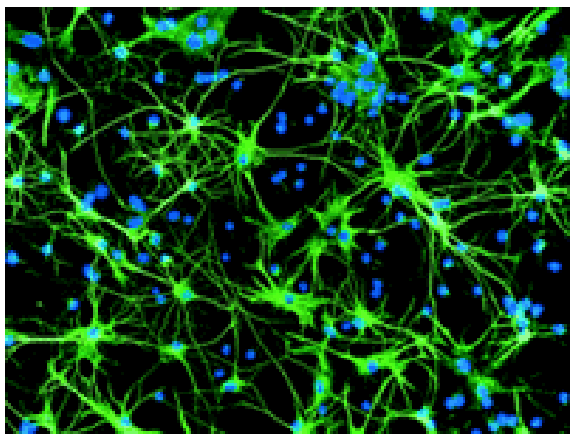
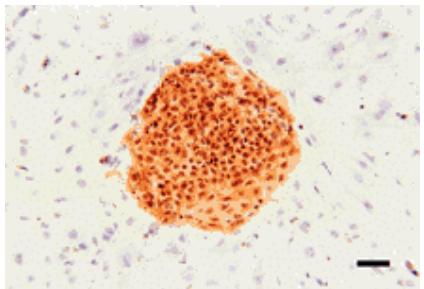
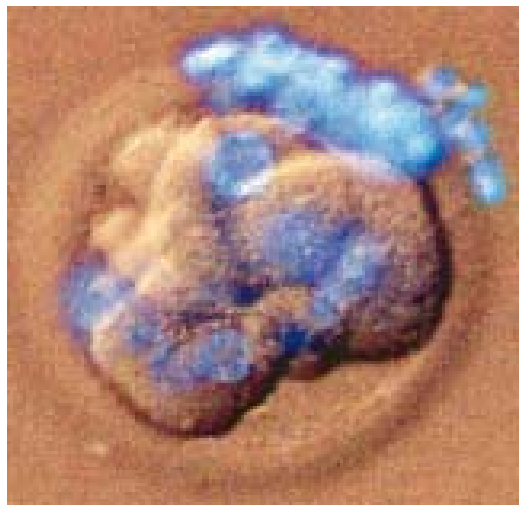
The Science of the Cloning Debate: Latest Developments







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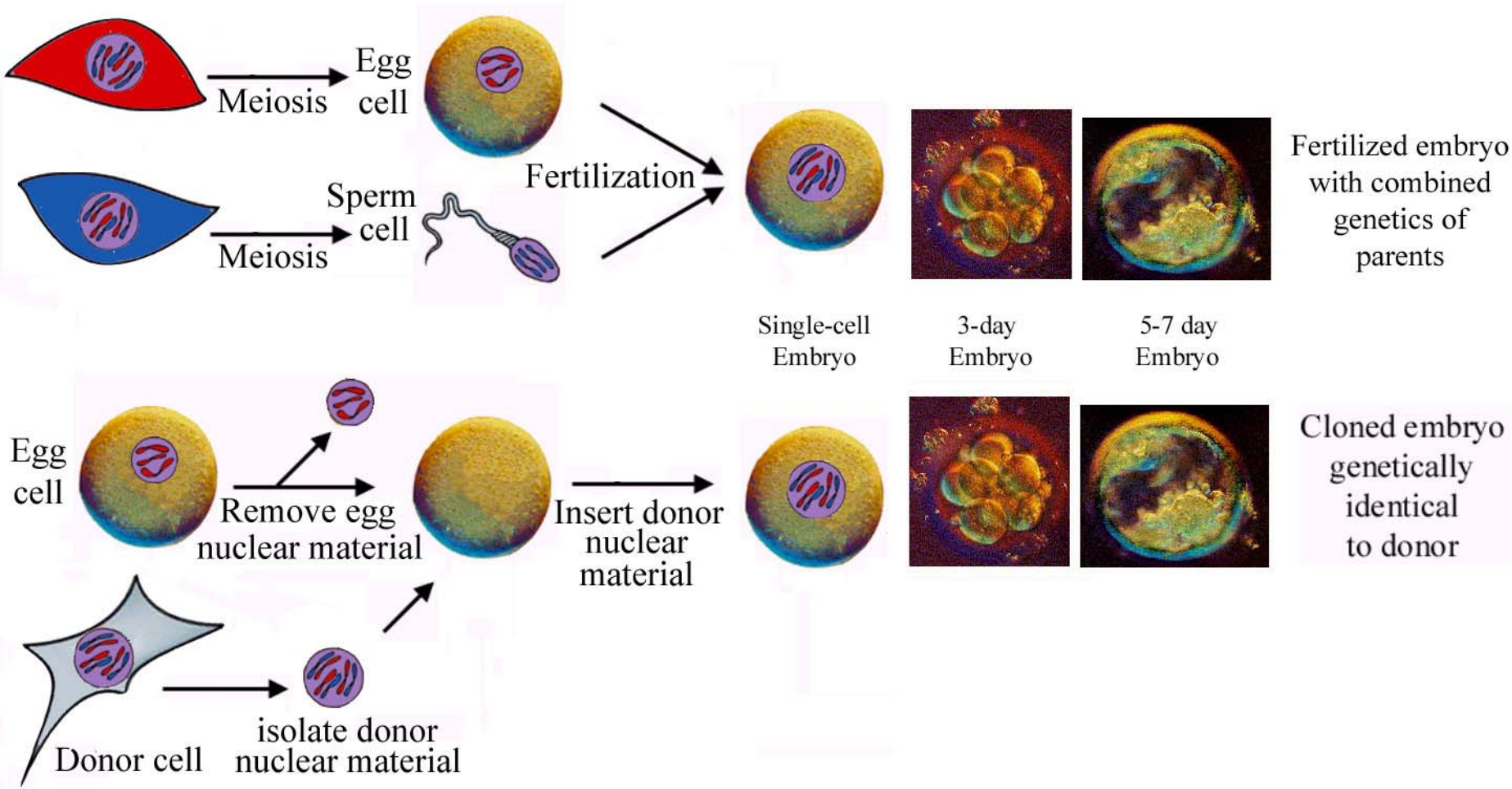
At an April 30 press conference, Senator Arlen Specter was asked by a reporter, within the context of human cloning, when life begins.

Senator Specter replied, “I haven’t found it helpful to get into the details.”

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



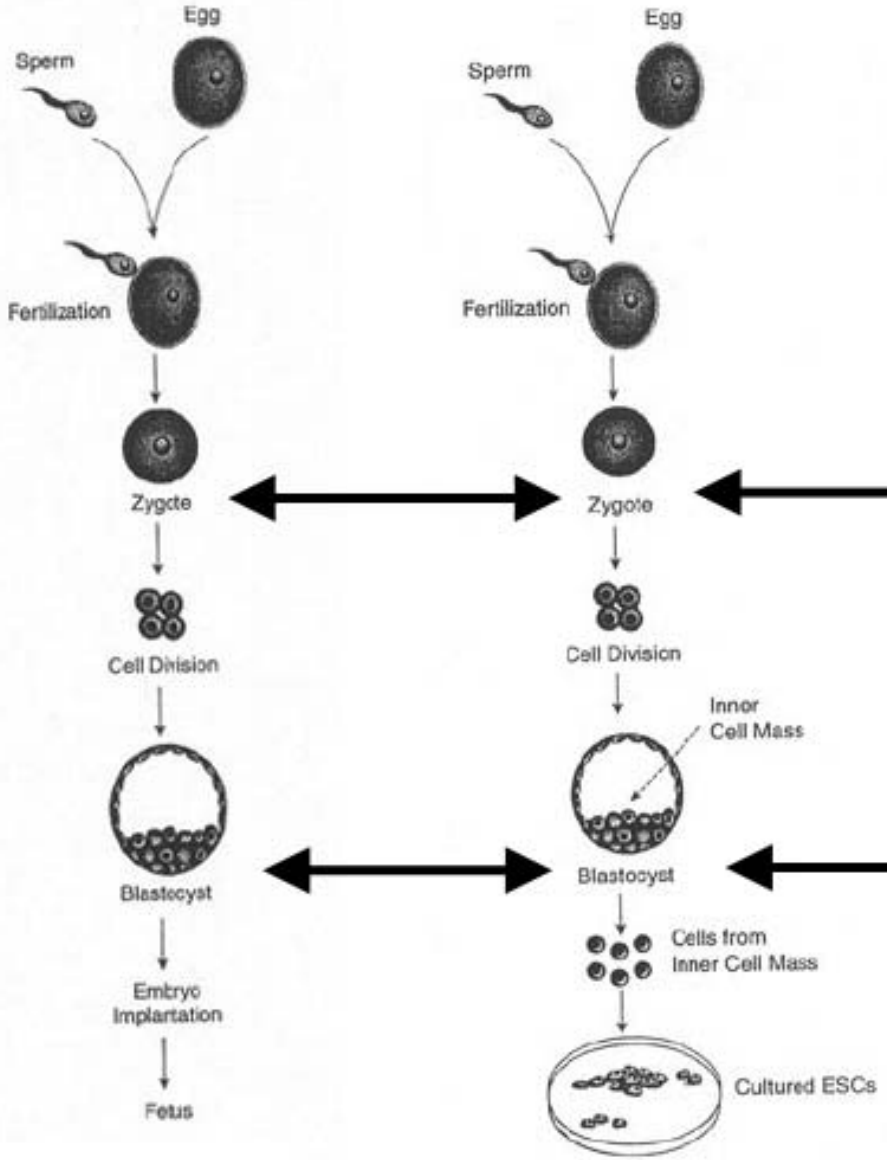


Figure 1 Stages of Development of the Human Embryo

Figure 2 Isolation and Culture of Human ESCs from Blastocysts

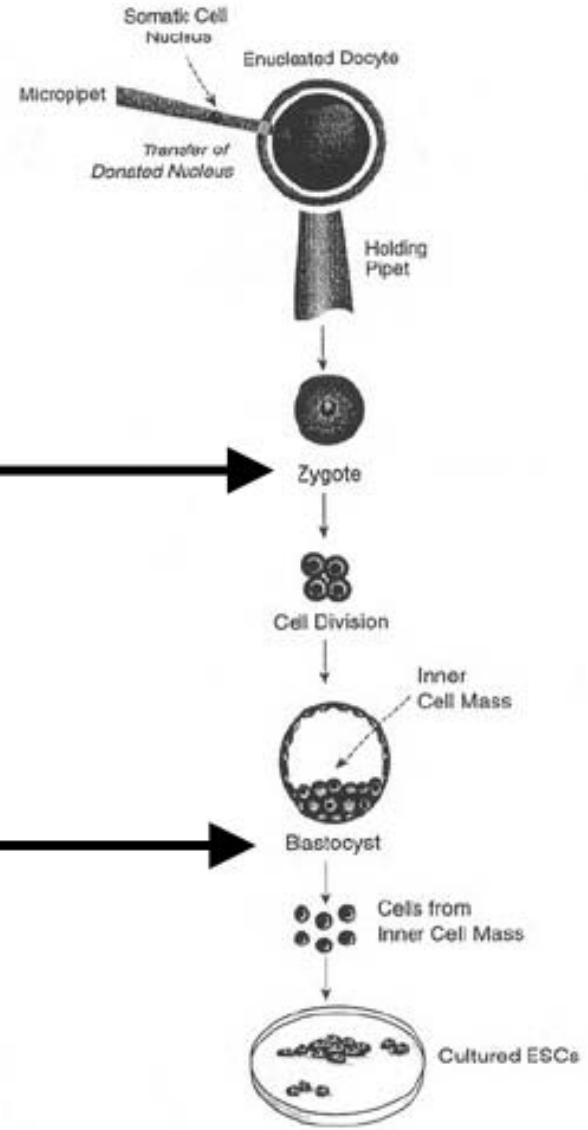


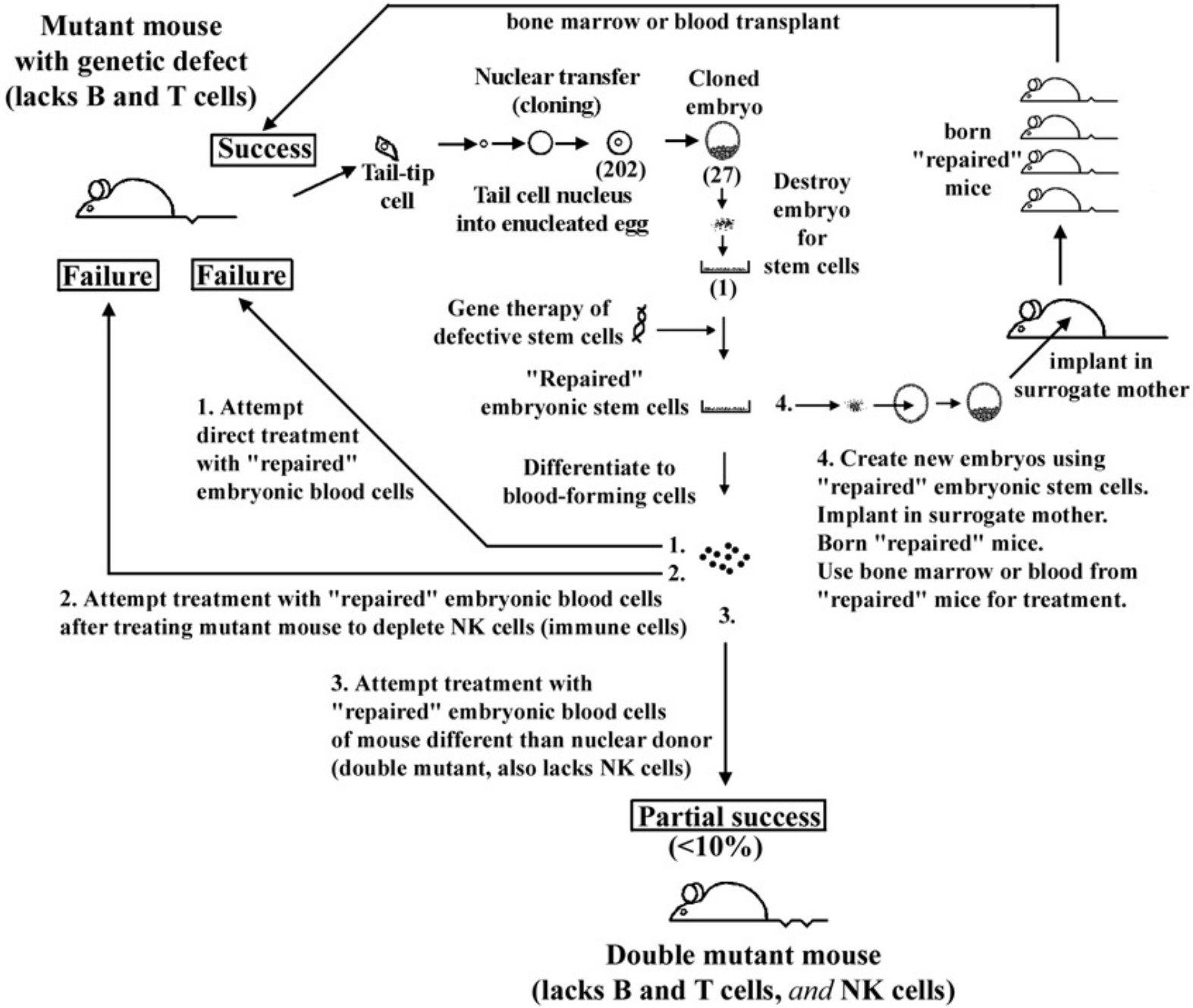
Figure 4 Somatic Cell Nuclear Transfer (SCNT).

[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]

Quotes regarding "therapeutic cloning"

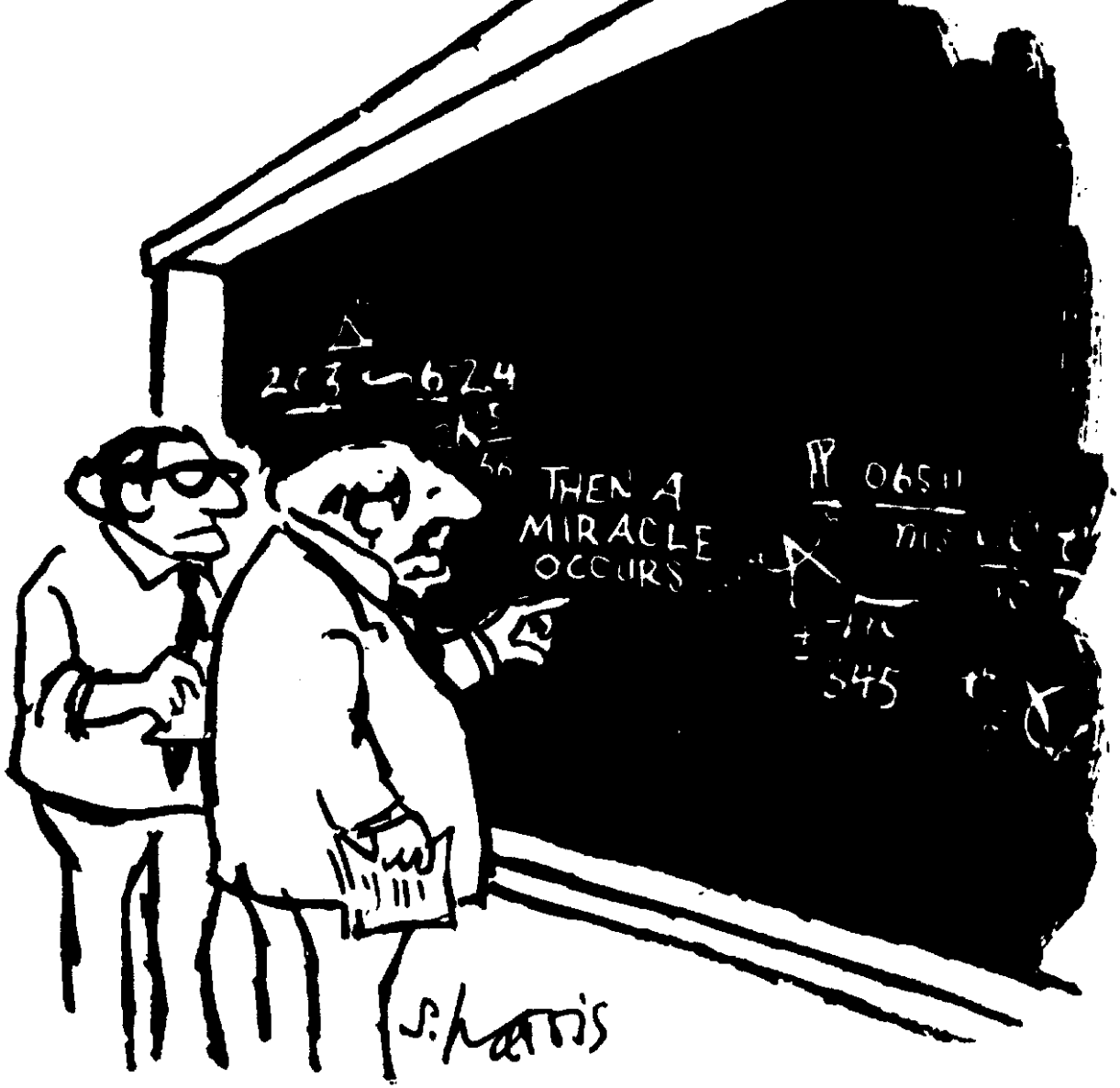
- "[John] Gearhart [of Johns Hopkins University] also says that many scientists 'feel there are ways of getting around [the rejection problem] without the nuclear transfer paradigm.' "
  Constance Holden, "Would cloning ban affect stem cells?", *Science* 293, 1025; Aug 10, 2001
- "[T]he poor availability 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
- "Moreover, because therapeutic cloning requires the creation and disaggregation ex utero of blastocyst stage embryos, this technique raises complex ethical questions."
  "CRNT [cell replacement through nuclear transfer, aka 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; Dec 27, 2000.

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



"I think you should be more explicit here in step two."

Adult stem cells show pluripotent capacity

Patients received hematopoietic stem cell transplant; stem cells also formed liver, skin, digestive tract.

--Korbling 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; March 7, 2002

Adult stem cells from bone marrow can form virtually all body tissues and be grown indefinitely.

--Schwartz RE *et al.*; "Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells"; *Journal of Clinical Investigation* 109,1291–1302; May 2002

--Zhao L-R et al.; "Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats", *Experimental Neurology* 174, 11-20; 2002.

--Reyes M *et al.*; "Origin of endothelial progenitors in human postnatal bone marrow"; *Journal of Clinical Investigation* 109, 337-346; Feb 2002.

--Reyes M *et al.*; "Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells"; *Blood* 98, 2615-2625; Nov 1, 2001

--Reyes M and Verfaillie CM; "Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells"; *Annals of the New York Academy of Sciences* 938, 231-233; June 2001

Adult stem cells can multiply almost indefinitely, providing numbers sufficient for clinical treatments

--Schwartz RE *et al.*; “Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells”; *Journal of Clinical Investigation* 109,1291–1302; May 2002
--Shi S *et al.*; "Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression"; *Nature Biotechnology* 20, 587-591; June 2002
--Simonsen JL *et al.*; "Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells"; *Nature Biotechnology* 20, 592-596; June 2002
--Neildez-Nguyen TMA *et al.*; “Human erythroid cells produced ex vivo at large scale differentiate into red blood cells in vivo”; *Nature Biotechnology* 20, 467-472; May 2002
--Zhao S *et al.*; “JAK2, complemented by a second signal from c-kit or flt-3, triggers extensive self-renewal of primary multipotential hemopoietic cells”; *EMBO J* 21, 2159-2167; May 1, 2002
--Antonchuk J *et al.*; “HOXB4-induced expansion of adult hematopoietic stem cells ex vivo”; *Cell* 109, 39-45; April 5, 2002
--Reyes M *et al.*; “Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells”; *Blood* 98, 2615-2625; Nov 1, 2001
--Krause DS; “Multipotent human cells expand indefinitely”, *Blood* 98, 2595; Nov 1, 2001

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 treating animal models of disease

Stroke—Adult bone marrow stem cells or umbilical cord blood stem cells, even delivered intravenously to brain tissue which has suffered stroke damage in rats, provide therapeutic benefit after stroke. The cells appeared to "home" to sites of damage.

--Zhao L-R et al.; "Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats", *Experimental Neurology* 174, 11-20; 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

Heart Damage—Bone marrow stem cells injected into heart, blood stream, or "mobilized" from the bone marrow, repair damage after heart attack.

--Toma C *et al.*; "Human Mesenchymal Stem Cells Differentiate to a Cardiomyocyte Phenotype in the Adult Murine Heart"; *Circulation*. 105, 93-98; Jan 1/8, 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, August 28, 2001.

--Jackson KA *et al.*; "Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells"; *Journal of Clinical Investigation* 107, 1395-1402; June 2001

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

Parkinson's Disease

Researchers at Chicago's Rush Hospital identified the signal to turn brain stem cells into dopamine neurons, then grafted the cells into brains of Parkinson's rats, effectively curing the animals' severe Parkinson symptoms.
Reported at the Experimental Biology Meeting in New Orleans, April 2002.

Using the patient's own adult neural stem cells, Dr. Michel Levesque, Los Angeles Cedars-Sinai Medical Center, reports a total reversal of symptoms in the first Parkinson's patient treated.
April 8, 2002, at the meeting of the American Association of Neurological Surgeons.

A team at Emory University School of Medicine has shown that implanting retinal cells into brains of advanced Parkinson's patients can improve motor function by 50%.
April 18, 2002 annual conference of the American Academy of Neurology, Denver.

Bone marrow stem cells formed neurons; delayed neurodegenerative disease.
Jin HK *et al.*; "Intracerebral transplantation of mesenchymal stem cells into acid sphingomyelinase–deficient mice delays the onset of neurological abnormalities and extends their life span"; *J. Clin. Invest.* 109;1183–1191; May 2002

Bone marrow forming blood vessels and neurons in brain.
Hess DC *et al.*; "Bone Marrow as a Source of Endothelial Cells and NeuN-Expressing Cells After Stroke"; *Stroke* 33,1362-1368; May 2002

Neural stem cells from brain can form functionally all neuronal types.
Song H-j *et al.*; "Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons"; *Nature Neuroscience* Published online: DOI: 10.1038/nn844; 15 April 2002,

Adult neural stem cells can functionally integrate into the brain
Using a mouse model, scientists from Princeton University and Sweden showed that neurons generated by adult stem cells functionally integrate into the synaptic circuitry of the brain.
Carlen M *et al.*; "Functional integration of adult-born neurons"; *Current Biology* 12, 606-608; April 2, 2002

Adult stem cells from fat can form nerves
Researchers from Duke University and Artecel transformed adult stem cells from liposuctioned fat into neuronal cells. The cells showed marked transformation within 3 hours of induction. Stem cells from fat may represent an alternative source of cells capable of neuronal differentiation, potentially enhancing their usefulness in the treatment of neurological disease.
Safford KM *et al.*; "Neurogenic differentiation of murine and human adipose-derived stromal cells"; *Biochemical and Biophysical Research Communications* 294, 371–379; May 31, 2002

Transplanted adult stem cells achieved re-growth of neurons 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; Feb 19, 2002

Adult bone marrow stem cells can form blood vessels

University of Florida scientists gave mice transplants of purified, genetically-engineered bone marrow stem cells; newly formed vessels could be tracked because they glowed green. In some experiments a single adult stem cell was transplanted. "Everything that glowed green in those animals, therefore, came from a single cell," said Edward Scott, one of the researchers. "That was our formal proof that it was indeed a blood stem cell that was making these blood vessels." "We're hoping we'll someday learn how to get your own stem cells to respond on their own and repair vascular damage, whether it's caused by a heart attack or the circulatory problems associated with diabetes," Scott said.

--Grant MB *et al.*; "Adult hematopoietic stem cells provide functional hemangioblast activity during retinal neovascularization"; *Nature Medicine* 8, 607-612; June 2002

Adult muscle stem cells form multiple tissues; regenerate dystrophic muscle

Scientists at the University of Pittsburgh and in Germany have isolated adult muscle stem cells that can grow for long periods in culture, and can transform into muscle, neural tissue, and blood vessels. The transformations could be accomplished both in the lab dish as well as in experimental mice, without immune rejection. The cells improved muscle regeneration in mouse models of muscular dystrophy.

--Qu-Petersen Z *et al.*; "Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration"; *Journal of Cell Biology* (on-line publication) doi: 10.1083/jcb.200108150; May 2002.

Bone marrow into functional lung cells.

Kotton DN *et al.*; "Bone marrow-derived cells as progenitors of lung alveolar epithelium"; *Development* 128, 5181-5188; Dec, 2001

Adult bone marrow stem cells form functional liver and kidney cells

Forbes SJ *et al.*; "Hepatic and renal differentiation from blood-borne stem cells"; *Gene Therapy* 9, 625-630; May, 2002

Adult bone marrow stem cells transformed into functional liver cells

Dr. Catherine Verfaillie's group at Minnesota continues to show more and more uses for the multipotent adult progenitor cells (MAPC) from bone marrow. The team has now shown that these adult stem cells can transform into functional liver cells. The adult stem cells also were grown in culture for over 100 generations, twice the length of time previously thought possible with adult cells.
--Schwartz RE *et al.*; "Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells"; *J. Clin. Invest.* 109,1291–1302; May 2002

Adult liver stem cells make pancreatic cells, reverse hyperglycemia in diabetic mice

Researchers at the University of Florida have transformed highly purified adult liver stem cells into pancreatic cells. The cells self-assemble in culture to form 3-dimensional islet structures, express pancreatic genes, produce pancreatic hormones, and secrete insulin. When implanted into diabetic mice, the transformed cells reversed their hyperglycemia in 10 days. Dr. Ammon Peck, one of the team leaders, said "Adult stem cells appear to offer great promise for the production of an almost unlimited supply of insulin-producing cells and islets of Langerhans... The ability to grow insulin-producing cells from liver stem cells shows the remarkable potential of adult stem cells for future cell therapy."
--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,* Online Early Edition; 10.1073/pnas.122210699; June 4, 2002

Adult Skin Cells Reprogrammed Without Cloning

A team of scientists from Norway has succeeded in coaxing one type of adult cell to start behaving like a completely different type of adult cell. The scientists have made human skin cells in a test tube behave as if they were immune system cells, by bathing the skin cells in extracts of the immune cells. In other work, they have been able to get skin cells to behave as if they were nerve cells.

"We can take a skin cell from your body and turn it directly into a cell type that you need to treat a particular disease," said Dr. Philippe Collas, the leader of the team, whose work was published May 1 in the respected journal *Nature Biotechnology*.

The technique being developed would allow skin cells from a patient to be turned directly into other types of cells without having to revert first to an embryonic state and without needing women's eggs. They told Reuters, "That's the beauty of our system -- we are not working with embryos or dealing with stem cells at all. You get around all these issues." "It would be a one-day procedure, in principal. The patient would come in and give a skin biopsy to the lab to reprogram and the day after you could put the cells back into the patient." The technique would have immediate applications in cancer. The group is also looking at making insulin-secreting pancreatic cells.

The approach will aid investigation of the mechanisms by which adult stem cells revert to cells capable of differentiating into other types of cells with potential use in therapies for conditions like diabetes, Parkinson's disease, and heart disease. From a clinical perspective, approaches based on this technology would allow replacement cells to be generated that are compatible with a patient's immune system, without the ethical problems of generating or destroying embryos.

Hakelien AM *et al.*; "Reprogramming fibroblasts to express T-cell functions using cell extracts"; *Nature Biotechnology* 20, 460-466; May 2002

Adult Stem Cells

www.stemcellresearch.org

<u>More promising</u> alternative for treatments

Vast biomedical potential

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

Avoid problems with tumor formation

Avoid problems with transplant rejection

Avoid ethical quandary