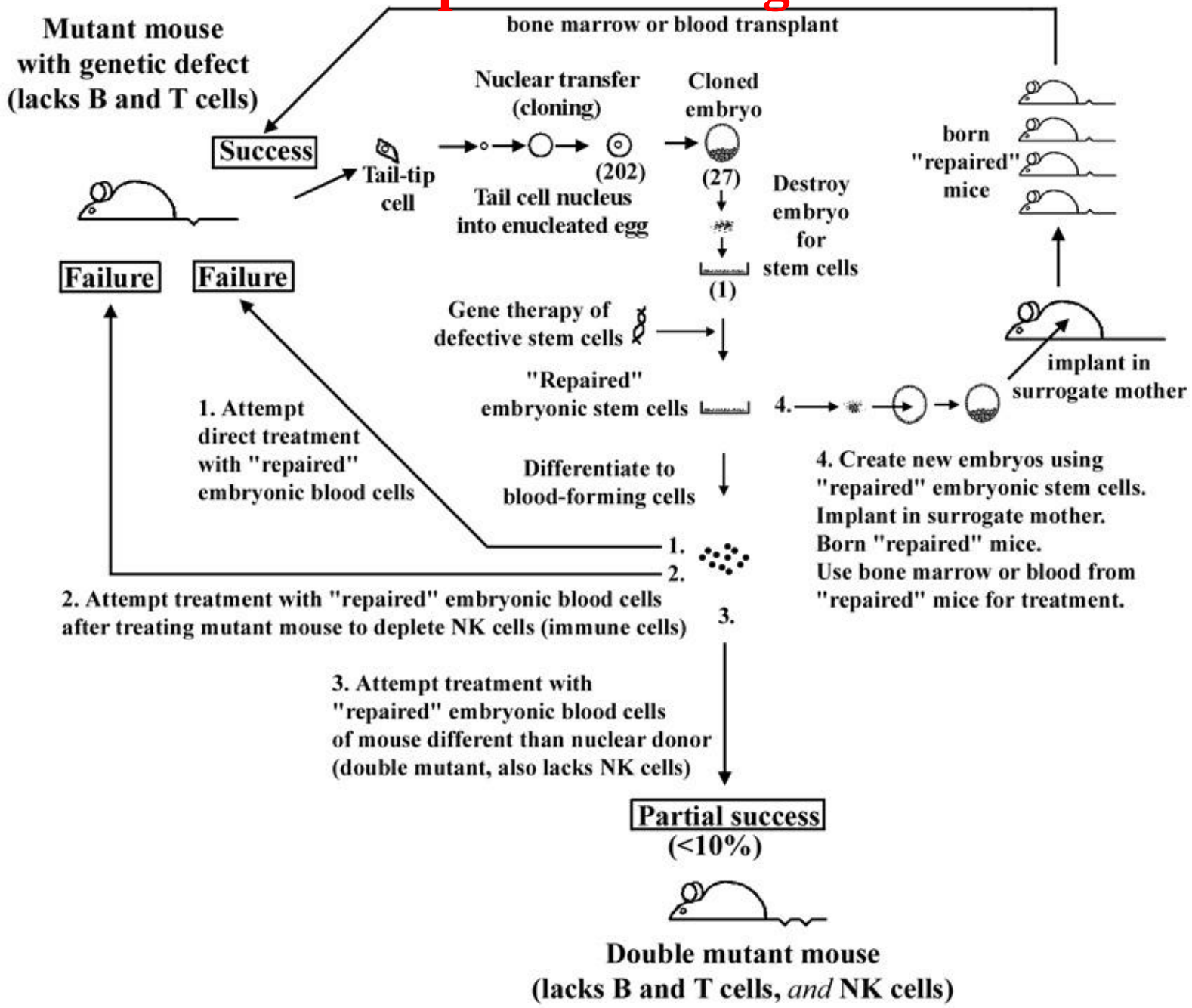
Will require *minimum* of 850 million eggs

Will require *minimum* 85 million women of childbearing age as donors

Significant Health Risks—High-dose hormone therapy and surgery to obtain eggs risks the donor's health and future reproductive success

Commercial Exploitation—disadvantaged women in U.S. and abroad

Therapeutic Cloning Unsuccessful



"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."

W.M. Rideout et al., "Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy," *Cell* Immediate Early Publication, published online March 8, 2002

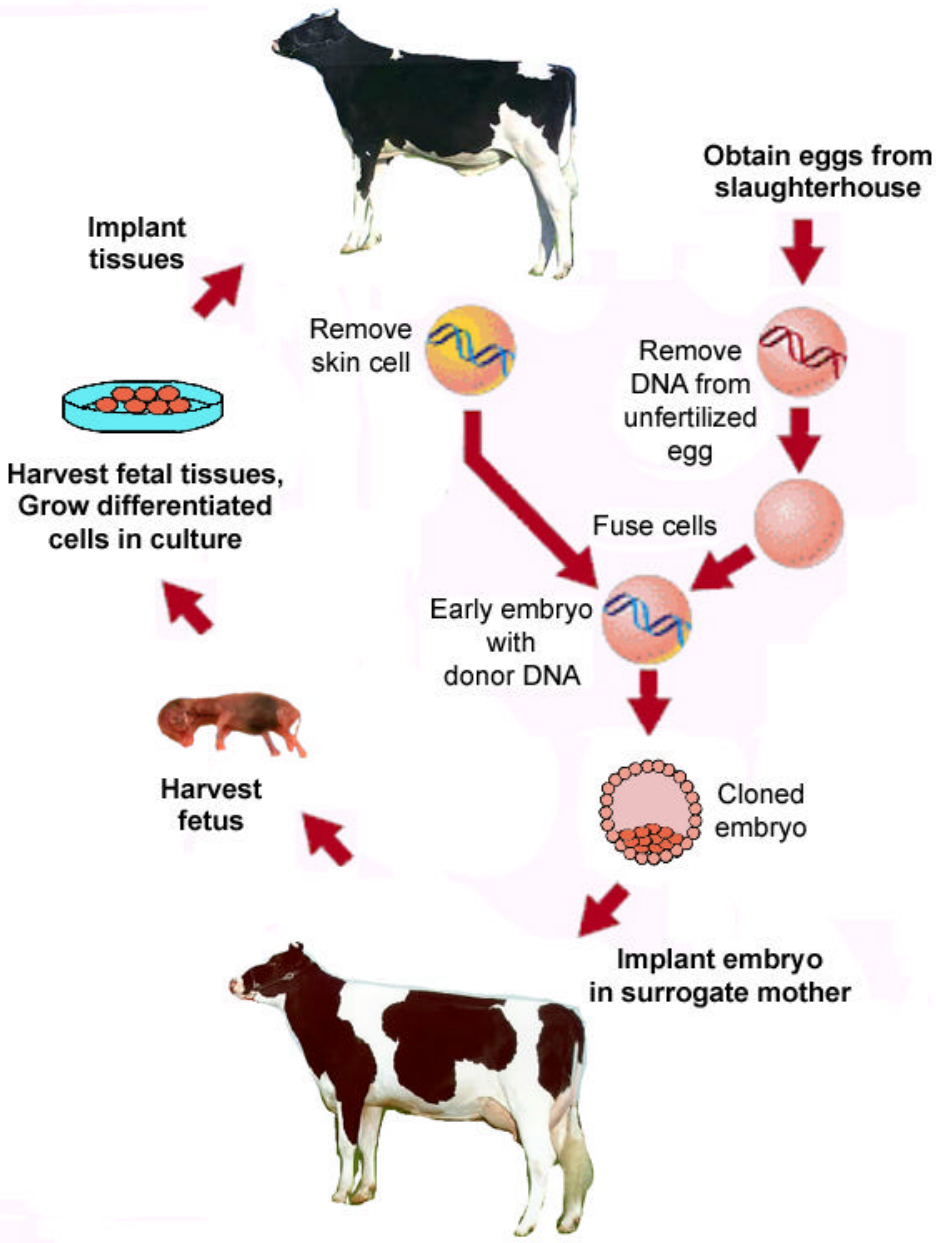
ACT experiment with cloned cow tissues

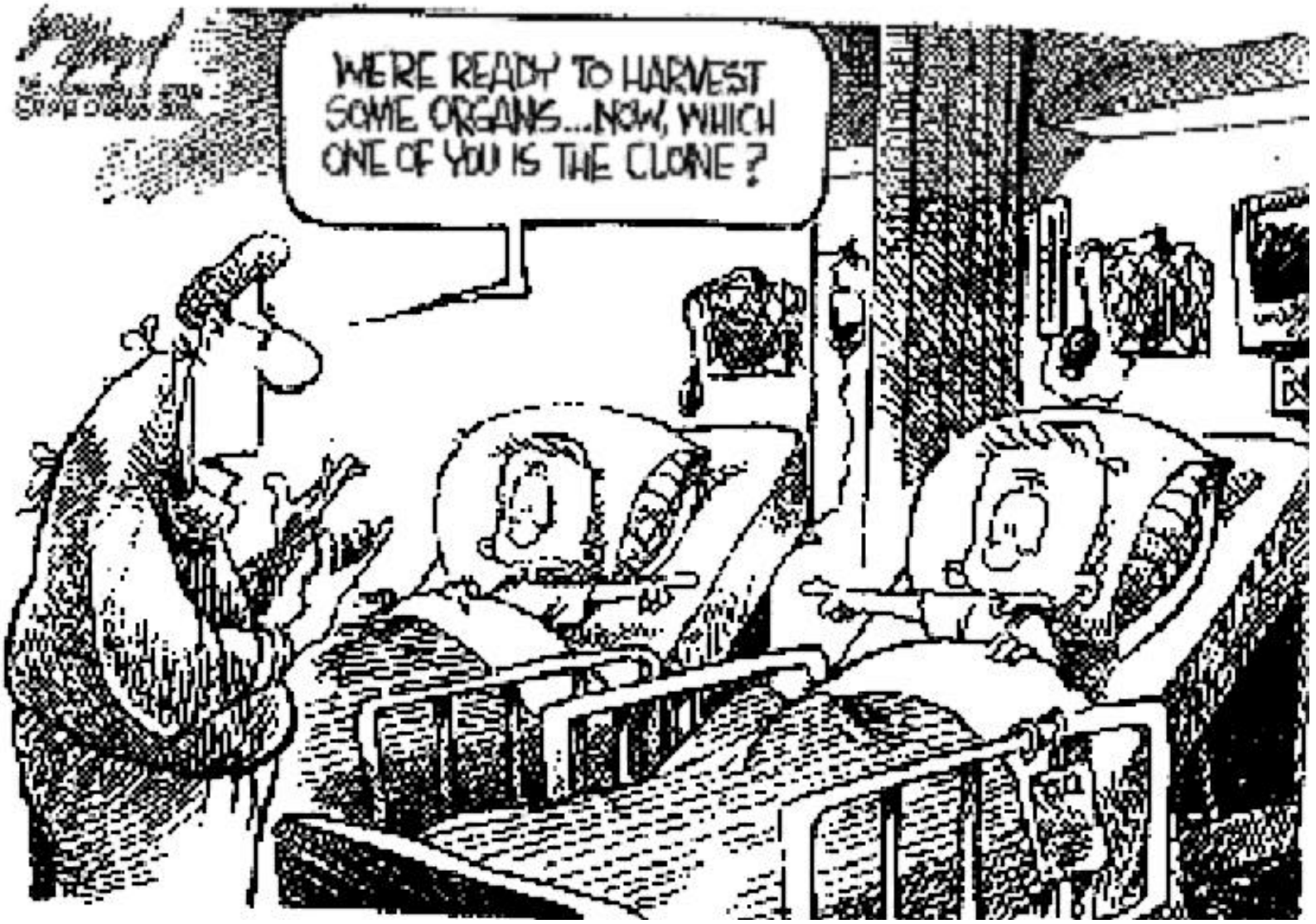
Matching cloned tissues…?

Not through use of embryonic stem cells, but by **gestation of clone to fetal stage before tissue harvesting**.

"Because cloned cells were derived from early-stage fetuses, *this approach is not an example of therapeutic cloning* and would not be undertaken in humans." (emphasis added)

Robert Lanza *et al*.; "Generation of histocompatible tissue using nuclear transplantation," *Nature Biotechnoology*, Advance Online Publication, June 3, 2002




WE'RE READY TO HARVEST SOME ORGANS...NOW, WHICH ONE OF YOU IS THE CLONE?

“CRNT [cell replacement through nuclear transfer, a.k.a. therapeutic cloning] requires the deliberate creation and disaggregation of a human embryo.”

“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; Dec 27, 2000.

Cloning will not provide the claimed medical treatments

Unlikely chance of success in clinical use:

Dr. James Thomson, USA—Odorico JS *et al.*; "Multilineage differentiation from human embryonic stem cell lines," *Stem Cells* 19, 193-204; 2001

Dr. Alan Trounson, Australia—Trounson AO; "The derivation and potential use of human embryonic stem cells", *Reproduction, Fertility, and Development* 13, 523-532; 2001

Transplant Rejection will still occur using cells from cloned embryos:

Dr. Irving Weissman—13 February 2002; before the President's Council on Bioethics

Dr. John Gearhart—25 April 2002; before the President's Council on Bioethics.

Cloning not commercially viable:

Thomas Okarma, chief executive officer, 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, May 10, 2002)

Cloning will not provide the claimed medical treatments

- "[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."
  "Although it is possible to customize ES cells by therapeutic cloning or cytoplasmic 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

Cloning will not prevent transplant rejection

- "**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)
- "**[John] Gearhart** [of Johns Hopkins University] also says that many scientists 'feel there are ways of getting around [the rejection problem] without the nuclear transfer paradigm.' "
  Constance Holden, "Would cloning ban affect stem cells?", *Science* 293, 1025; Aug 10, 2001
- "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; transcript of the April 25, 2002 meeting of the President's Council on Bioethics; p.47; http://www.bioethics.gov/meetings/200204/0425.doc
- **"**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, February 13, 2002, before the President's Council on Bioethics.

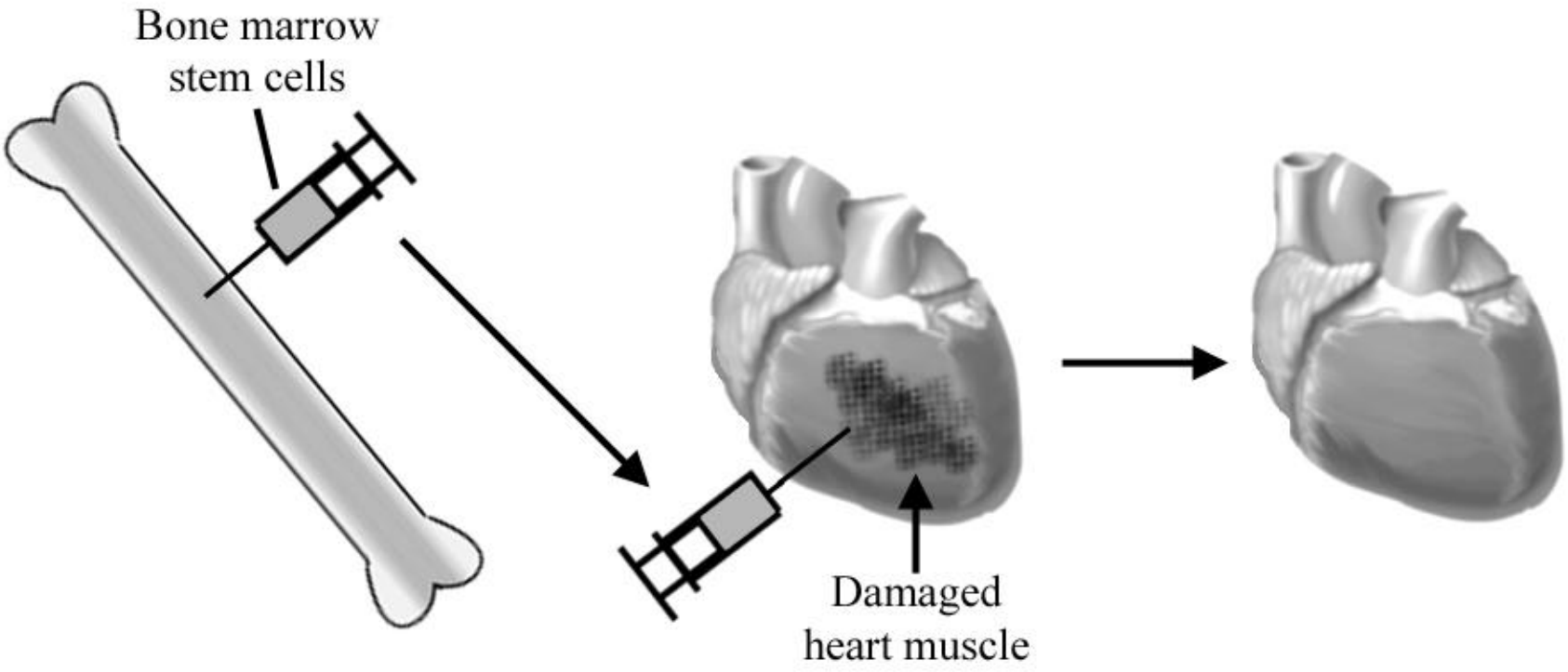
“Cloning Unnecessary and Obsolete”

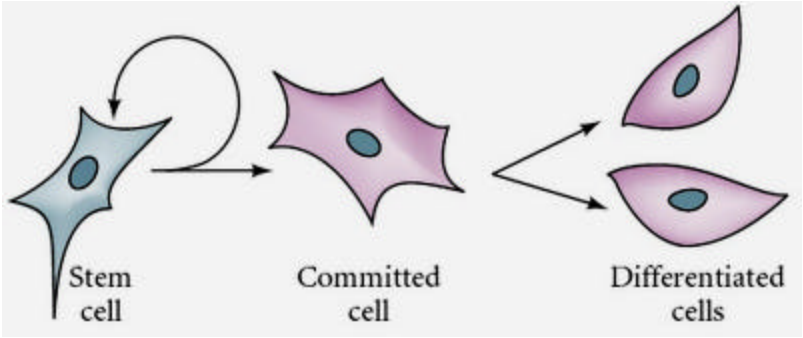
--leading embryonic stem cell expert

- **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

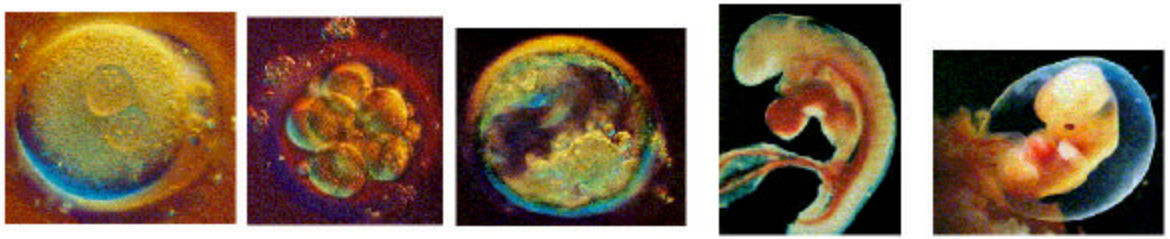
Regenerative Medicine with Stem Cells





Stem Cells

Human Developmental Continuum →

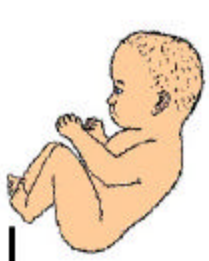


Single-cell Embryo | 3-day Embryo | 5-7 day Embryo | 4-week Embryo | 6-week Embryo

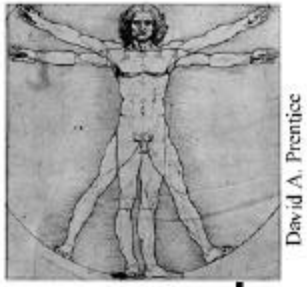
Embryonic Stem (ES) cells
Totipotent

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

Promises, Premises, and Published Data…

Claims for embryonic stem cells unsubstantiated

Current and potential embryonic stem cell problems:

- No current clinical treatments
- Few successes in animal models
- Difficulty in obtaining pure cultures in the dish
- Questions regarding functional differentiation
- Difficult to establish and maintain
- Problem of immune rejection
- Potential for tumor formation and tissue destruction
- Genomic instability
- Ethically contentious

Ample Evidence that Adult Stem Cells show Pluripotent Capacity

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

Transplantation of adult bone marrow stem cells can repair patients' hearts.

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

Patients receiving adult bone marrow stem cell transplant—stem cells also formed liver, skin, digestive tract.

Körbling MK *et al.*; "Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells"; *New England Journal of Medicine* 346, 738-746; 7 March 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 from brain can grow into a wide variety of organs—heart, lung, intestine, kidney, liver, nervous system, muscle, and other tissues.

Clarke DL *et al.*; "Generalized potential of adult neural stem cells"; *Science* 288, 1660-1663, 2 June 2000.

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

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

CORD BLOOD

Various Tissues

David A. Prentice

Adult stem cells effective in tissue repair

Stroke—Adult stem cells from brain, bone marrow, and umbilical cord blood provide therapeutic benefit after stroke. The cells appear to "home" to sites of damage.

*Arvidsson A *et al.*; "Neuronal replacement from endogenous precursors in the adult brain after stroke"; *Nature Medicine* published online; 5 August 2002

*Riess P *et al.*; "Transplanted neural stem cells survive, differentiate, and improve neurological motor function after experimental traumatic brain injury"; *Neurosurgery* 51, published online October 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

Spinal Cord Injury—Adult stem cells capable of re-growth and reconnection in spinal cord.

*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; 19 February 2002

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

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

Diabetes—Liver or pancreatic adult stem cells can form insulin-secreting islets.

*Horb ME *et al.*; Experimental conversion of liver to pancreas. *Current Biology*, 13, 105–115; 21 Jan 2003

*Abraham *et al.*; "Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells"; *Endocrinology* 143, 3152-3161; August 2002

*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

*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 and muscle stem cells repair damage after heart attack.

*****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

*****Toma C *et al.*; "Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart"; *Circulation*. 105, 93-98; 1/8 January 2002

*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.

*Orlic D *et al.*; "Bone marrow cells regenerate infarcted myocardium"; *Nature* 410, 701-705; 5 April 2001

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

*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.

American Association of Neurological Surgeons meeting, 8 April 2002

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 or patient's own neural stem cells
- **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

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

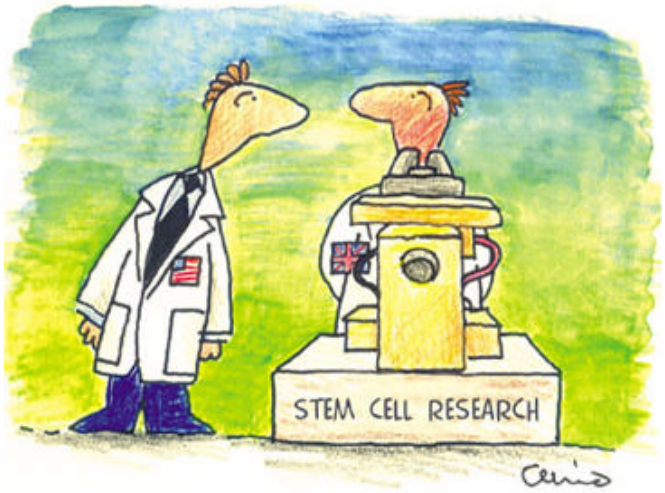
Ability to “home in” on damage

Avoid problems with tumor formation

Avoid problems with transplant rejection

Avoid ethical quandary

What are other countries doing?



- United Kingdom
  - Research on embryos allowed up to 14 days after conception, using embryos created for reproduction or solely for research purposes
  - "Therapeutic cloning" approved, **currently on hold and under judicial review**
- France, Australia, Canada—debates underway on proposals
  - Permits use of human embryonic stem cells and derivation from embryos not needed by genetic parents for reproduction; no creation of embryos for research
  - Proposed cloning ban (**Australia--cloning ban passed**)
- Germany
  - Prohibits derivation of human embryonic stem cells; importation allowed with limitations
  - Cloning ban
- Other countries, as well as EU and UN, debating stem cell research and cloning

Arguments Against Human Cloning

- **No evidence that cloning is necessary or useful for medical treatments**
- **Cloning research will divert resources and delay cures**
- **Banning only implantation is unenforceable**
- **Creates a class of humans who exist only as means to achieve the ends of others**
- **Risking health and exploitation of women**
- **Leading to commodification, commercialization of human life**
- **Gateway to genetic manipulation and control of human beings**

Unsafe, Unethical, Unnecessary