Stem Cell Therapy In Diabetes Mellitus

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- For many years, there has been great interest in approaches to the replacement of insulin-producing beta cells in individuals with diabetes
- Cellular replacement therapies carry the hope to achieve physiologic glucose control, thereby reducing or eliminating the need for daily medication, as well as preventing long-term diabetic complications

- Cellular treatments seem specifically suited for type 1 diabetes because primary destruction of beta cells is the central pathological process in this disease
- In addition, patients with advanced type 2 diabetes and only relative beta cell insufficiency could benefit from replacement therapy

Transplantation of Whole Donor Pancreas

- It has been successfully performed in a large number of patients with type 1 diabetes
- The need for intense immunosuppression, as well as the difficulties associated with drainage of exocrine pancreatic fluid, result in undesirable morbidity

The Transplantation Of Isolated Pancreatic Islets

- The transplantation of isolated pancreatic islets into the livers of type 1 diabetic patients had been largely unsuccessful for many years
- Recently, the newly devised "Edmonton Protocol" for islet transplantation provided unprecedented positive results (Shapiro et al, 2000)

Scarcity of Islet Cells For Transplantation

- The amount of donor islet tissue is severely limited
- The Edmonton Protocol requires the utilization of islets from 2 to 4 donor pancreata for the successful transplantation of a single patient
- Using current protocols, less than 0.5% of needy recipients can be treated.

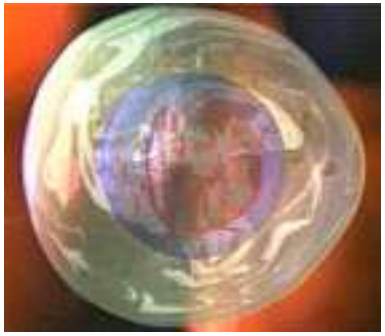
Possible Approaches To Overcome The Shortage Of Donor Pancreata

- Direct expansion of beta cells *in vitro* for use in transplantation (limited proliferative potential of fully differentiated beta cells)
- Genetic manipulation of an unrelated cell type to secrete insulin in a glucose responsive manner (difficult, to accurately mimic the complex regulatory circuits of a beta cell)
- Expansion and subsequent differentiation of stem or progenitor cells

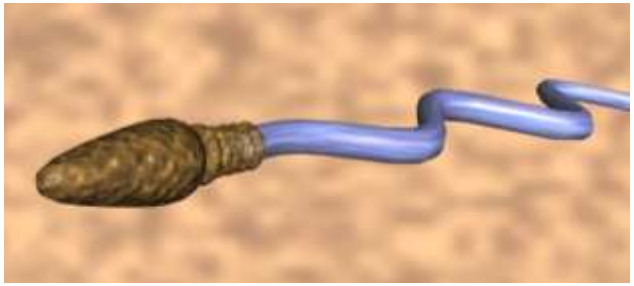
Expansion And Subsequent Differentiation Of Stem Or Progenitor Cells

- This approach seems promising
- The proliferative capacity of beta cells *in vivo* is limited and that new beta cells are mainly generated via their differentiation from undifferentiated progenitor cells
- The formation of new islet tissue via the differentiation of stem/progenitor cells in adult pancreata is referred to as islet neogenesis

In the beginning...

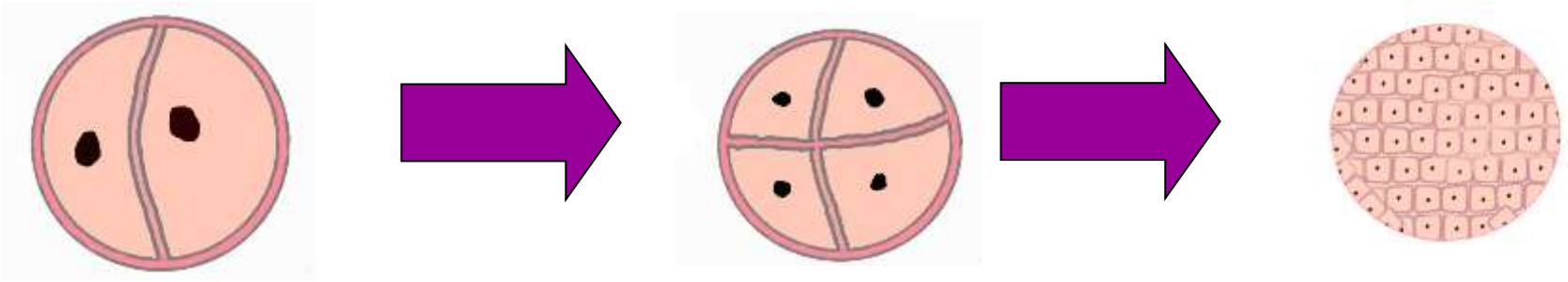


Egg



Sperm

Cell division...

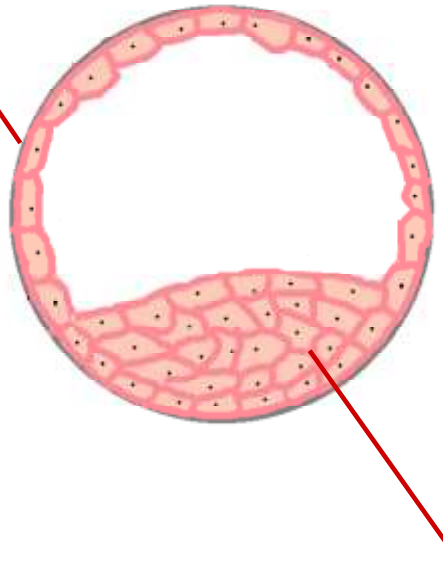


zygote

morula

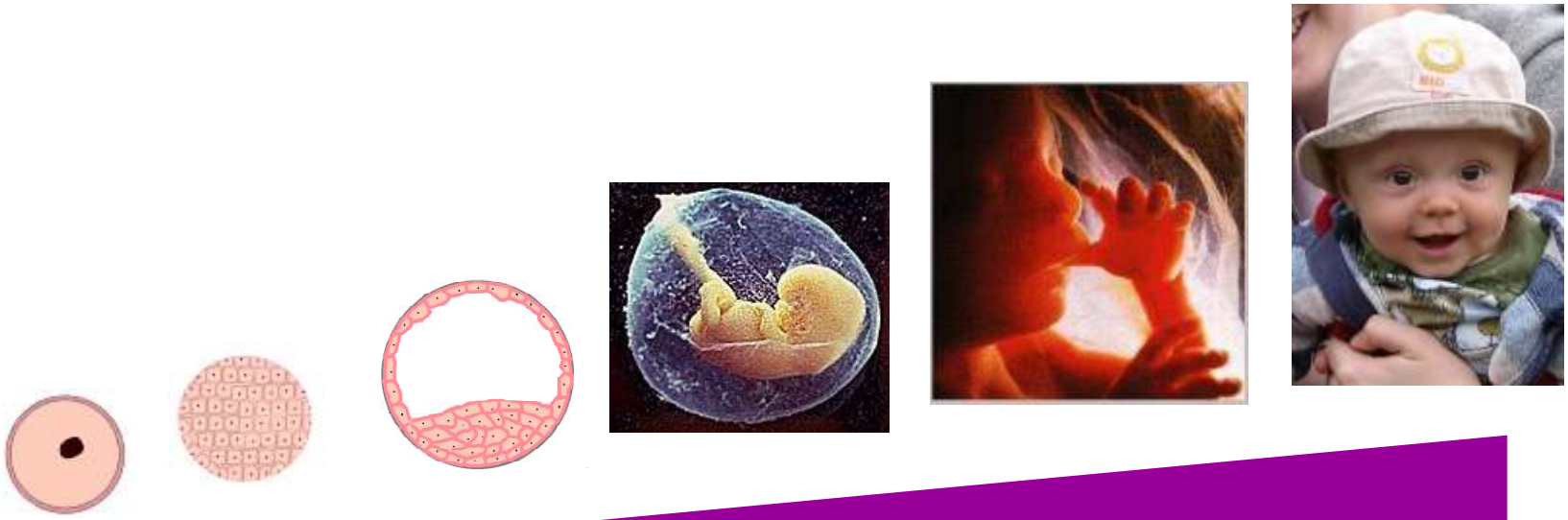
The Blastocyst

Outer cell layer ⇒ placenta

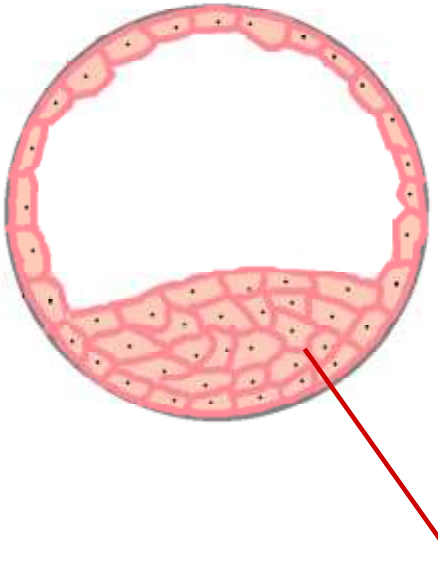


Inner mass ⇒ baby

And then...

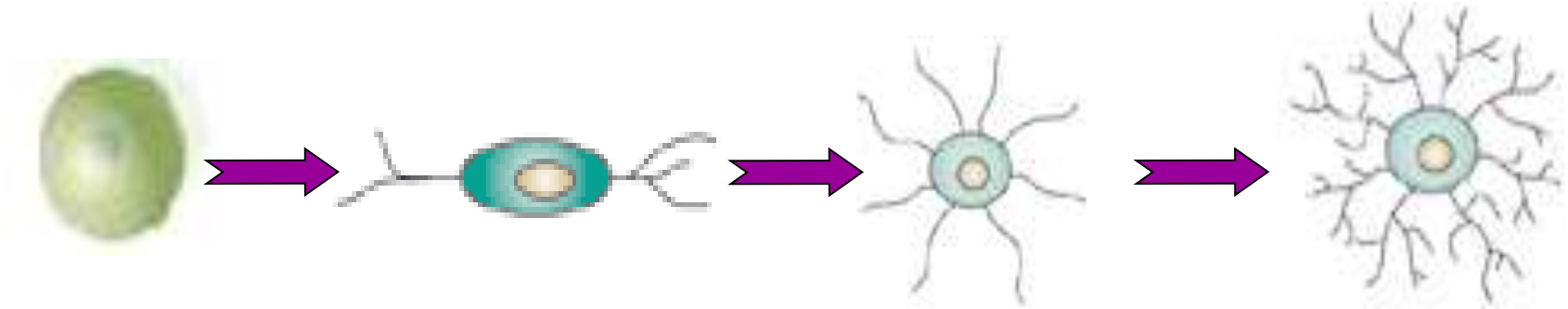


The Blastocyst



Inner mass ⇒ embryonic stem cells

Cell Development



Plastic

SPECIALIZED

- Totipotent stem cells -cells produced by the first few divisions of the cell . So can form any cell of the embryo as well as the placenta.

- Pluripotent – these cells differentiate into cells derived from the three germ cell layers.

- Eg: embryonic stem cell, embryonic germ cell and embryonic carcinoma cells.

- **Multipotent** – these cells can produce cells of a closely related family of cells.

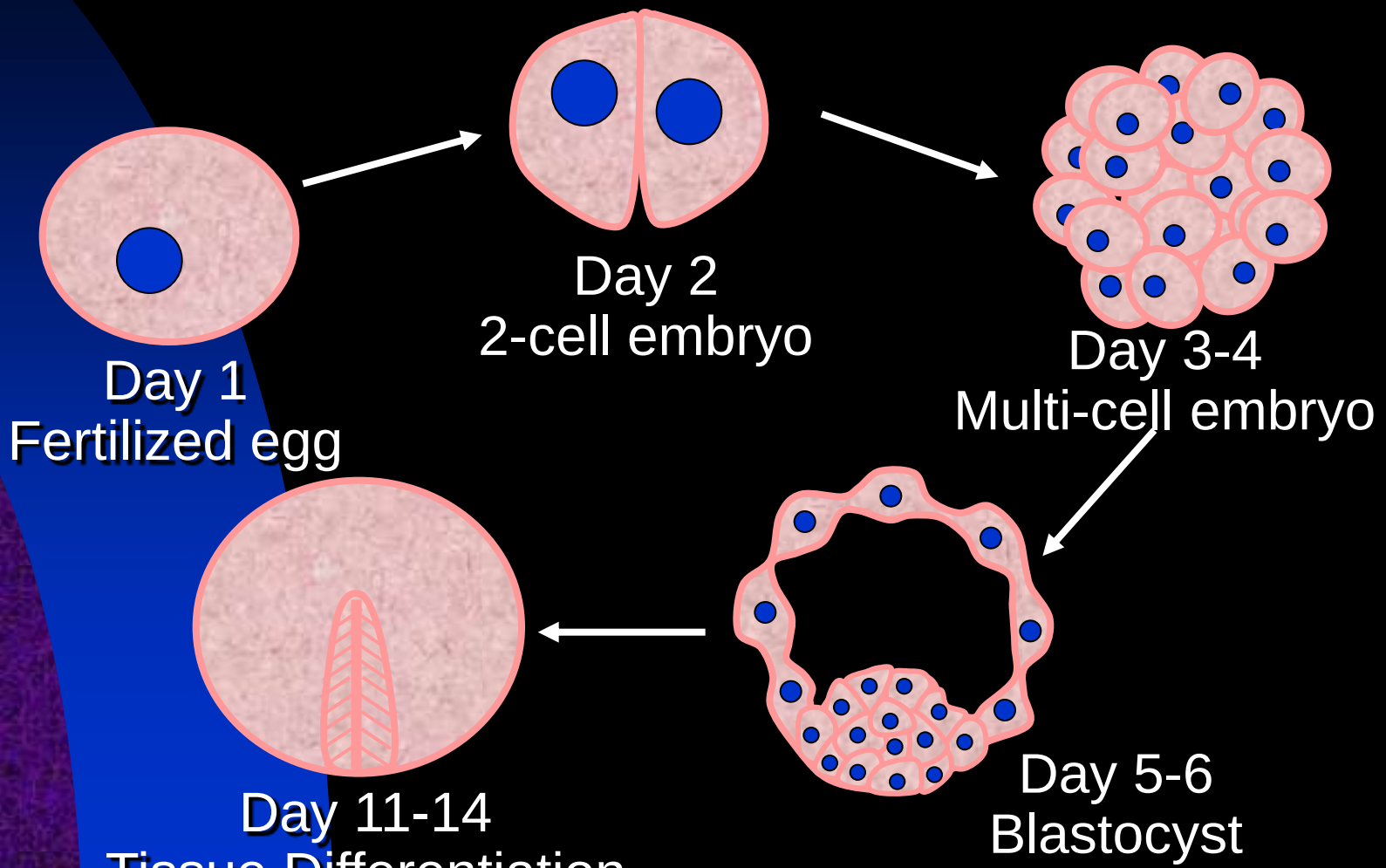
- Eg: haematopoeitic stem cells, neural and mesenchymal stem cells

- **Unipotent** – these cells only produce one cell type., but have the property of self renewal which distinguishes them from the non stem cells.

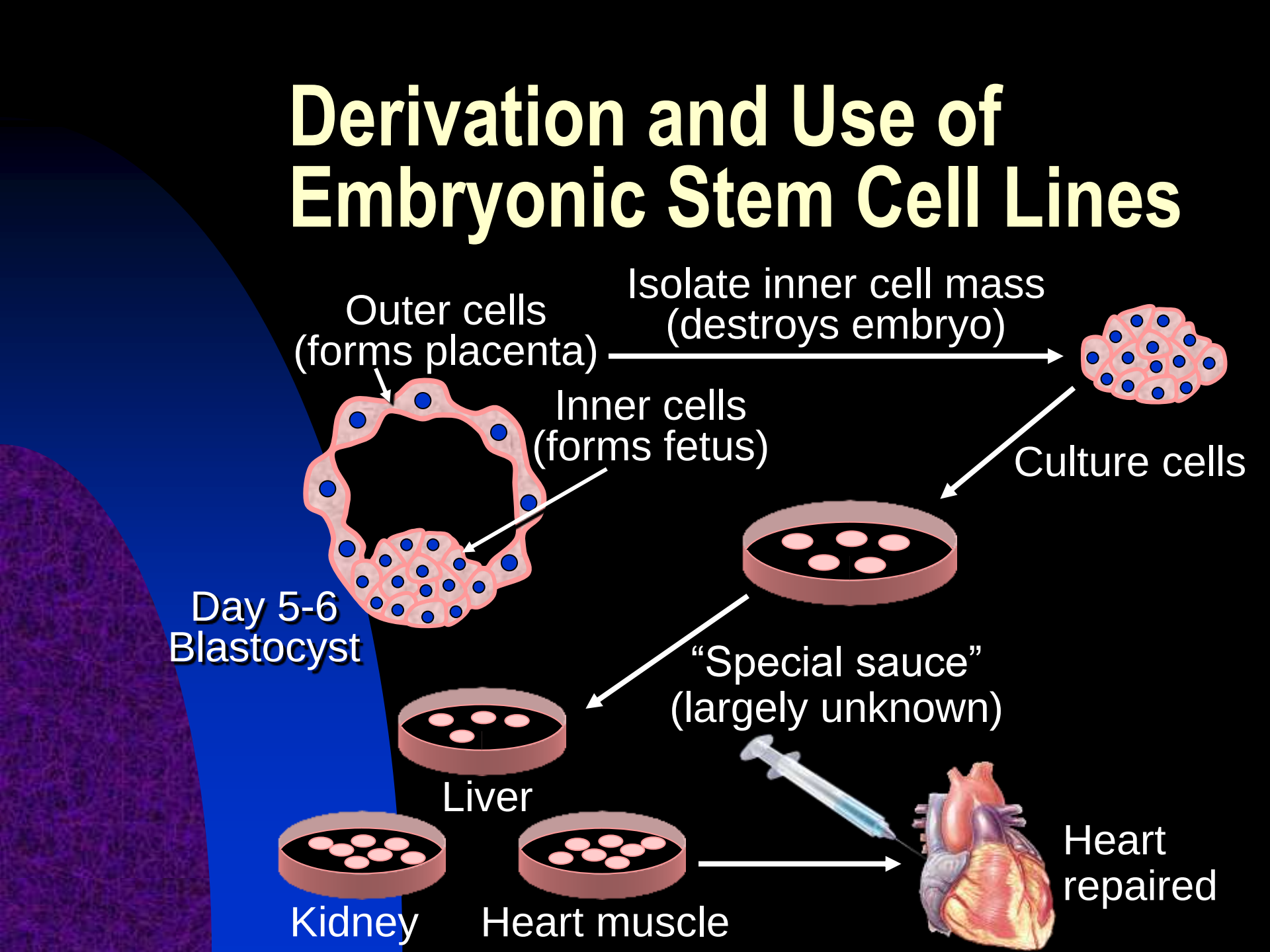
Kinds of Stem Cells

Stem cell type	Description	Examples
Totipotent	Each cell can develop into a new individual	Cells from early (1-3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5 to 14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and adult stem cells

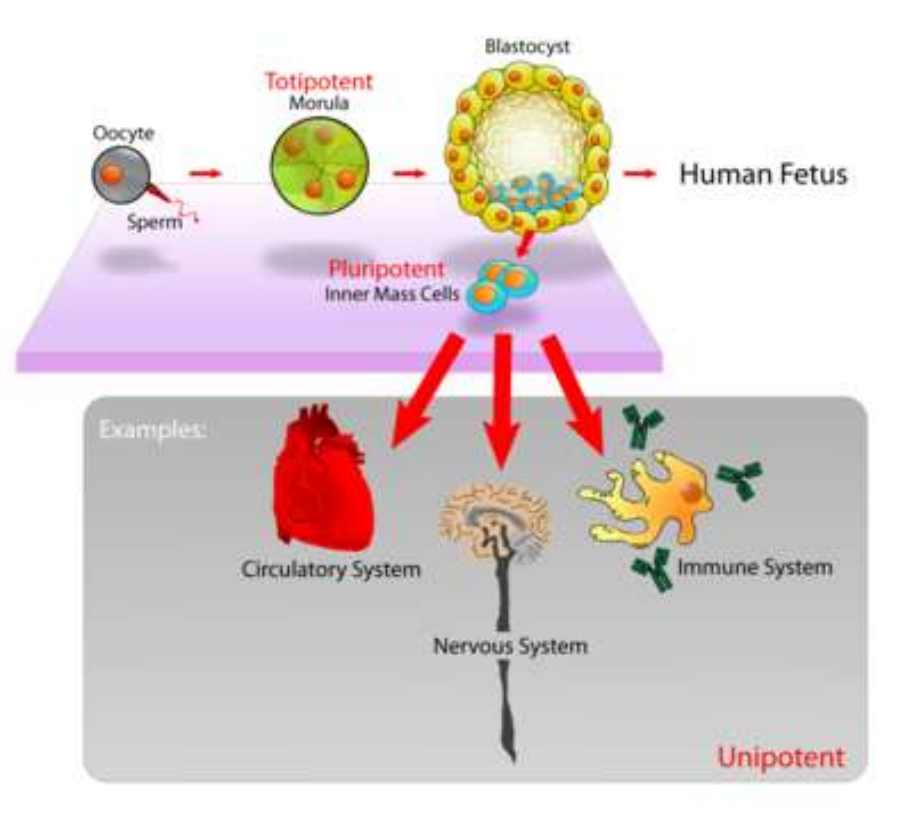
Stages of Embryogenesis



Derivation and Use of Embryonic Stem Cell Lines

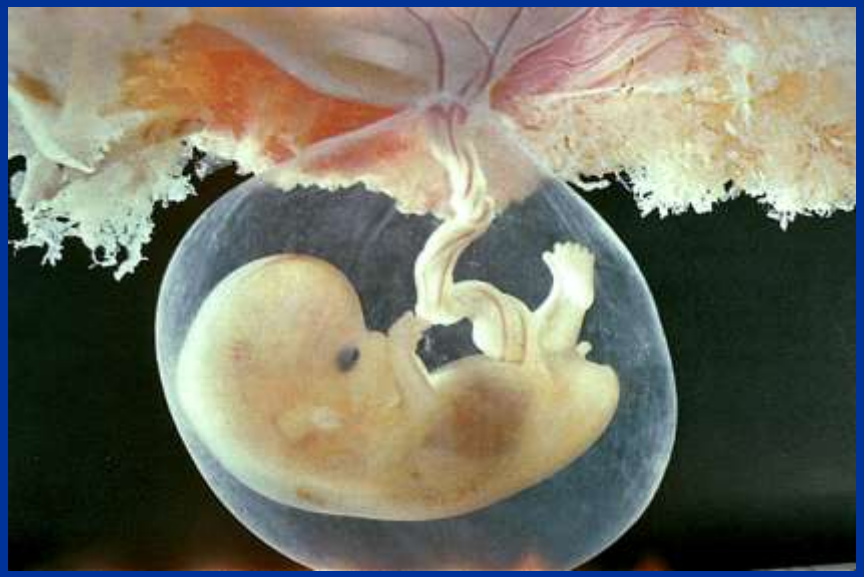


Cell Potency Types



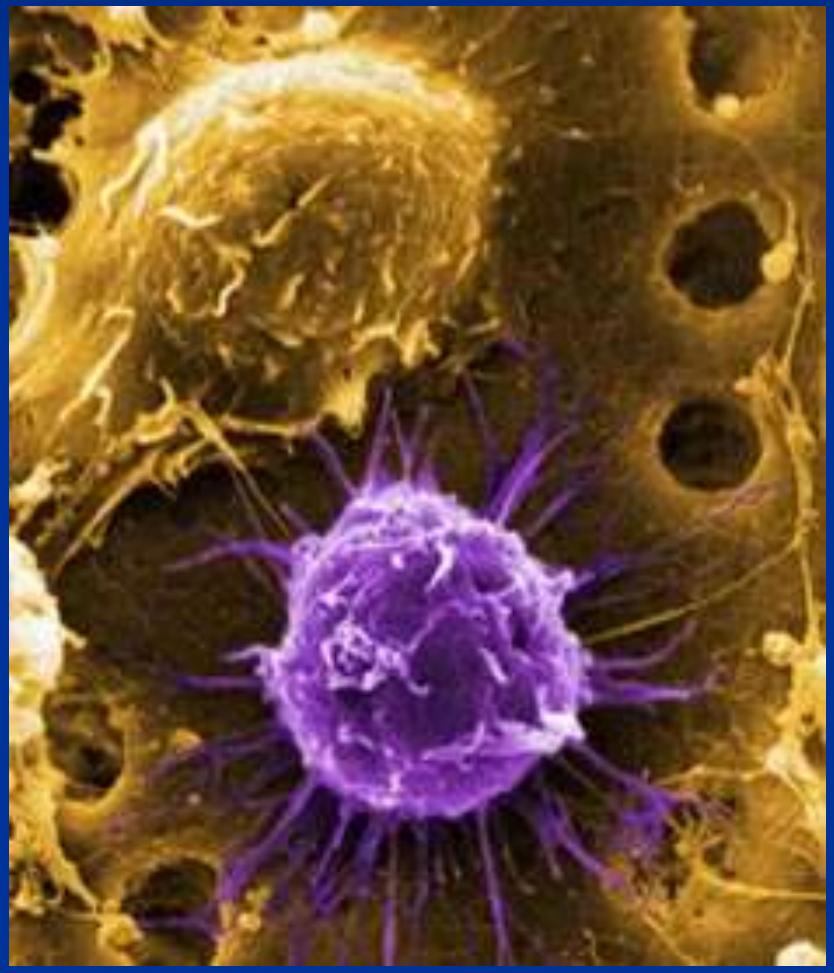
- Totipotent - fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent.
- Pluripotent stem cells are the descendants of totipotent cells and can differentiate into cells derived from any of the three germ layers. (embrionic stem cells)

Embryonic Stem Cells



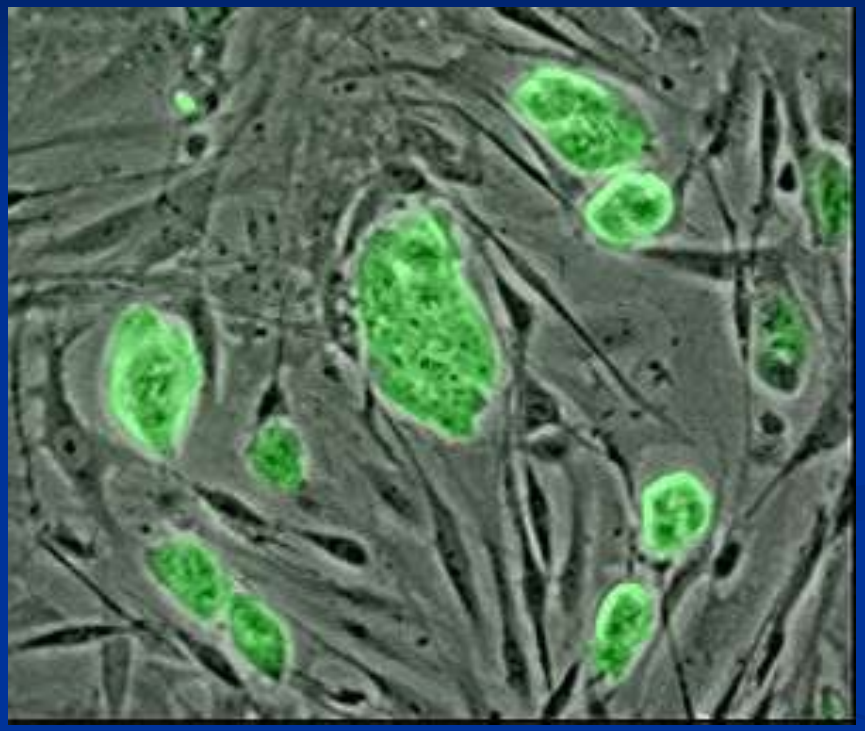
- Obtained when cultures of cells are taken from the epiblast tissue of the inner cell mass of a blastocyst or earlier stage embryos.
- A blastocyst is an early stage embryo approximately 4 to 5 days old in humans and consisting of 50–150 cells.

Embryonic Stem Cells Cont...



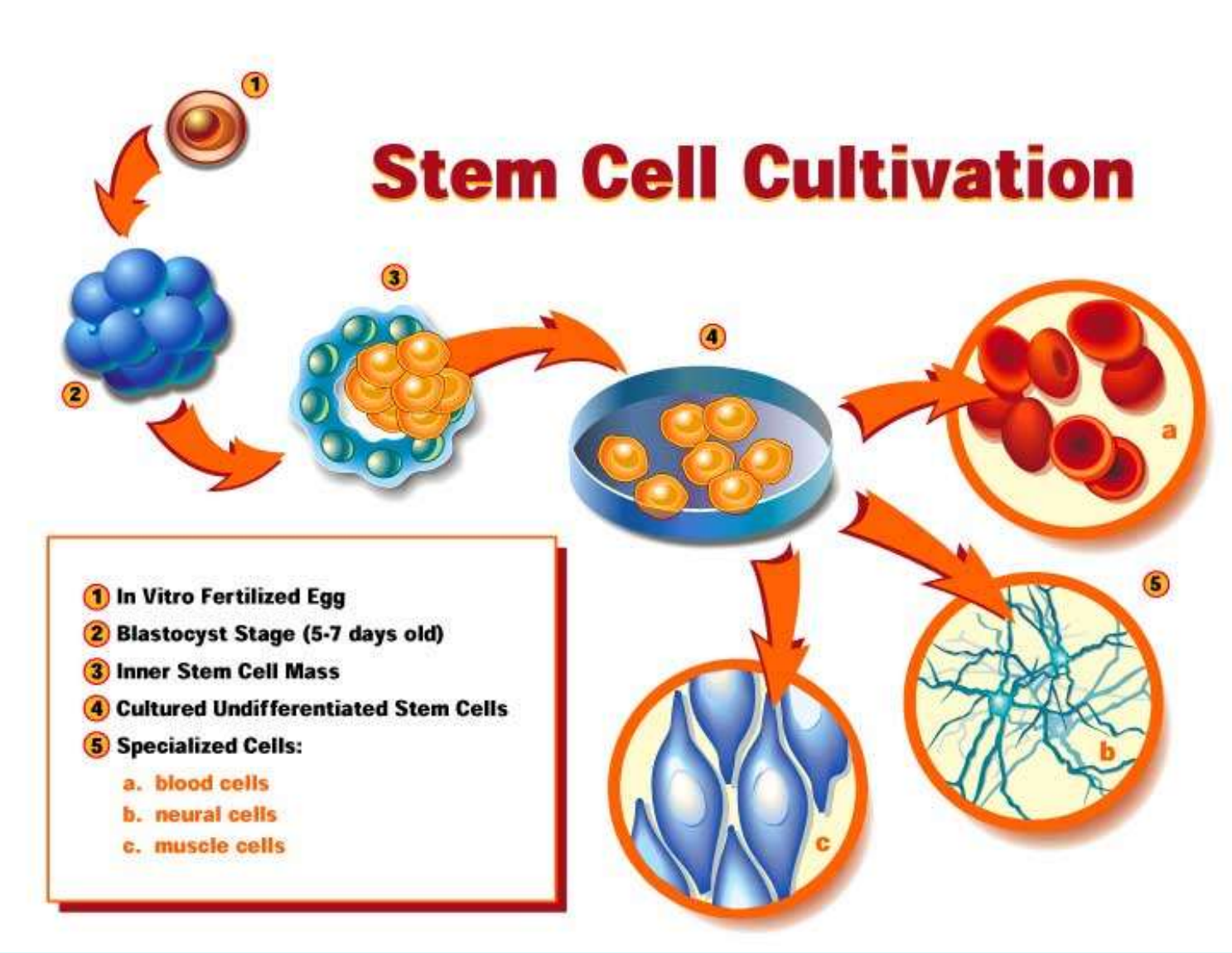
- Can be coaxed into developing all 220 types of cells found in the human body (e.g. blood cells, heart cells, brain cells, nerve cells, etc).
- Derived from human embryos in a process that causes the death of the embryos.

Mice Embryonic Stem Cells

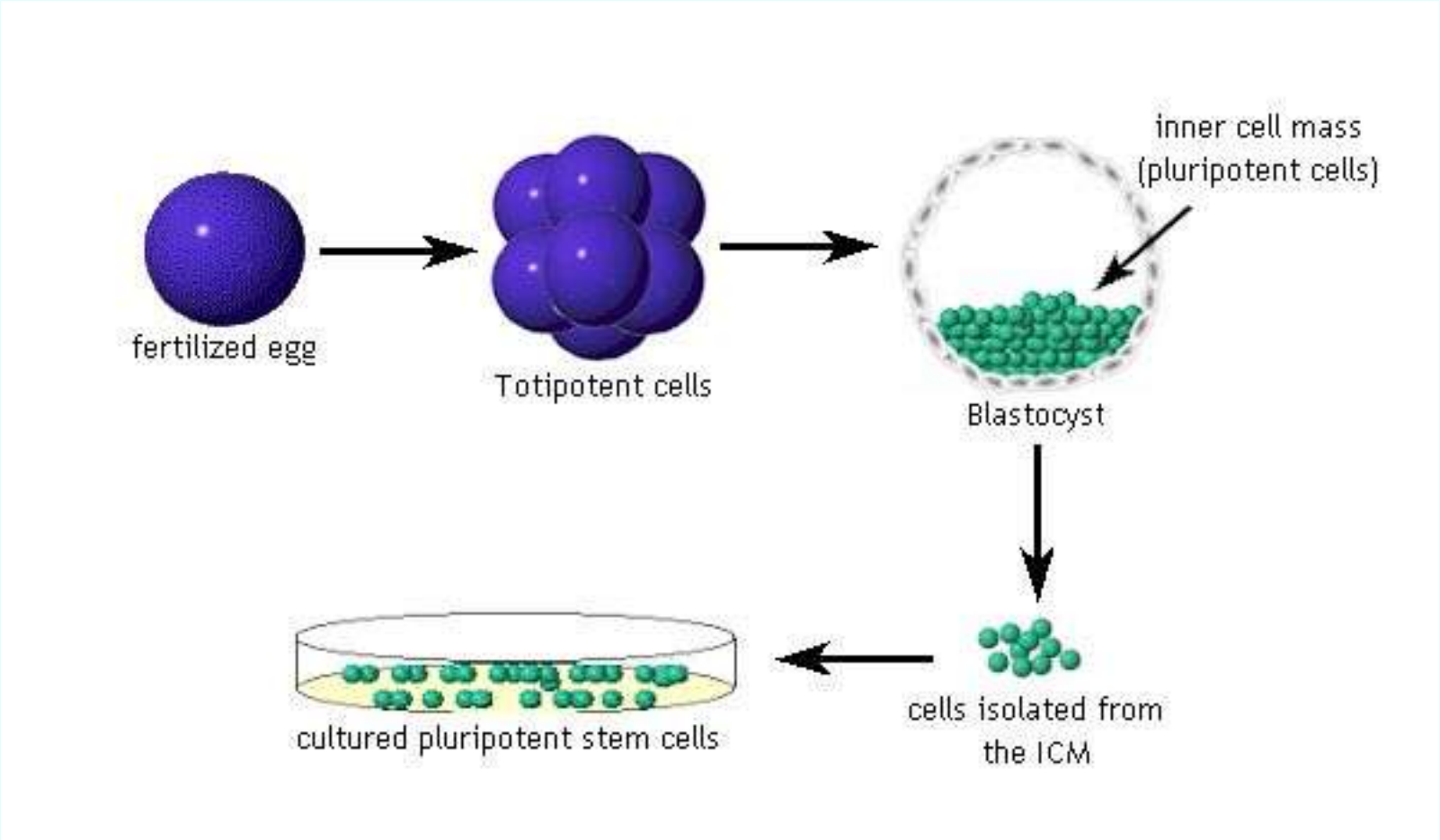


- Mouse embryonic stem cells with fluorescent marker.

Stem Cell Cultivation



Blastocyst Diagram

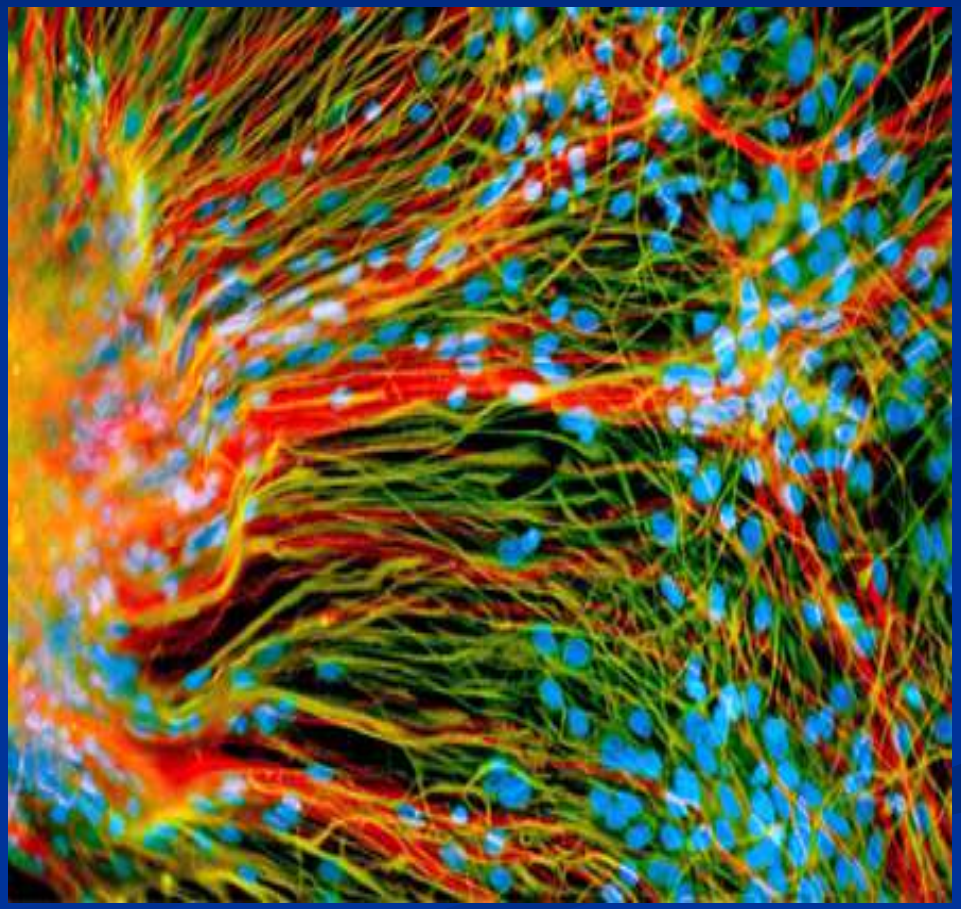


Five Day Pre-Embryo

http://www.nationalgeographic.com/ngm/

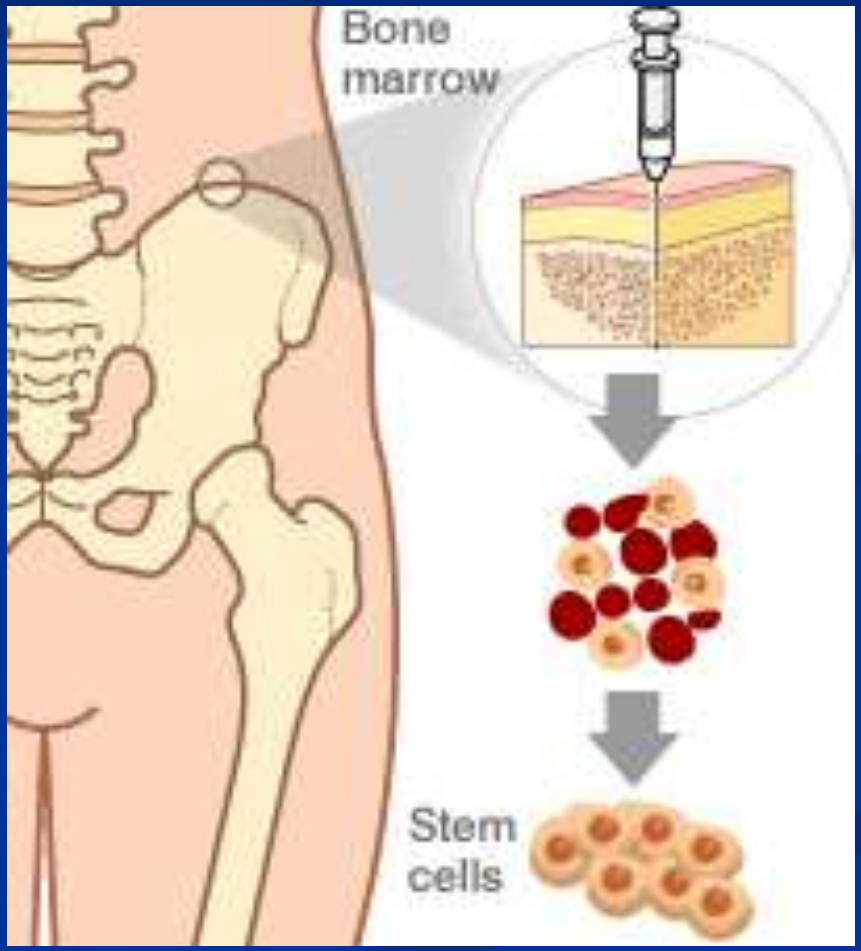
Adult Stem Cells

- Not as versatile for research purposes specific to certain cell types, such as blood, intestines, skin, and muscle.
- Extracted from an umbilical cord, or a child's or adult's body.
- The term "adult stem cell" may be misleading because both children and adults have them.



Adult Stem Cells Continued

- Difficult to extract, yet plentiful.
- Taken from the patient's own body, ensuring an exact DNA match so the body's immune system never rejects them.



Benefits of Stem Cell Research:

***Possible Treatments for:**

- Alzheimer's
- Parkinson's
- Spinal Cord injuries
- Heart Damage
- Muscle Damage
- Brain Damage
- Stroke Damage
- Sickle Cell Anemia
- Surface Wound Healing
- Jawbone Replacement
- Skull Replacement

More Benefits:

- Stem Cell Research may provide treatment to over 26 different types of Cancers including:
  - Lymphoma
  - Leukemia
  - Brain Cancer
  - Breast Cancer
  - Ovarian Cancer
  - Testicular Cancer
- Can provide treatment to illnesses ranging from Cardiovascular diseases to Bladder diseases.

Potential U.S. Patient Populations for Stem Cell-Based Therapies

Medical Condition	Number of Patients
Cardiovascular disease	58 million
Autoimmune disease	30 million
Diabetes	16 million
Osteoporosis	10 million
Cancer	8.2 million
Alzheimer's disease	5.5 million
Parkinson's disease	5.5 million
Burns (severe)	0.3 million
Spinal-cord injuries	0.25 million
Birth defects	0.15 million/year

Source: Derived from the National Academy of Sciences web site.

Characteristics Of Stem, Progenitor And Precursor Cells

	Differentiation potential	Proliferation potential	Examples
Stem cell	Pluripotential, can differentiate into tissues from multiple germ layers	Unlimited capacity for self renewal	ES cell Hemopoietic stem cell
Progenitor cell	Multipotential, can differentiate into several tissue or cell types from one germ layer	Intermediate capacity for self renewal	Intestinal crypt progenitor
Precursor cell	Lineage restricted; can differentiate into one type of tissue or cell	Limited capacity for self renewal	Erytroblast

Specific Pancreatic Progenitor Cells

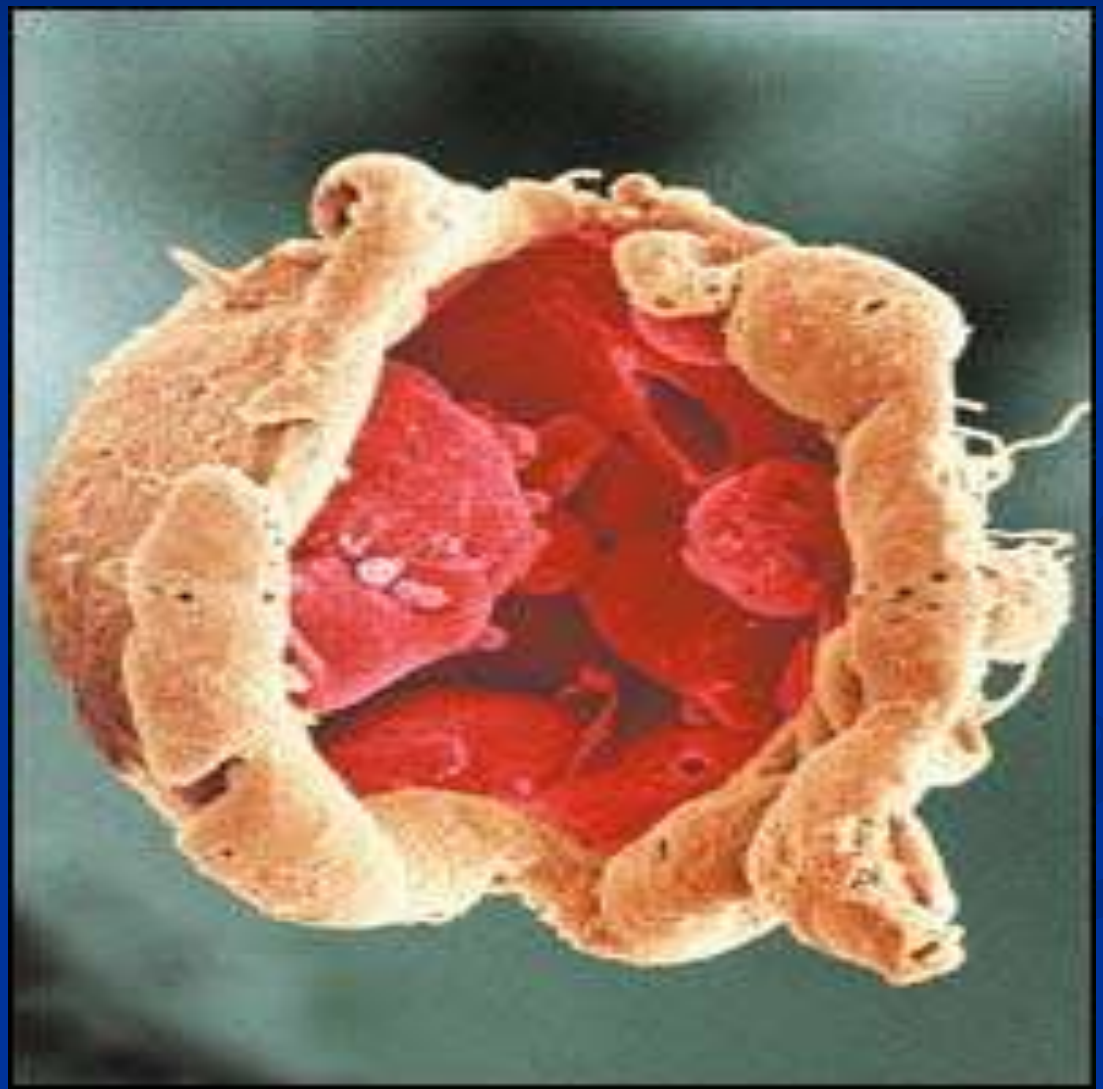
- The first specific progenitor cells for the pancreas are characterized by the expression of the transcription factor Pdx-1
- Pdx-1 is expressed early in development (embryonic day 8.5 in the mouse) and its expression is required for the initial pancreatic anlage to bud from the endodermal epithelium.

- The expression of neurogenin 3 (Ngn3), a basic helix-loop-helix transcription factor, at embryonic development day 9.5 defines the first definitive endocrine cell precursors
- From then on, a cascade of several transcription factors leads to the formation of all endocrine lineages of the islets of Langerhans

Embryonic Stem Cells

- ES cells are derived from the inner cell mass (ICM) of the embryonic blastocyst.
- Mouse ES cells were first isolated 20 years ago, while human and other primate ES cells were isolated more recently, in 1998.

The Blastocyst



- Because the establishment of ES cell lines *in vitro* involves the destruction of a potentially viable embryo, the use of human ES cells for such purposes is ethically controversial.

Selective ESC Differentiation

- The basic concept of stem cell biology is that an undifferentiated cell can be isolated at some stage of development, expanded along a differentiation pathway until the desired type of cell or tissue is achieved
- Embryonic stem cells (ESC) can be differentiated into insulin-producing cells by manipulating culture conditions

- Selection for nestin-expressing ESC in culture, can be stimulated to differentiate towards a ß-cell-like phenotype
- The use of pax4 or pdx-1 (transcription factors associated with ß-cell lineage) can yield promising results

Challenges For Utilizing ES Cells As A Source Of Beta Cells For Transplantation

- Unregulated differentiation into other cell types
- Teratoma formation from remaining undifferentiated cells have to be prevented after transplantation
- Maturity of the cells generated
- Safety (lack of tumorgenicity) of ES cell derived transplants

Fetal Stem Cells

- Fetal islet-like clusters (ILCs), obtained from both human and porcine pancreata, have been evaluated for their potential as a source of beta cells
- These ILCs contain a large proportion of undifferentiated progenitor cells that only differentiate into fully mature β-cells after transplantation

- The fetal human pancreas could become a valuable source of expandable beta cell progenitors in the future
- Similarly, attempts are under way to isolate a multipotential liver/pancreas stem cell from the fetal liver

Adult Stem Cells

- Attention is currently focused on 2 sets of cells within the pancreas that could be candidates for a therapeutic application as islet progenitors:

1. Cells of the pancreatic ducts
2. Nestin-positive islet-derived progenitor cells (NIPS)

1. Cells Of The Pancreatic Ducts

- The expansion of cells from a crude preparation of mouse pancreatic ducts derived from nonobese diabetic (NOD) mice generated insulin producing cells (ILCs)
- Upon transplantation into diabetic NOD mice, these ILCs significantly lowered the plasma glucose levels of the animals.
- However, the specific cells in the pancreatic ducts that are the progenitors and give rise to the insulin-producing cells remain to be identified and characterized

2. Nestin-positive Islet-derived Progenitor Cells (NIPS)

- Cells expressing the intermediate filament protein nestin, a marker of neural stem cells, can be isolated from human and rodent islets and expanded *in vitro*
- Insulin, glucagon and Pdx-1 expression, as well as low-level insulin secretion, can be detected in these cultures after the addition of differentiating cytokines and growth factors
- These cells also form ILCs *in vitro*, a process that is markedly enhanced by the addition of the insulinotropic, neogenic hormone glucagon-like peptide-1 (GLP-1)

Hepatic Oval Cells

- The close anatomical proximity of pancreas and liver development in the primitive foregut during embryogenesis has prompted attempts to isolate pancreatic progenitor cells from a subpopulation of cells in adult liver
- Recently, Yang et al (2002) reported the *in vitro* generation of ILCs from rat liver cell preparations enriched for hepatic oval cells

Adult Stem Cell Plasticity "Transdifferentiation"

- It was commonly accepted until recently that tissue development is a unidirectional pathway in which cells become increasingly restricted in their differentiation potential
- Several studies done both *in vitro* and *in vivo* suggest that transformation of one adult cell type into a completely unrelated tissue is possible

Multipotent Adult Progenitor Cells MAPCs

- The most striking results with regard to adult stem cell plasticity were recently presented in a cell population from human and rodent bone marrow (multipotent adult progenitor cells, MAPCs)
- MAPCs demonstrated an unlimited lifespan and differentiation potential reminiscent of embryonic stem cells
- Functional hepatocytes could be generated from MAPCs *in vitro*, and so, the derivation of pancreatic endocrine cells seems possible

Advantages of Adult Stem Cells

- The use of adult tissue-derived stem cells would be preferable over ES cells, not only for ethical reasons, but also because the tumorgenicity of adult cells appears to be far lower than that of ES cells.
- It might also be possible to harvest adult stem cells from patients for expansion *ex vivo* and then transplantation into the patients as isografts, thus avoiding the need for donor recruitment and immunosuppression.

Stem Cells Derived From Haemopoietic Organs

- Bone marrow harbors cells that can become parenchymal cells after entering the liver, intestine, skin, lung, skeletal muscle, heart muscle, and central nervous system, in rodent models and in human recipients of marrow transplantation.
- 1-2 months after bone-marrow transplantation, donor-derived cells are found in pancreatic islets of recipient mice
- These cells express insulin and genetic markers of ß cells

- In overtly diabetic mice whose ß cells have been destroyed by streptozotocin, bone-marrow transplantation normalized blood glucose and insulin concentrations

Bone-marrow Transplantation As A Therapeutic Approach For ß-cell Replacement

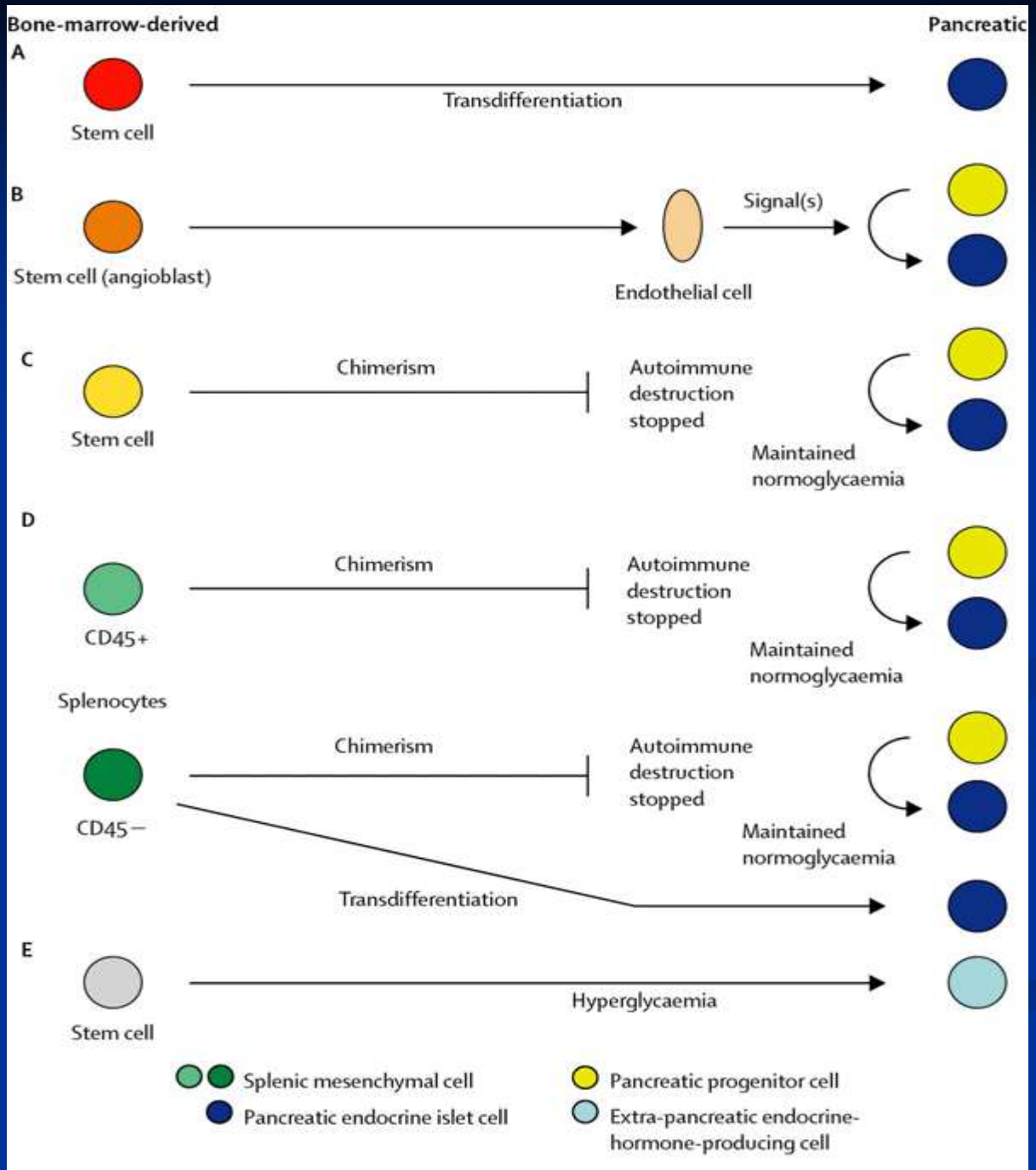
- Transplanted hemopoietic stem cells can transdifferentiate into pancreatic islet cells
- In islets, marrow-derived cells can differentiate into endothelial cells which stimulate the proliferation of local pancreatic progenitors to insulin-producing cells

Bone-marrow Transplantation As Immune Therapy For Type 1 DM

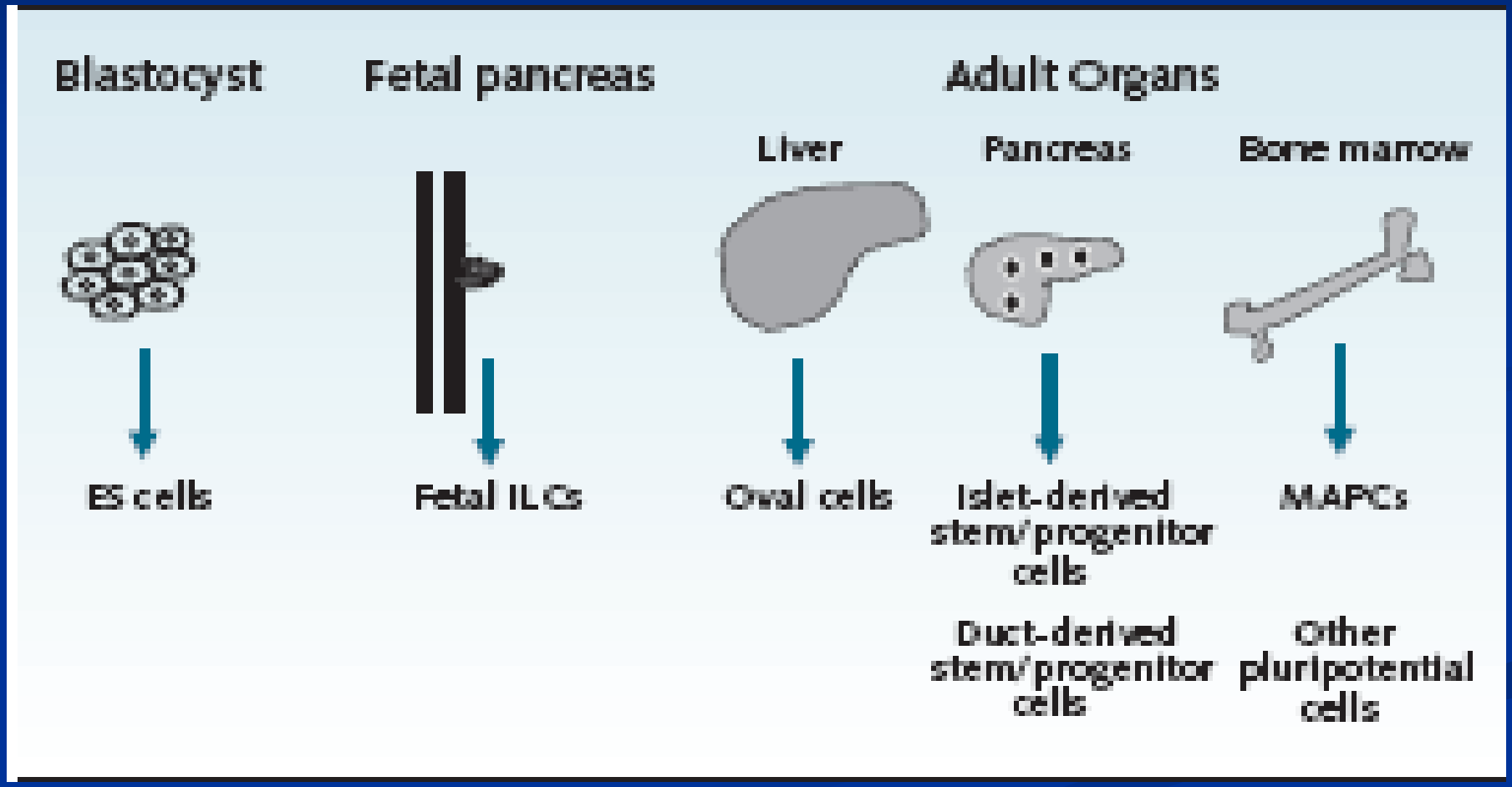
- Bone-marrow transplantation induces microchimerism
- In non-obese diabetic (NOD) mice, an autoimmune model of type 1 diabetes, transplanted with marrow before development of autoimmune diabetes, chimerism prevents diabetes mellitus
- Donor immunoregulatory cells may have prevented the host cells from becoming autoreactive against ß cells

Splenic Mesenchymal Cells

- Transplantation of mesenchymal cells from the spleen combined with complete Freund's adjuvant led to reversal of diabetes accompanied by regeneration of insulin-producing islets
- The transplanted splenic mesenchymal cells differentiate into ß cells
- Thus splenic mesenchymal cells transplanted under certain conditions seem not only to keep immune destruction of islets in check, but also can transdifferentiate into pancreatic ß cells


Bone-marrow-derived
Pancreatic
A
Transdifferentiation
Stem cell
B
Signal(s)
Stem cell (angioblast)
Endothelial cell
C
Chimerism
Autoimmune destruction stopped
Stem cell
Maintained normoglycaemia
D
Chimerism
Autoimmune destruction stopped
CD45+
Maintained normoglycaemia
Splenocytes
Chimerism
Autoimmune destruction stopped
CD45−
Maintained normoglycaemia
Transdifferentiation
E
Hyperglycaemia
Stem cell
Splenic mesenchymal cell
Pancreatic endocrine islet cell
Pancreatic progenitor cell
Extra-pancreatic endocrine-hormone-producing cell

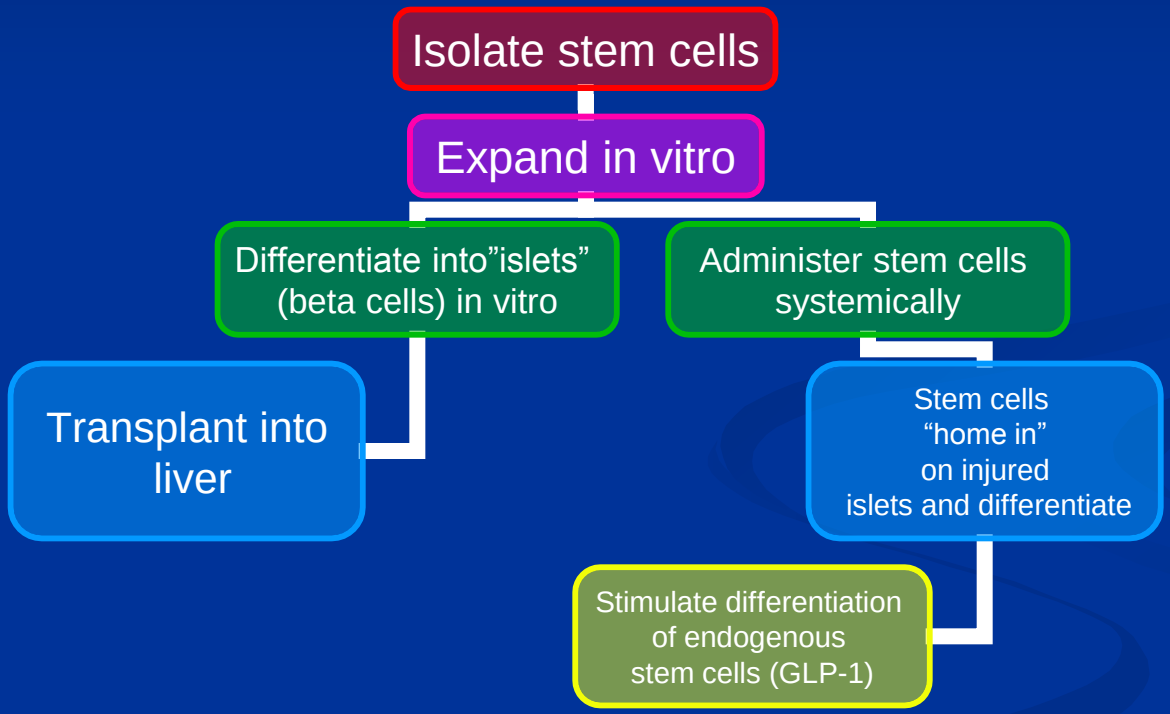
Potential Stem/Progenitor Cells For The Treatment Of Diabetes



Approaches For The Use Of Stem Cells In The Treatment Of Diabetes

- Stem cells isolated from embryonic or fetal tissues and adult organs are expanded *in vitro*.
- They may then either be differentiated *in vitro* into glucose-responsive insulin-producing “islets” for transplantation into the liver of diabetic individuals or
- Administered into the circulation of diabetic patients where they “home in” on injured islets and differentiate into insulin-producing cells.
- Another approach is to administer “stem cell stimulators” such as drugs, growth factors, or hormones (GLP-1) to stimulate endogenous stem cells to differentiate into insulin-producing cells.

Different Approaches For The Use Of Stem Cells In The Treatment Of Diabetes



Conclusions

- Cellular replacement therapy may offer the best approach to achieve physiologic glucose control in diabetic patients
- The expansion and subsequent differentiation of stem cells, be they of embryonic, fetal, or adult origin, appear to have considerable potential to overcome the shortage of donor organs

Thank You