

Stem Cells and The Endometrium

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Nothing to disclose

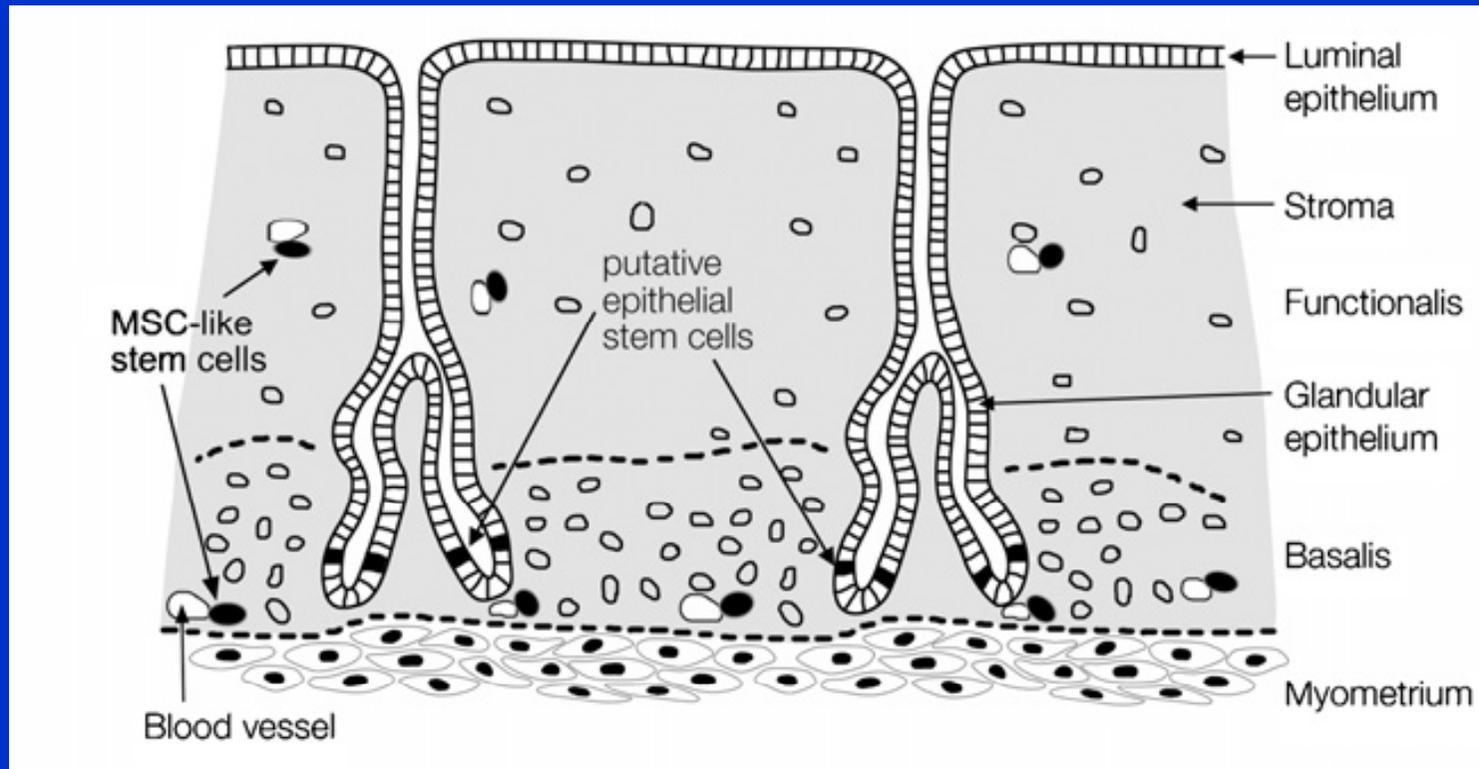
Stem Cells

Cells that are capable of both self-renewal and have broad potential to differentiate into multiple adult cell types. These cells may be derived from the inner cell mass or may be found in the adult. Bone marrow is one source of multipotent adult stem cells.

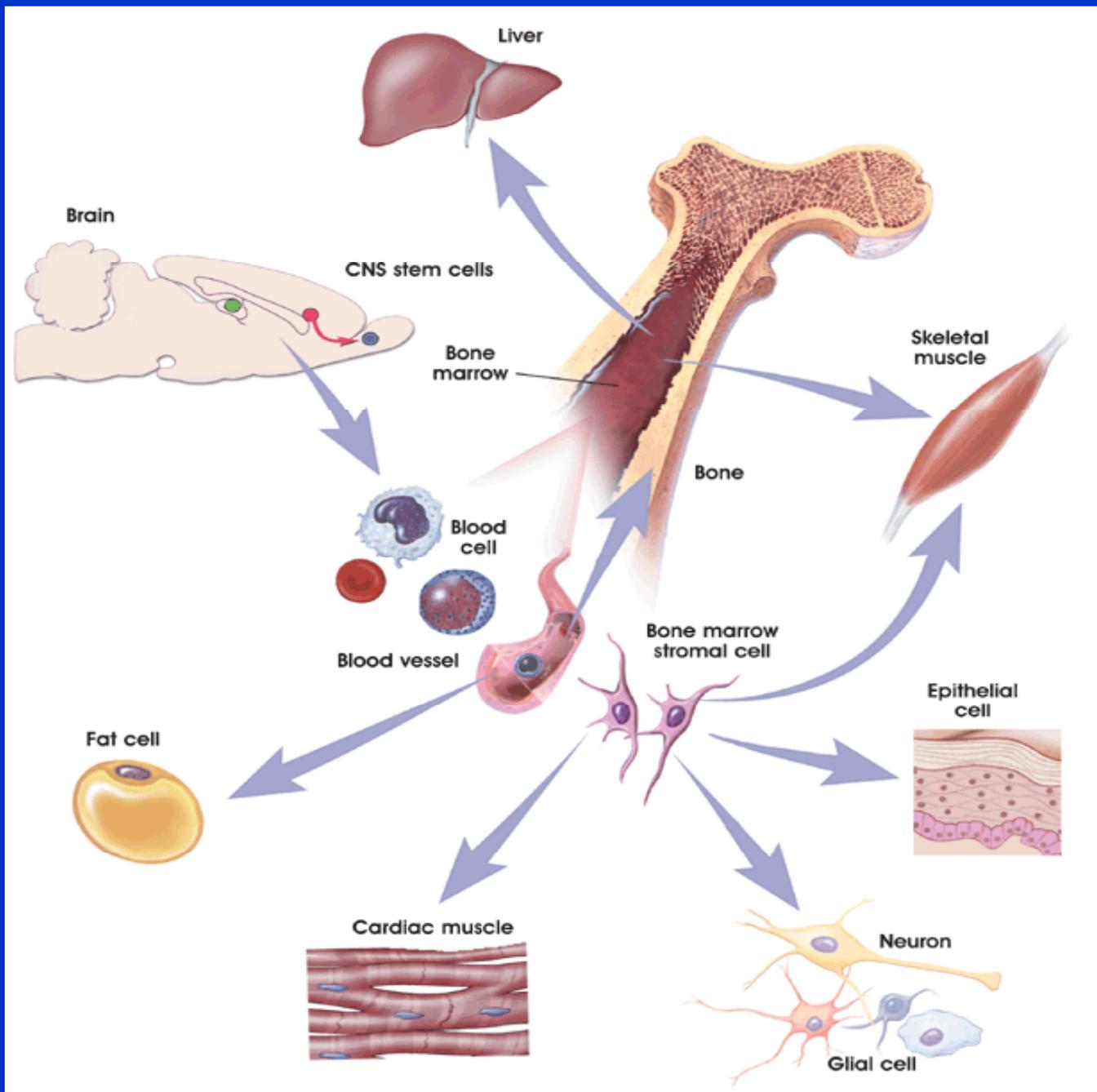
Endometrium

- The endometrium, is known for its remarkable regenerative capacity as it undergoes dynamic changes each menstrual cycle.
- Need for stem cells to replenish the endometrium.

Endometrial Progenitor Stem Cells



- In the last 20 years , multipotent stem cells have been isolated multiple tissues.
- They are few in number. About 1 stem cell found in 100,000 cells circulating in the blood.
- Furthermore, they resemble the other cells that surround them.



Can Bone Marrow Derived
Cells
Differentiate Into
Endometrium?

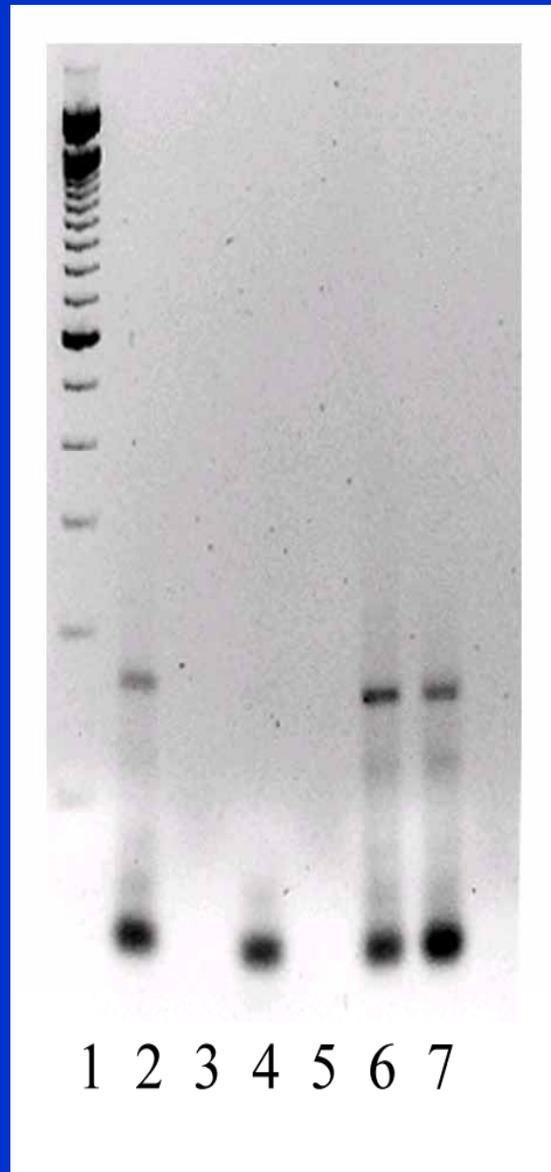
Subjects

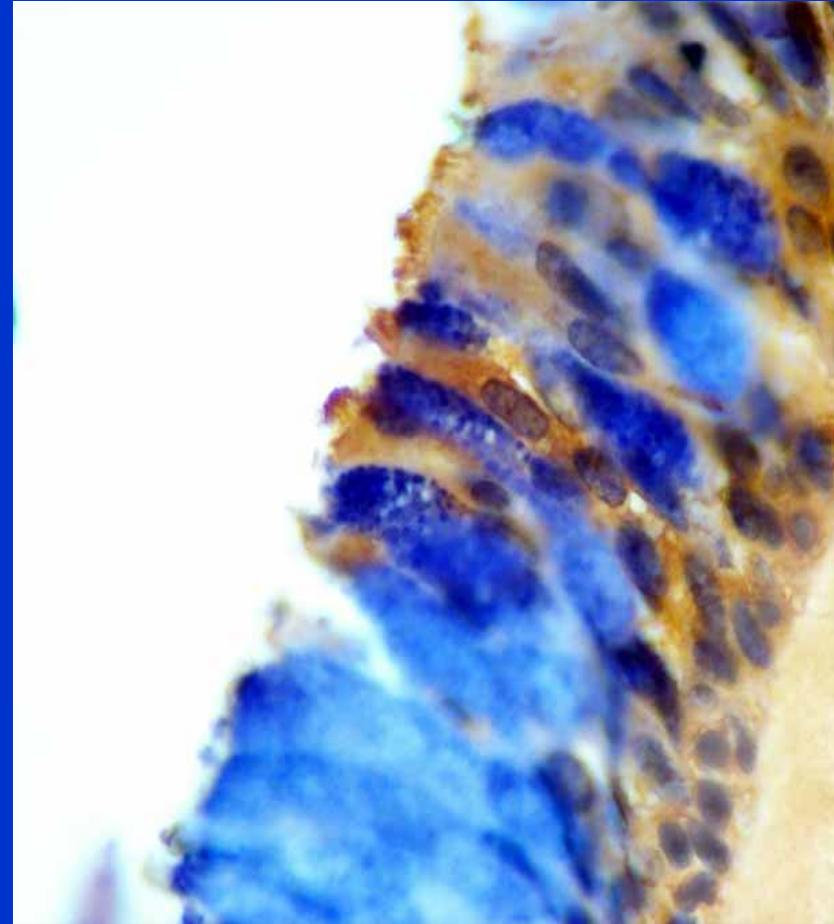
- Four bone marrow transplant recipients
- HLA type that allowed determination of the origin any cell
- Age 28-43
- Rx Chemotherapy and TBI

Methods

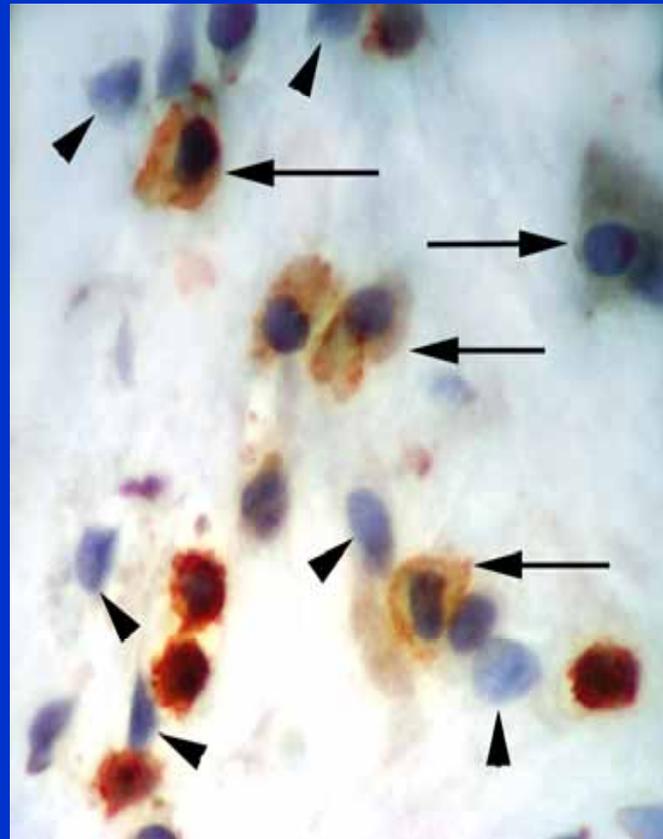
- Endometrial biopsy
- RT-PCR for HLA
- Immunohistochemistry for HLA and CD45

RT-PCR
amplifying
HLA A11

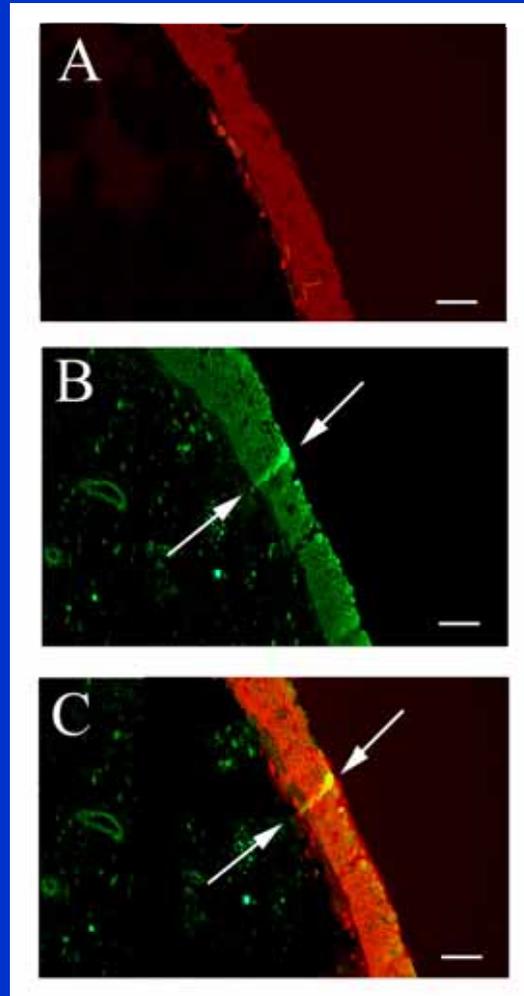




Serial IHC for HLA and CD45



Marker of Differentiation



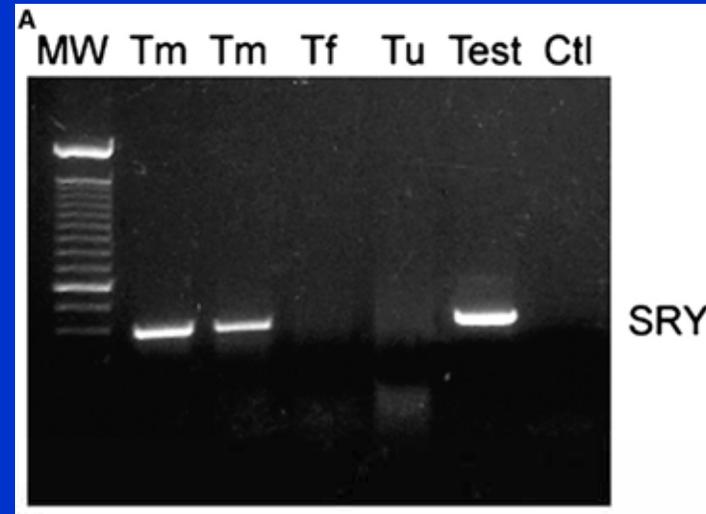
Calcitonin

HLA

Merge

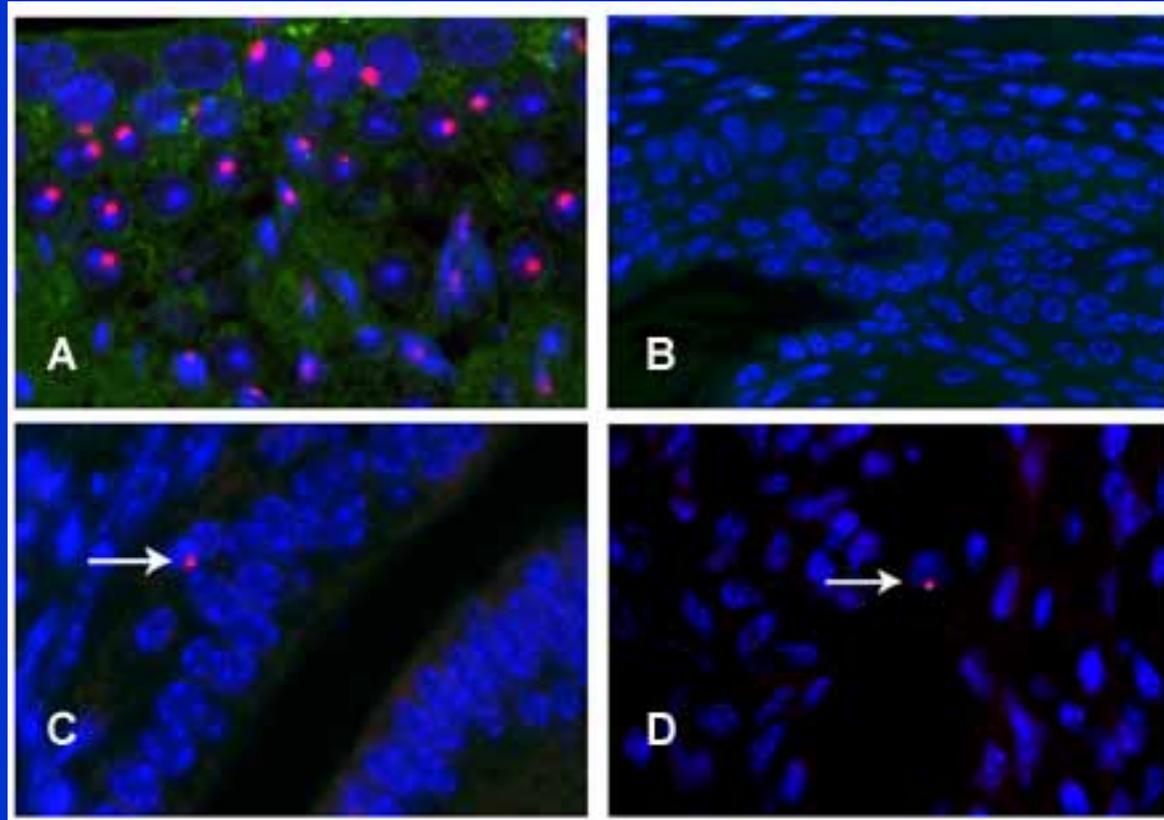
Do Stem Cells Contribute
to Endometrium in
a murine model?

Identification of bone marrow-derived cells in murine-endometrium



Transplant of Male bone marrow into female mice. Assessment of SRY gene expression and Y chromosome by FISH

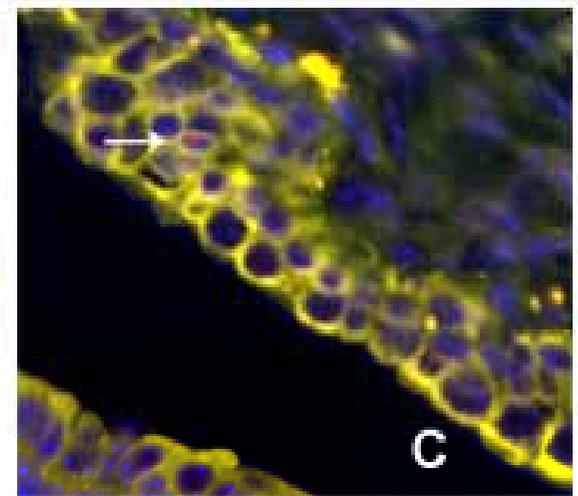
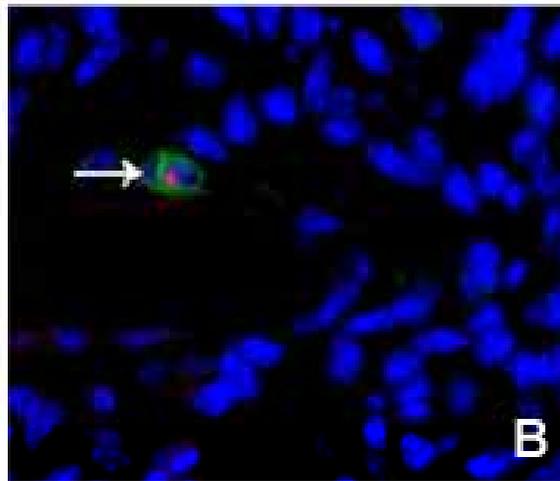
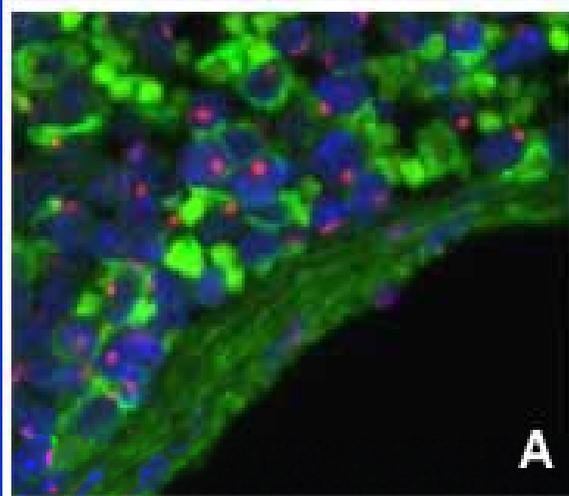
Stem cell origin of endometrium in mouse



Male

CD45

Cytokeritin



Stem Cells and Disease

Can Stem Cells Contribute
to Endometriosis ?

Support of Sampson's theory

- Dependent distribution
- Common occurrence of retrograde menstruation
- High incidence with outflow tract obstruction
- Tubal patency common
- Risk factors that include frequent menstruation and early menarchy.
- Animal models involving peritoneal transplant

Sampson's Theory does not explain the presence of endometriosis outside of the peritoneal cavity or in men.

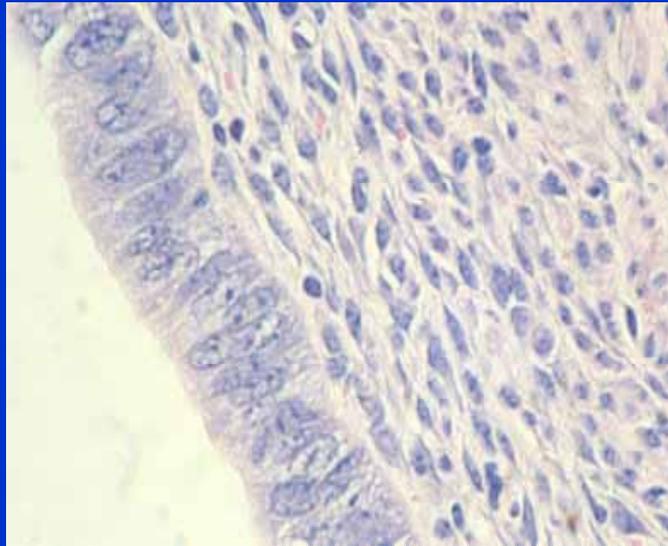
A Novel Origin of Endometriosis

- Do stem cells contribute to murine endometriosis?

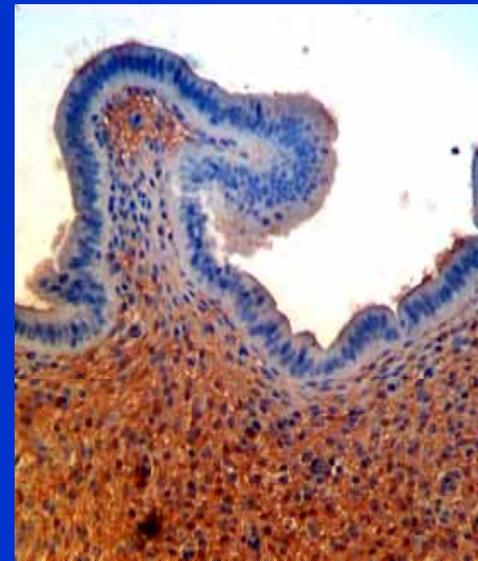
Methods

- Wild Type and LacZ transgenic mice
- Hysterectomy
- Uterine transplant to ectopic location
- Beta-Galactosidase activity and expression

IHC using anti Beta-galacotosidase antibody

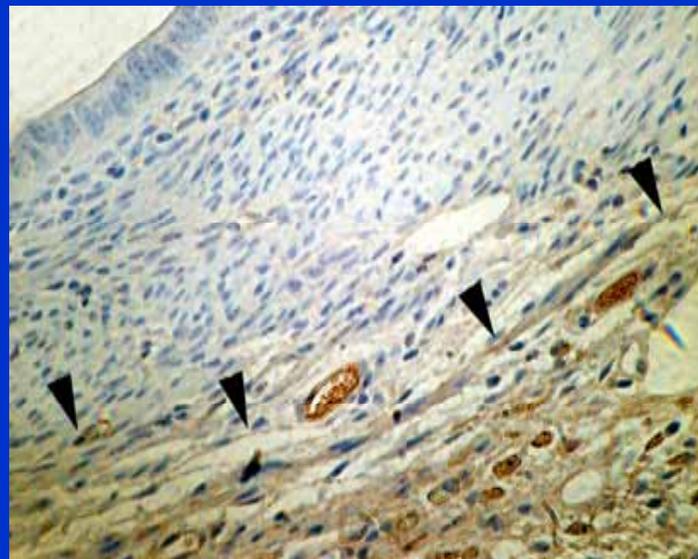


Wt control



LacZ transgenic

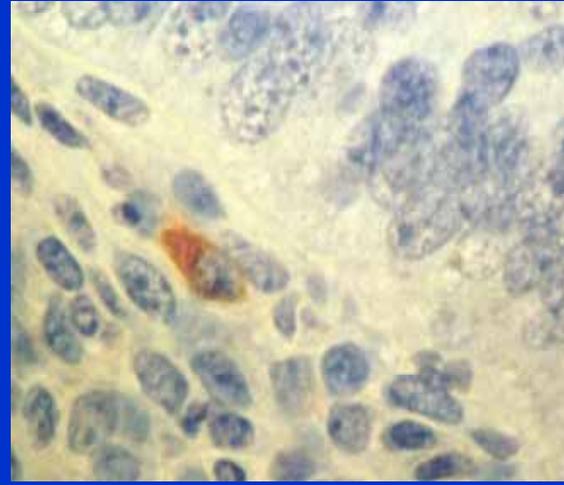
Wt transplanted
to LacZ transgenic



Du, H. and Taylor H.
Stem Cells 2007;25:2082-2086

IHC using anti Beta-galactosidase

Stromal Cells

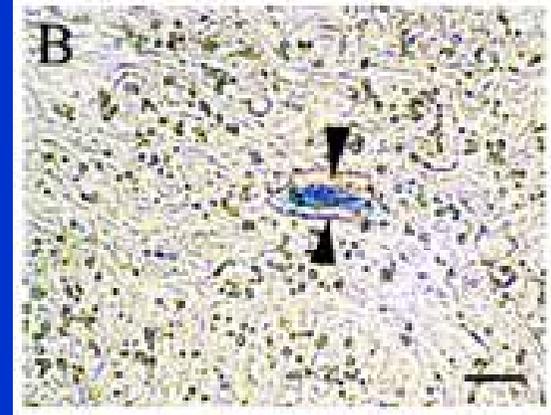


X-GAL staining of Beta-Galactosidase activity

Glandular cell



Stromal Cell



- Novel mechanism of disease-
Ectopic transdifferentiation of stem cells.

Bone Marrow and Endometrial Stem Cells

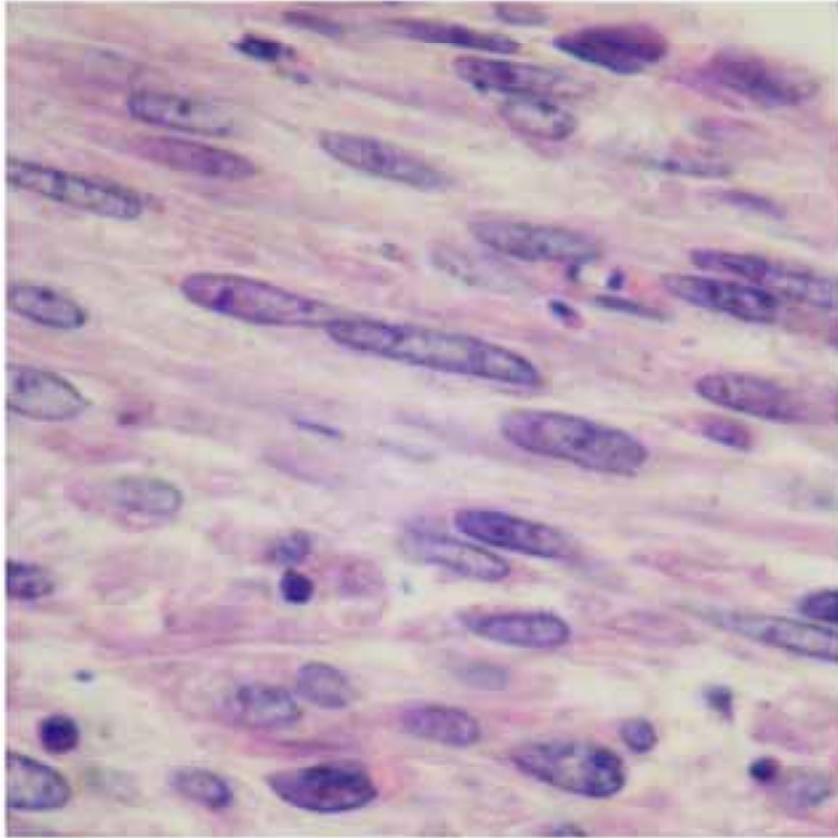
- Potential for endometrial repair after disease.
- Explains failure of ablative therapies for abnormal bleeding.
- Novel mechanism of disease-
Ectopic transdifferentiation of stem cells.

Does the Endometrium
Contain Stem Cells that can
Differentiate into Non-
endometrial Cell Types?

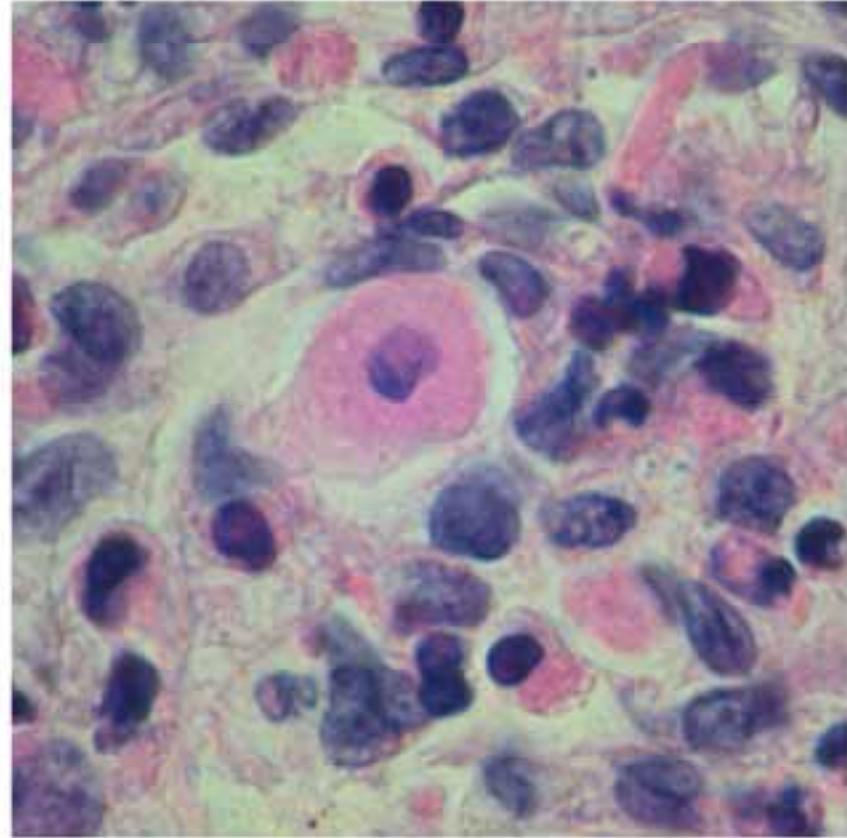
Methods

- Reproductive aged women
- Endometrial biopsy
- Endometrial stromal cell culture
- Differentiation protocols

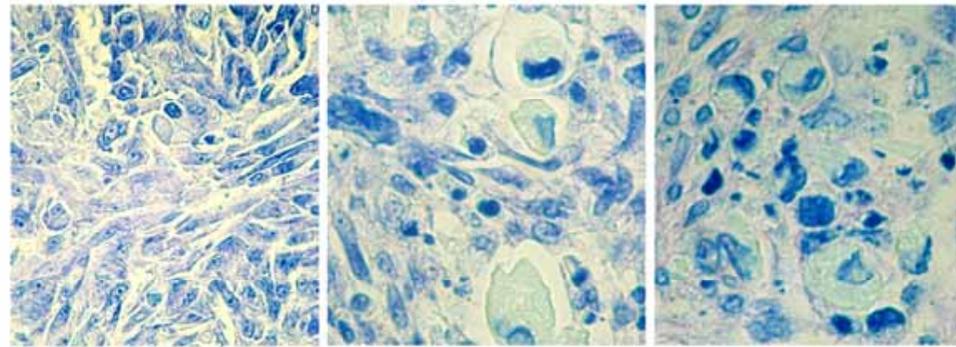
Chondrocytes



Control



CM



3

14

21

Days

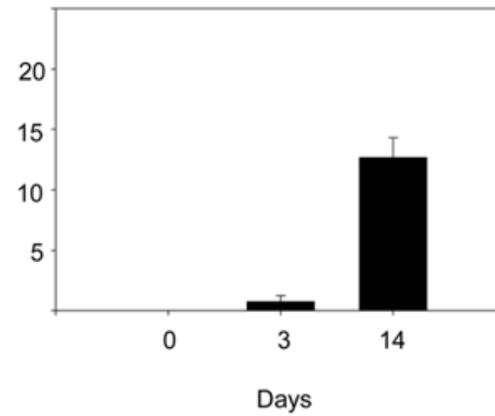
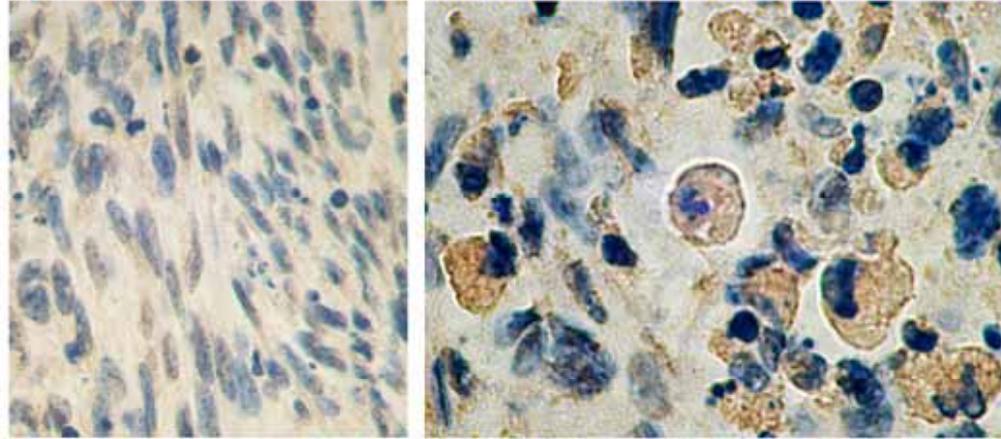


Fig 2

Type II Collagen

A

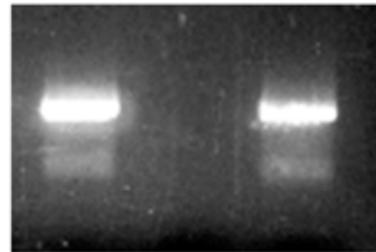


Control

CM

B

CAR CTL CM

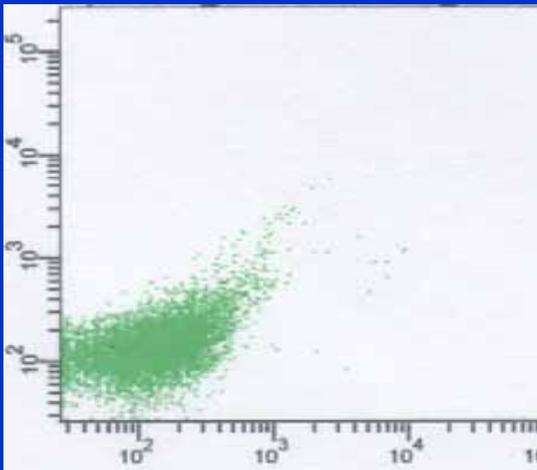


Collagen II

Neurogenic Differentiation

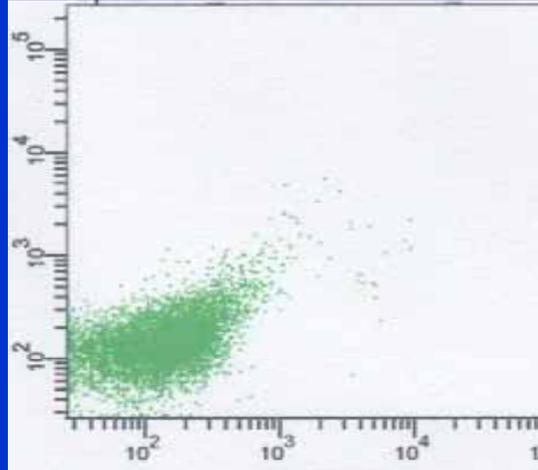
Flow cytometry: Human Endometrial Derived Stem Cells

CD45⁺(0.3%)



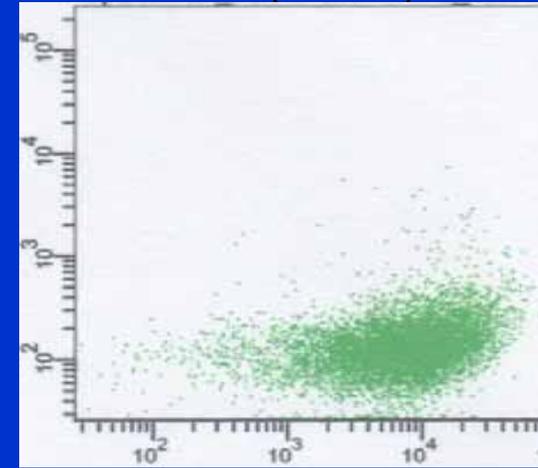
Leukocyte

CD31⁺(1.4%)



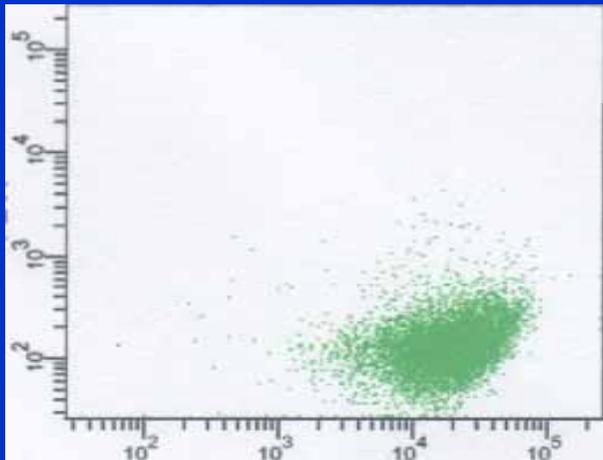
Endothelial cell

CD90⁺(99.6%)

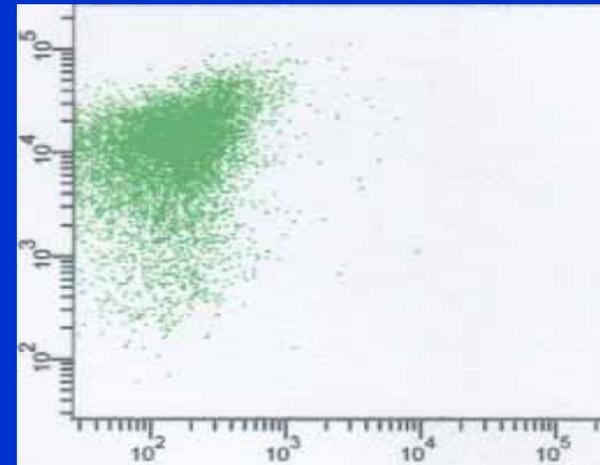


Stromal cell

PDGFR β ⁺(99.7%)

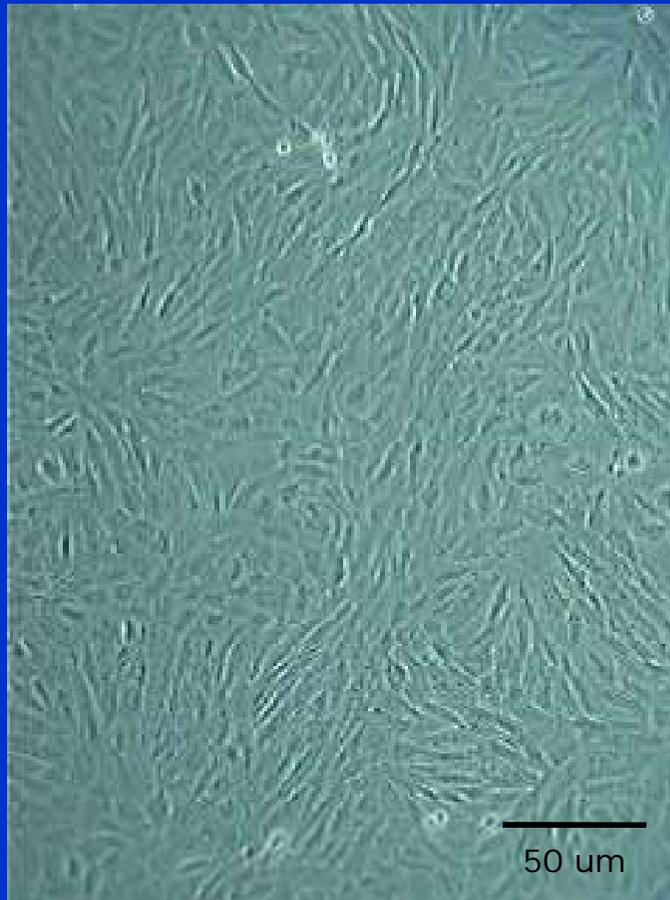


CD146⁺(99.7%)



in vitro

- Control Endometrial Stromal Cells



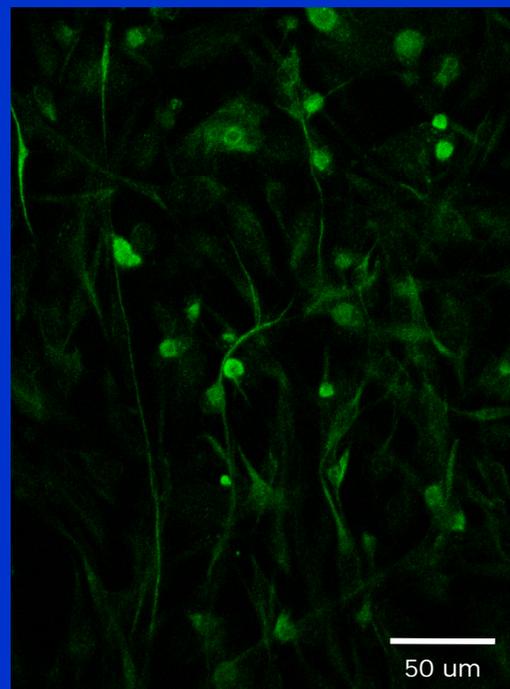
- Neurogenic Differentiated



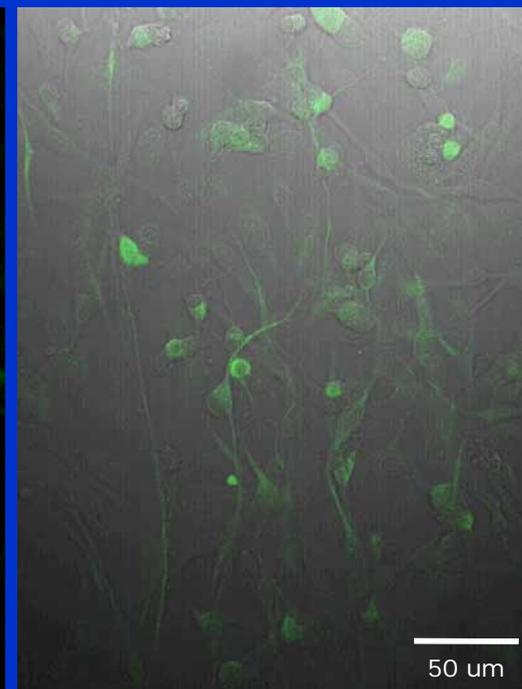
in vitro Nestin Expression



DIC

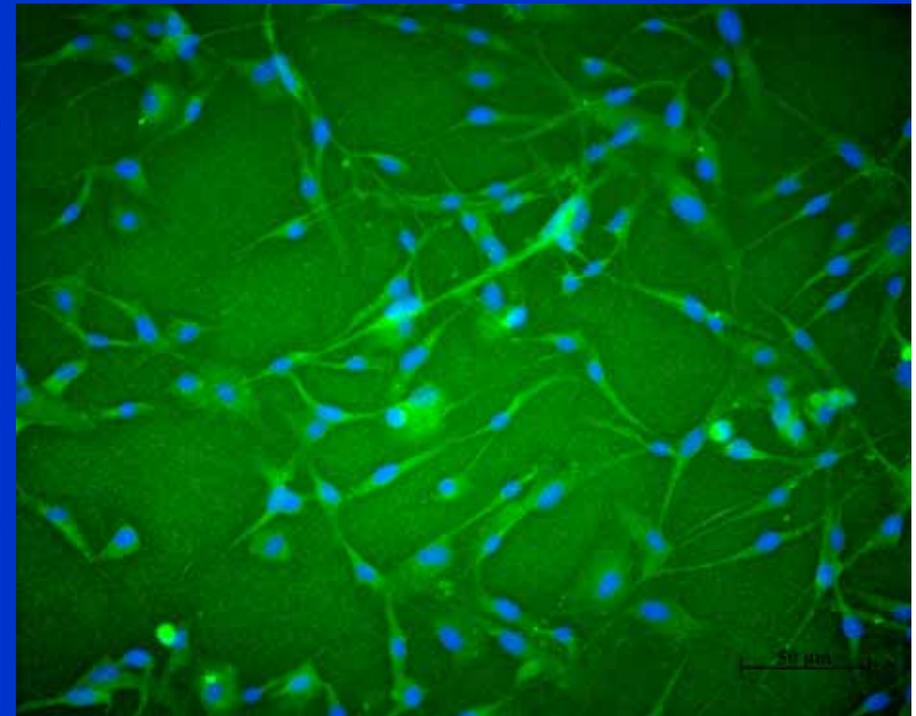
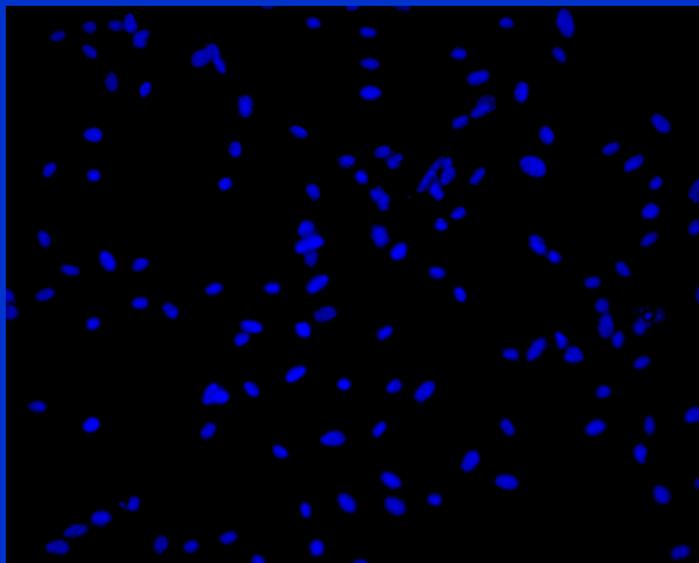
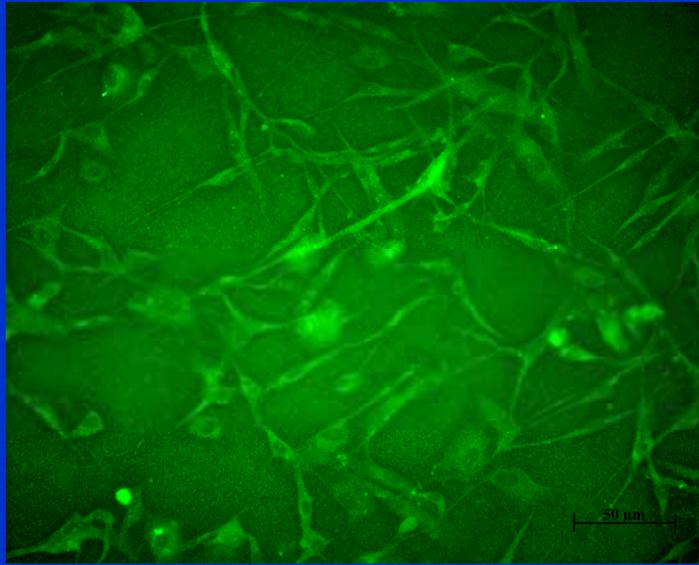


Immunofluorescence



Merge

in vitro Tyrosine Hydroxylase Expression

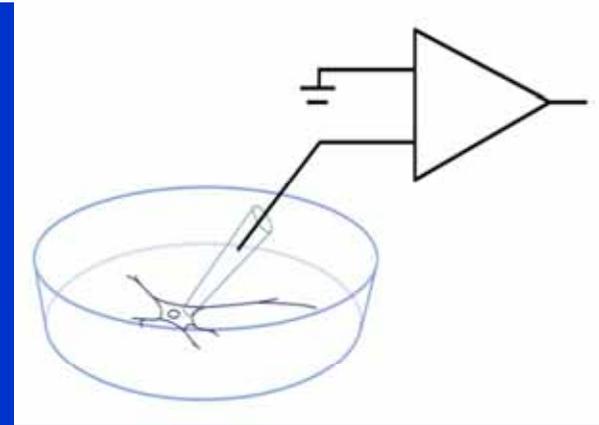


Merge

in vitro Dopamine Synthesis: Metabolites Present in Media

	Control	Neurogenic
DOPAC (Dihydroxy-phenylacetic acid)	0.2 pg/ml	1.2 pg/ml

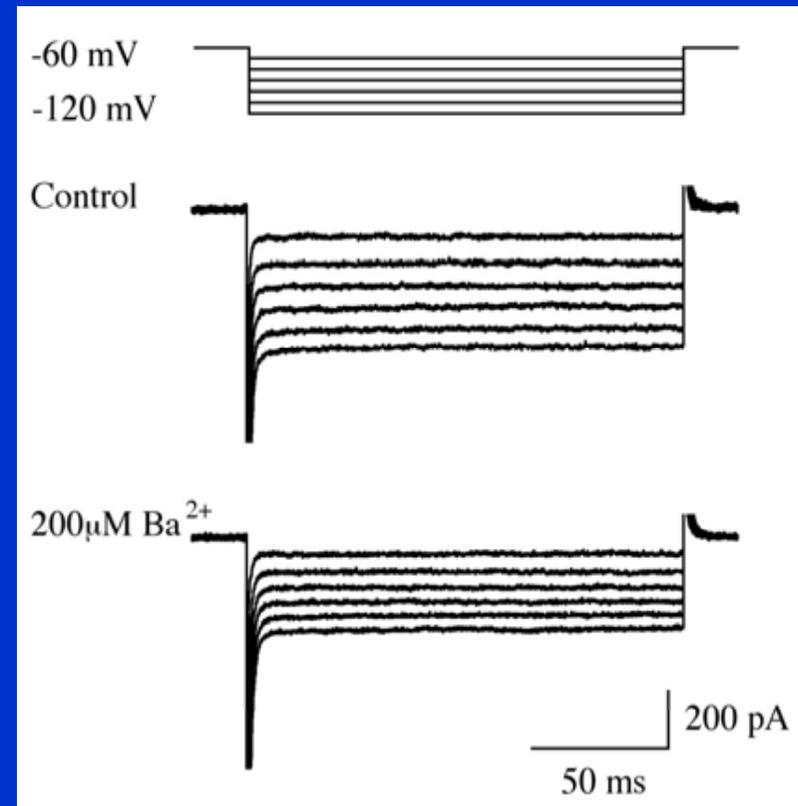
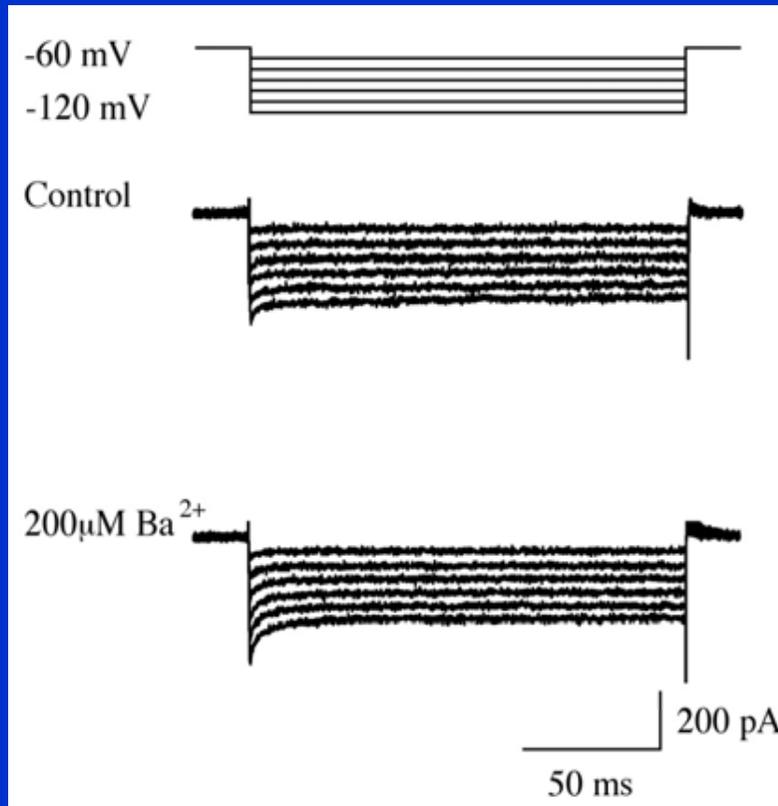
Ba²⁺ sensitive K⁺ currents: GIRK2 Channels



Undifferentiated HEDSC

Neurogenic

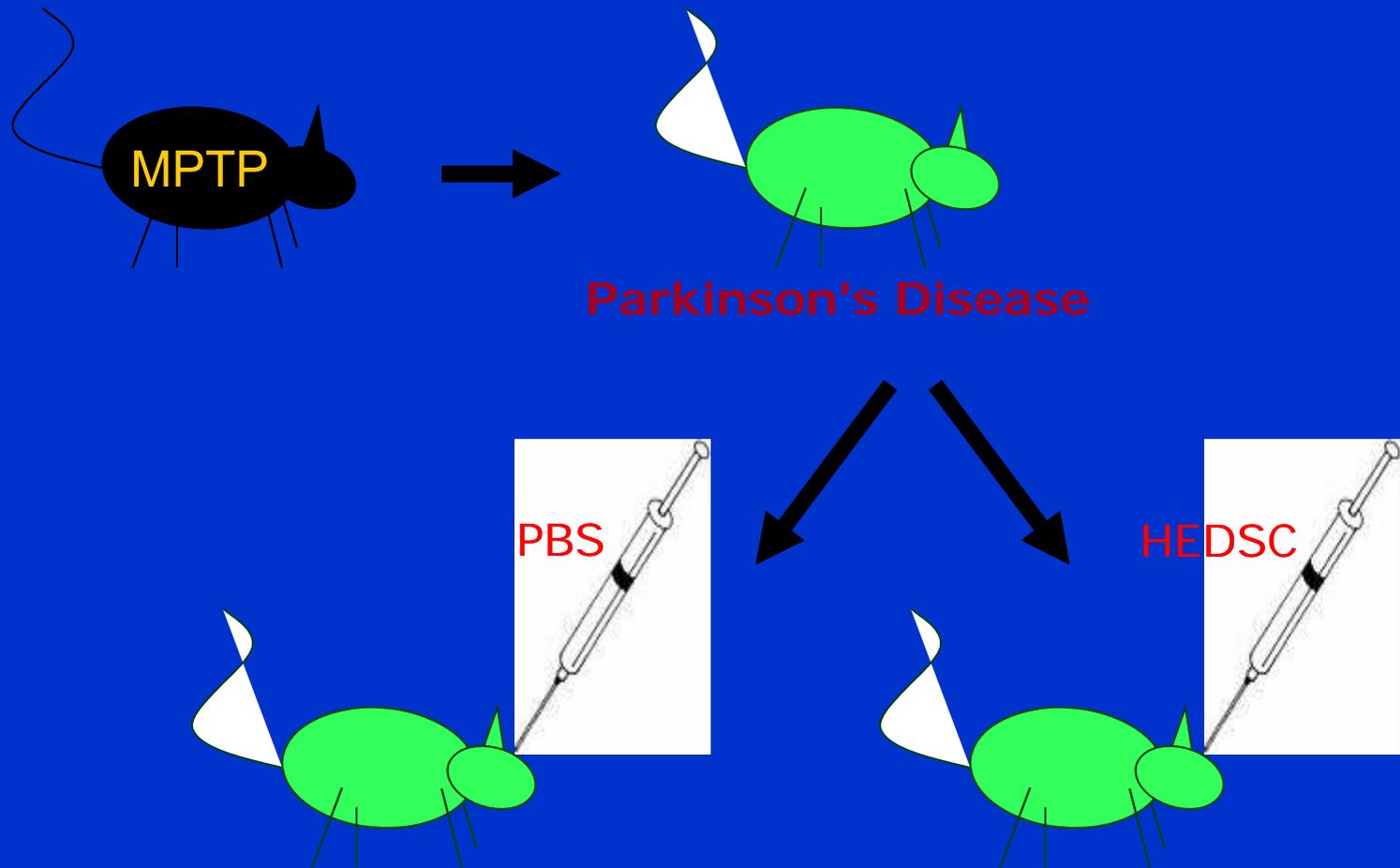
Baseline



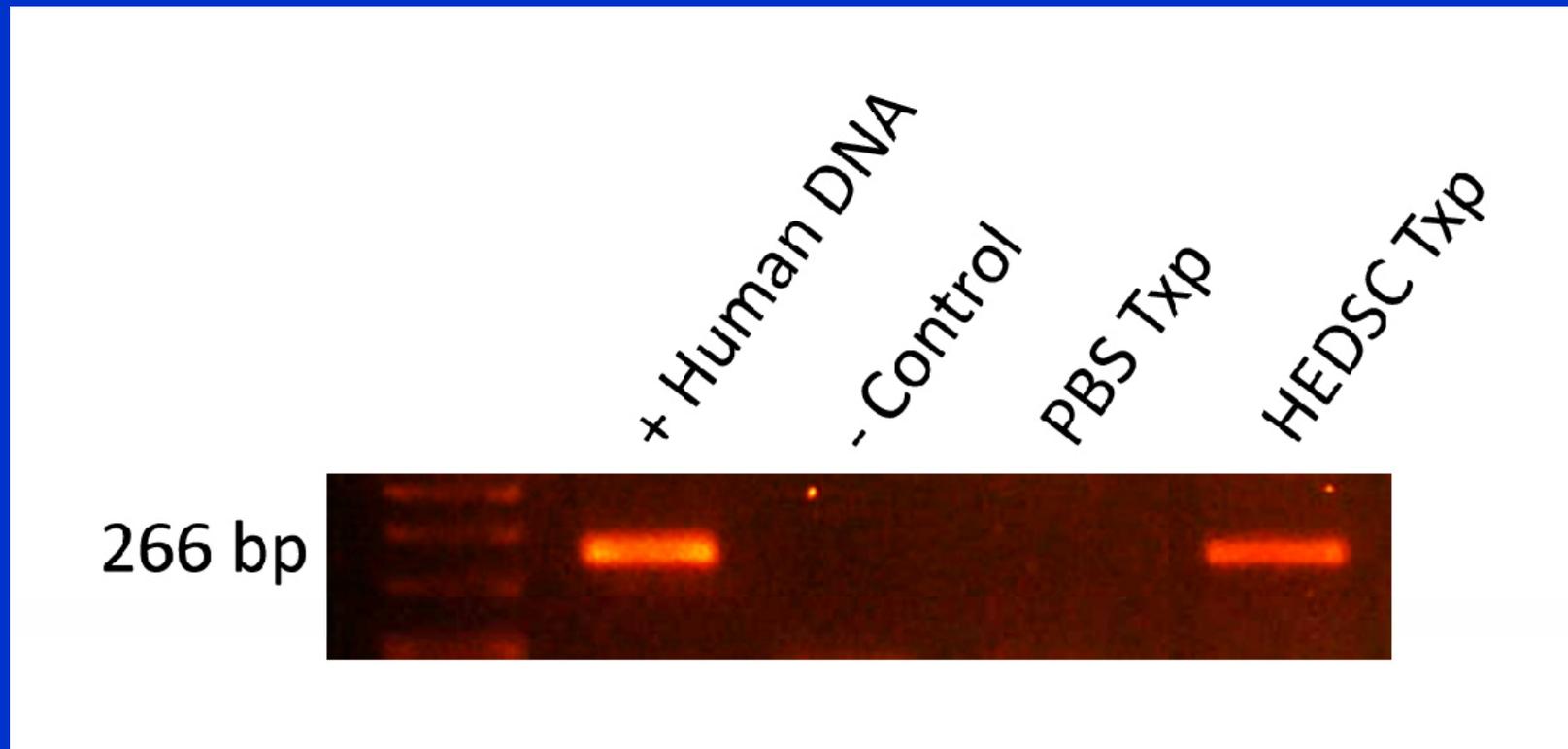
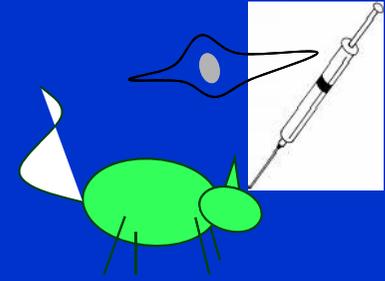
Parkinson's Disease (PD) Model

- PD is a degenerative disorder of the central nervous system that impairs movement and speech
- PD is caused by deficiency of Dopamine production in the Substantia Nigra of the brain
- MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine) is a neurotoxin of dopamine producing cells
- MPTP induces a permanent model of PD

Human Endometrial Derived Stem Cells (HEDSC) Transplantation

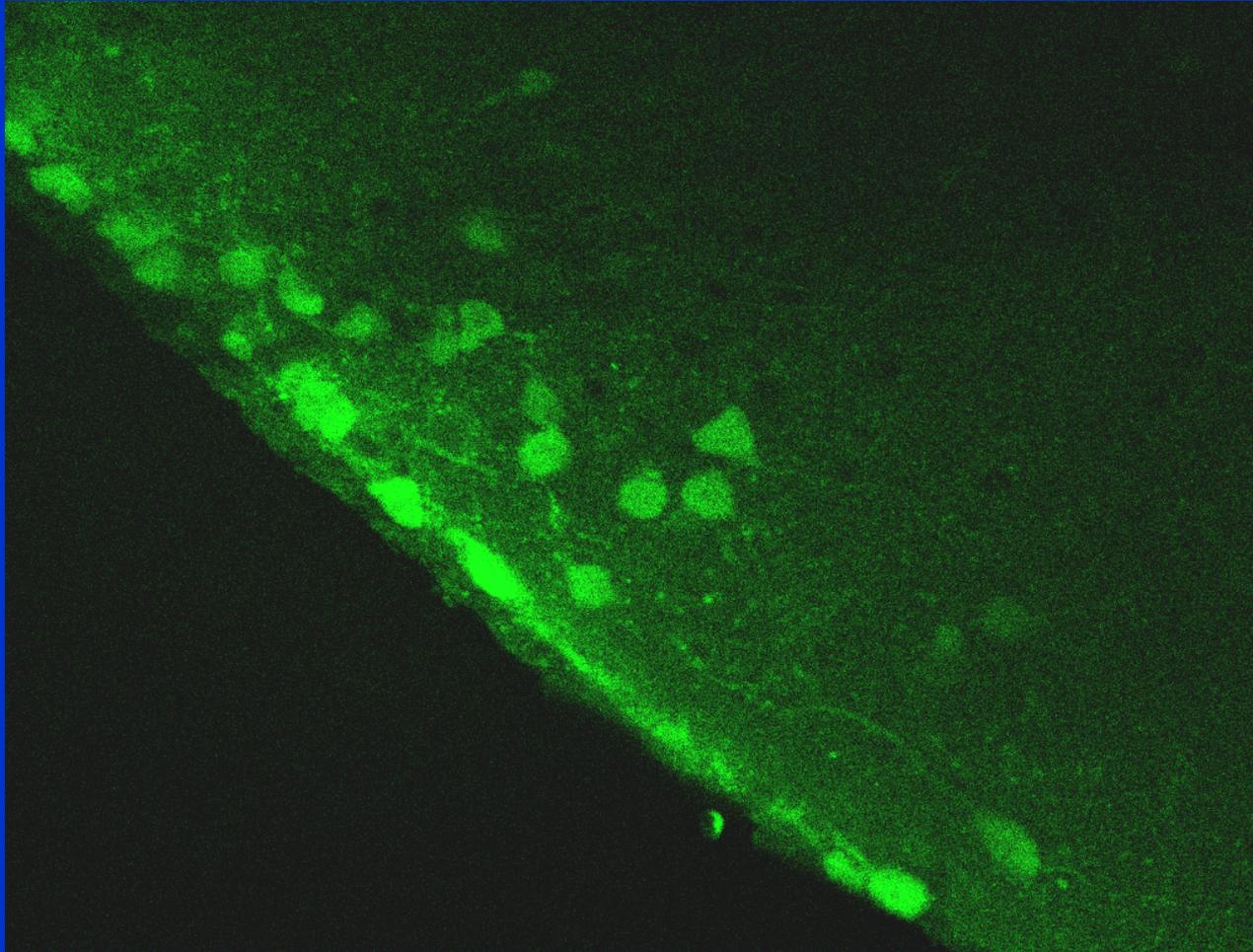
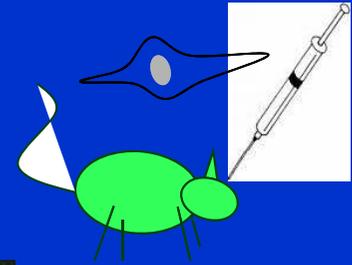


HEDSC Engraftment



PCR for Human DNA in
Transplanted Brains

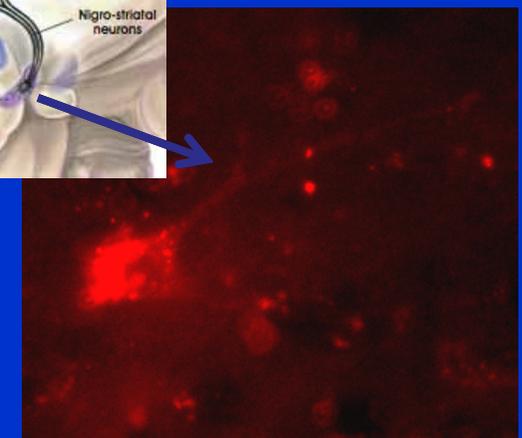
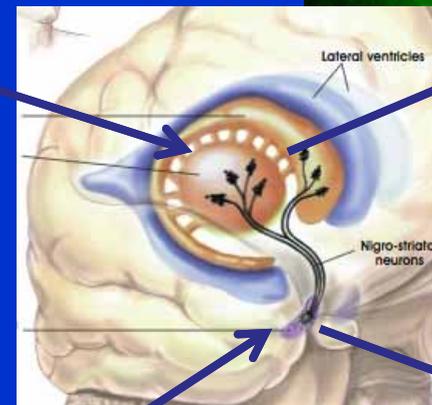
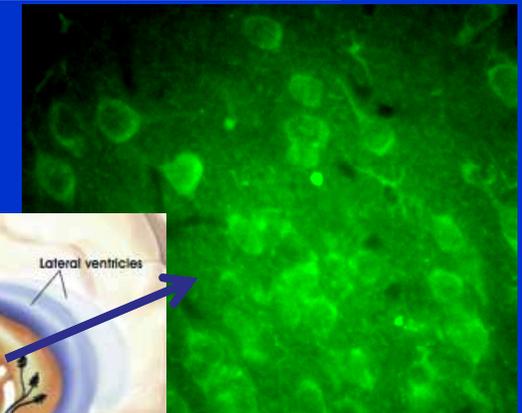
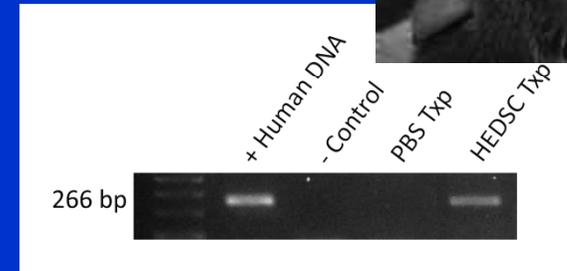
HEDSC Engraftment



Human Nestin Antibody

EDSC Neurotransplantation

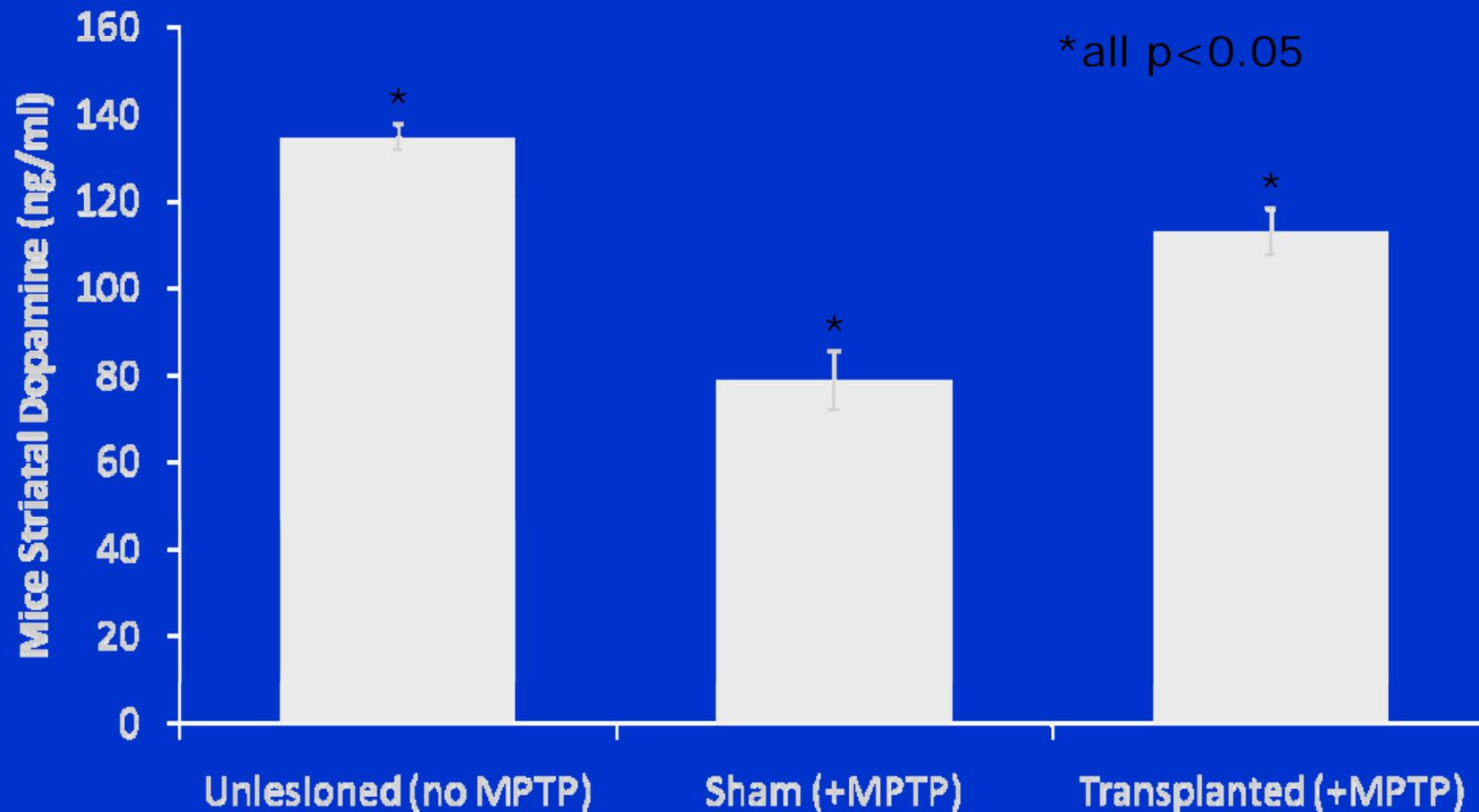
- Engraft in mice brains
 - PCR detects human DNA
- Engraft in mice brains
 - Striatum (transplant site)
- Migrate & Differentiate morphologically *in vivo*
 - Substantia nigra (lesion site)



ESC Neurotransplantation



Rescues dopamine concentrations

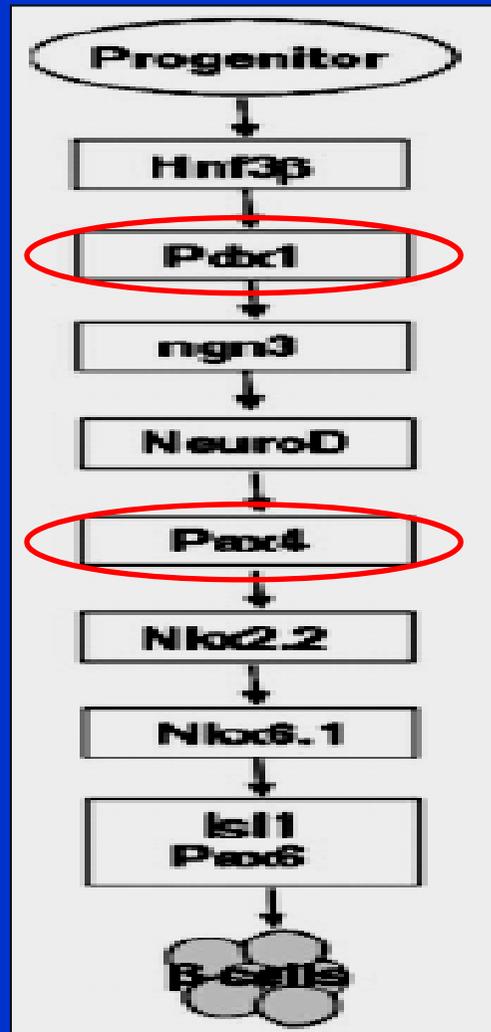


Pancreatic Beta Cells From Endometrial Stem Cells

Methods:

- Human endometrial tissue from subjects undergoing hysteroscopy/hysterectomy at P2-P5 (n=7)
- Differentiation following a 3 step-protocol
- SCID mouse model

β -pancreatic Gene Expression During Development



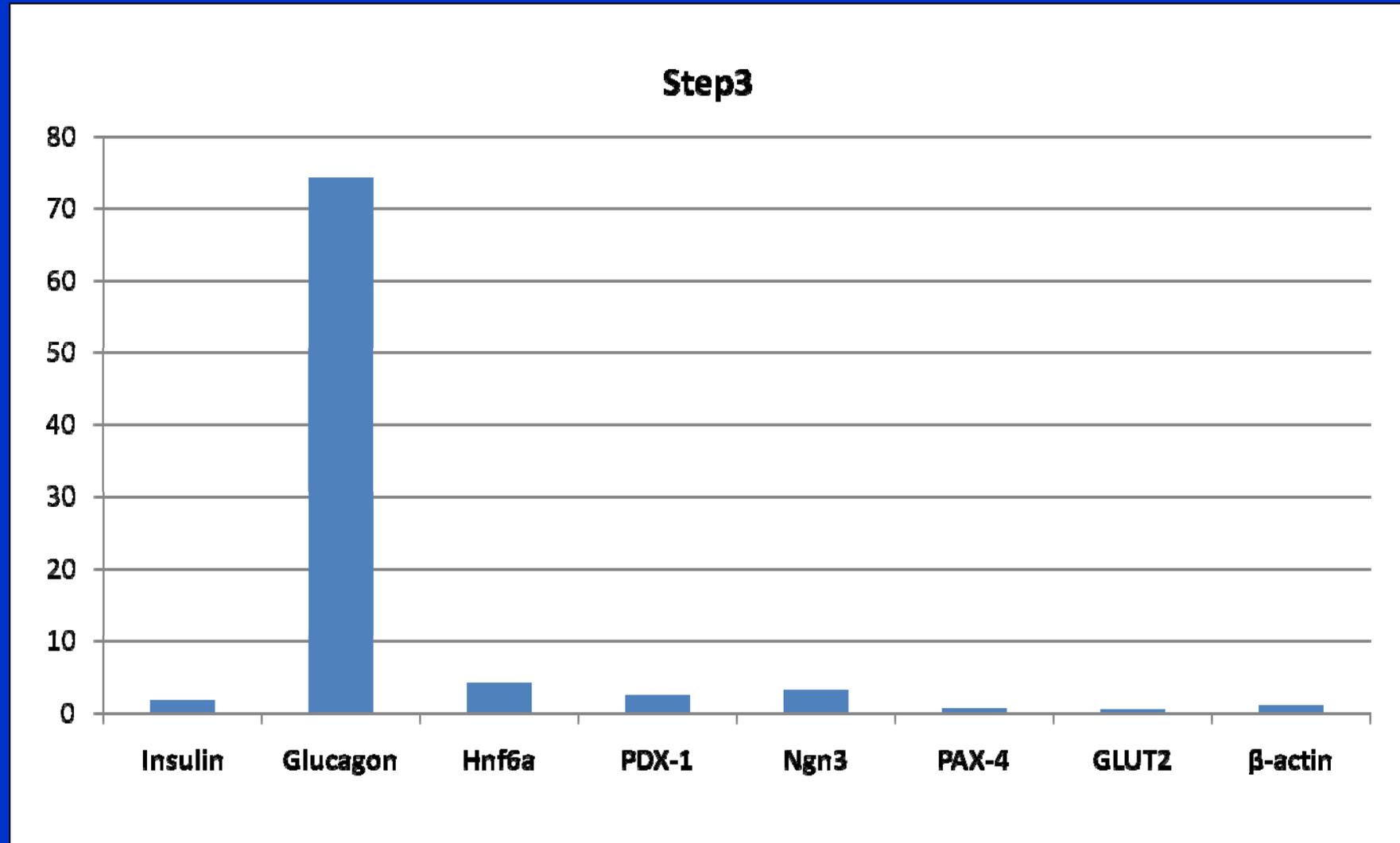
Chandra V et al. *Stem Cells*2009
Aug;27(8):1941-53

Differentiation Protocol

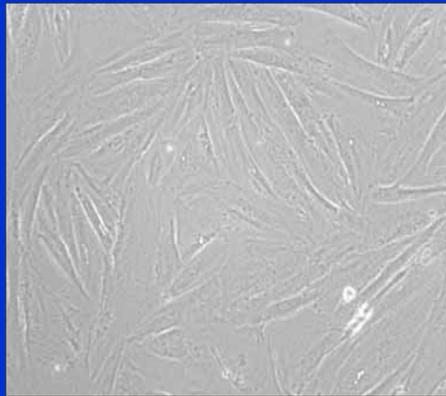
➤ Standard protocol:

- In ECM gel
- Step 1: H-DMEM+10%FBS+ 10^{-6} M RA for 24 hours, H-DMEM+10%FBS for 48 hours
- Step 2: L-DMEM+10%FBS+10mmol/L nicotinamide+ 20ng/mL EGF for 7 days
- Step 3: L-DMEM+10%FBS+10 nmol/L exendin 4 for 7 days

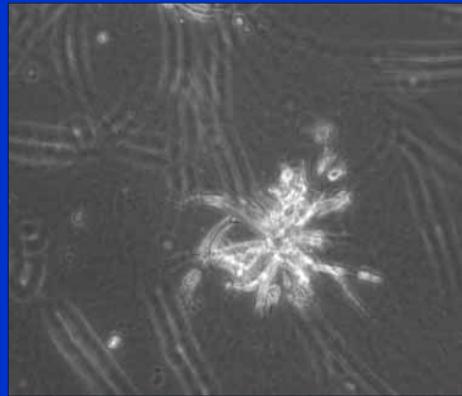
Gene expression using standard protocol



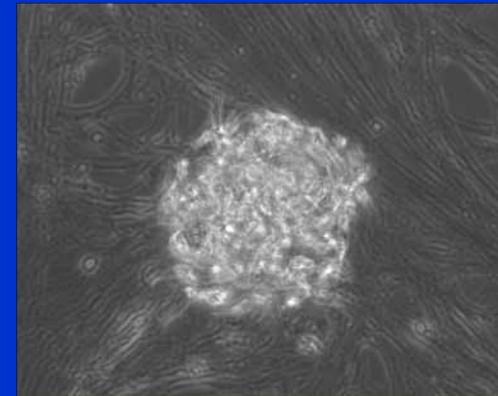
In vitro differentiation



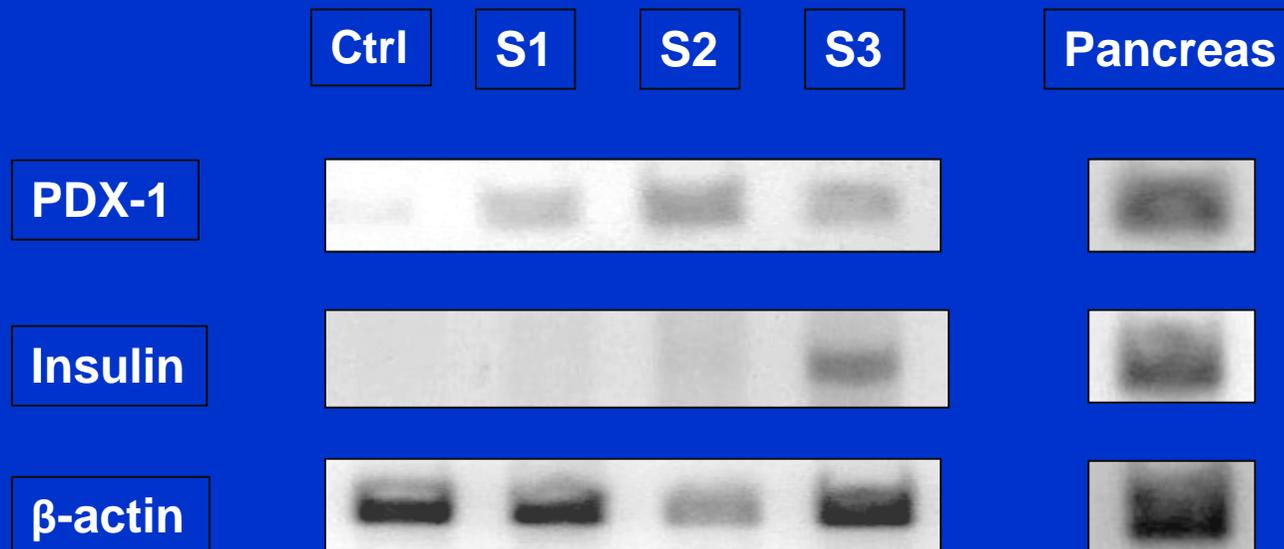
Undifferentiated



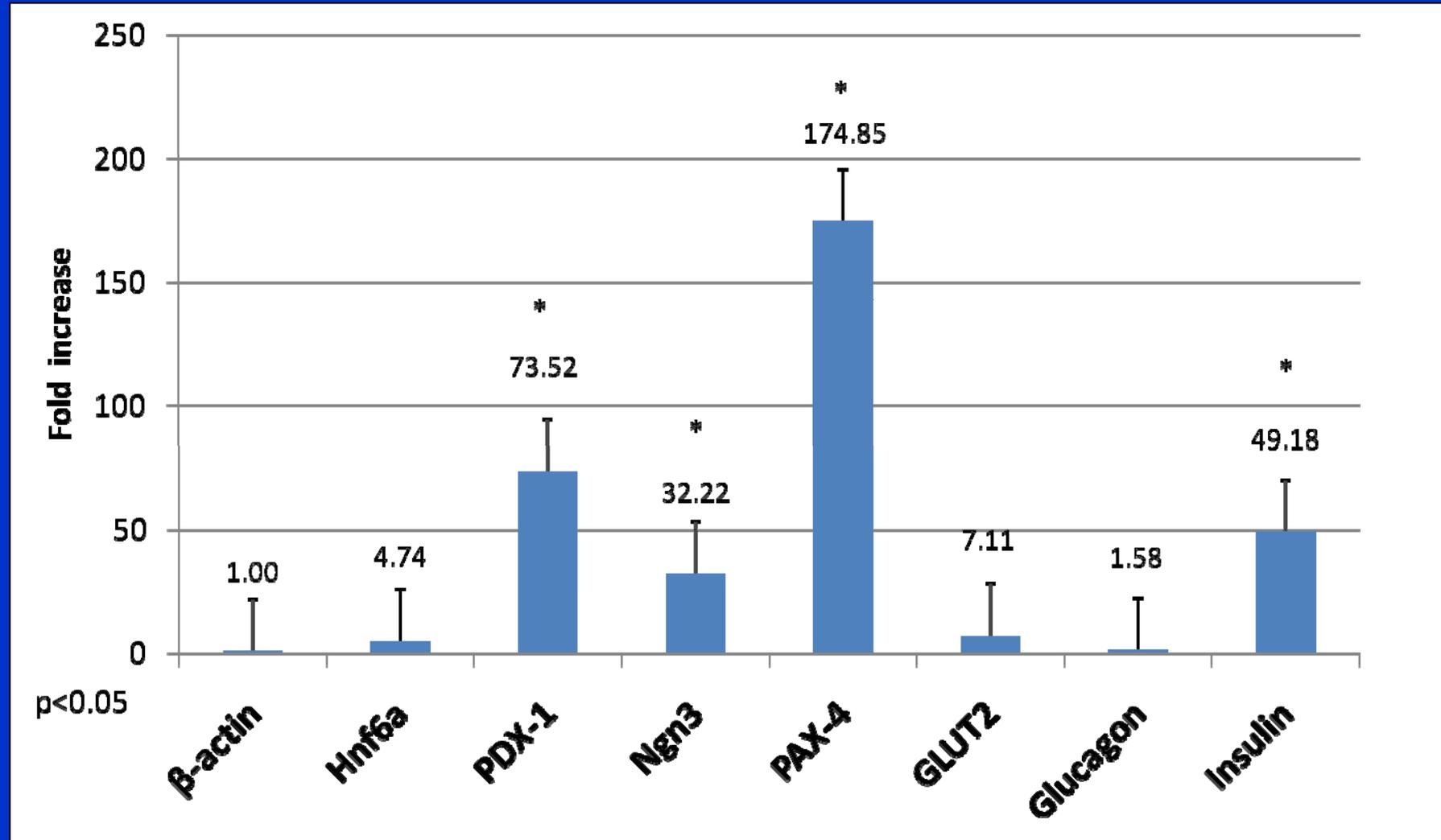
Step 2



Step 3

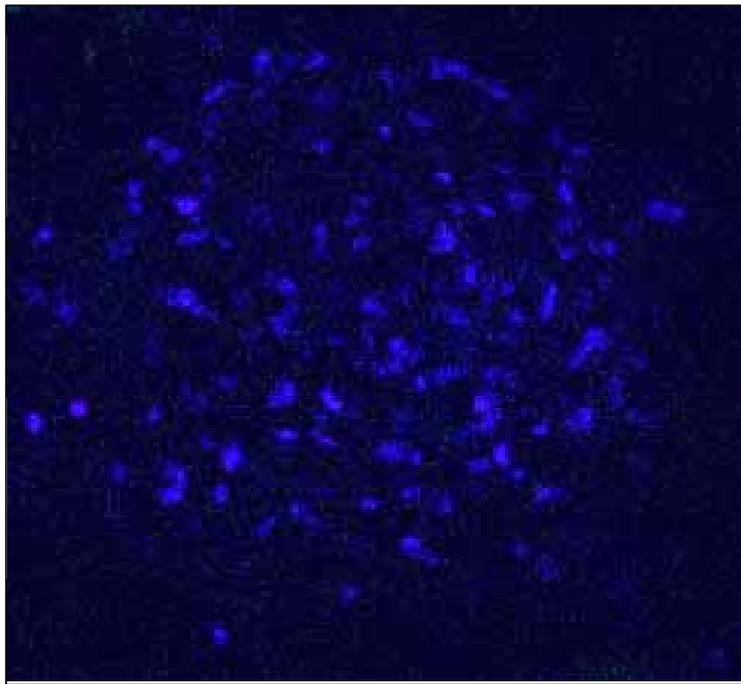


Modified Protocol Step 3

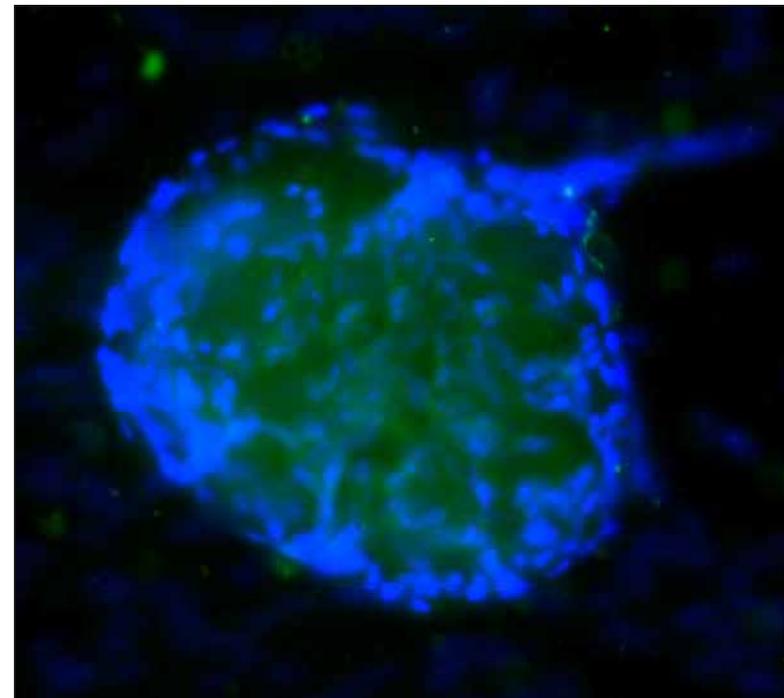


Immunofluorescence

— Insulin
— DAPI

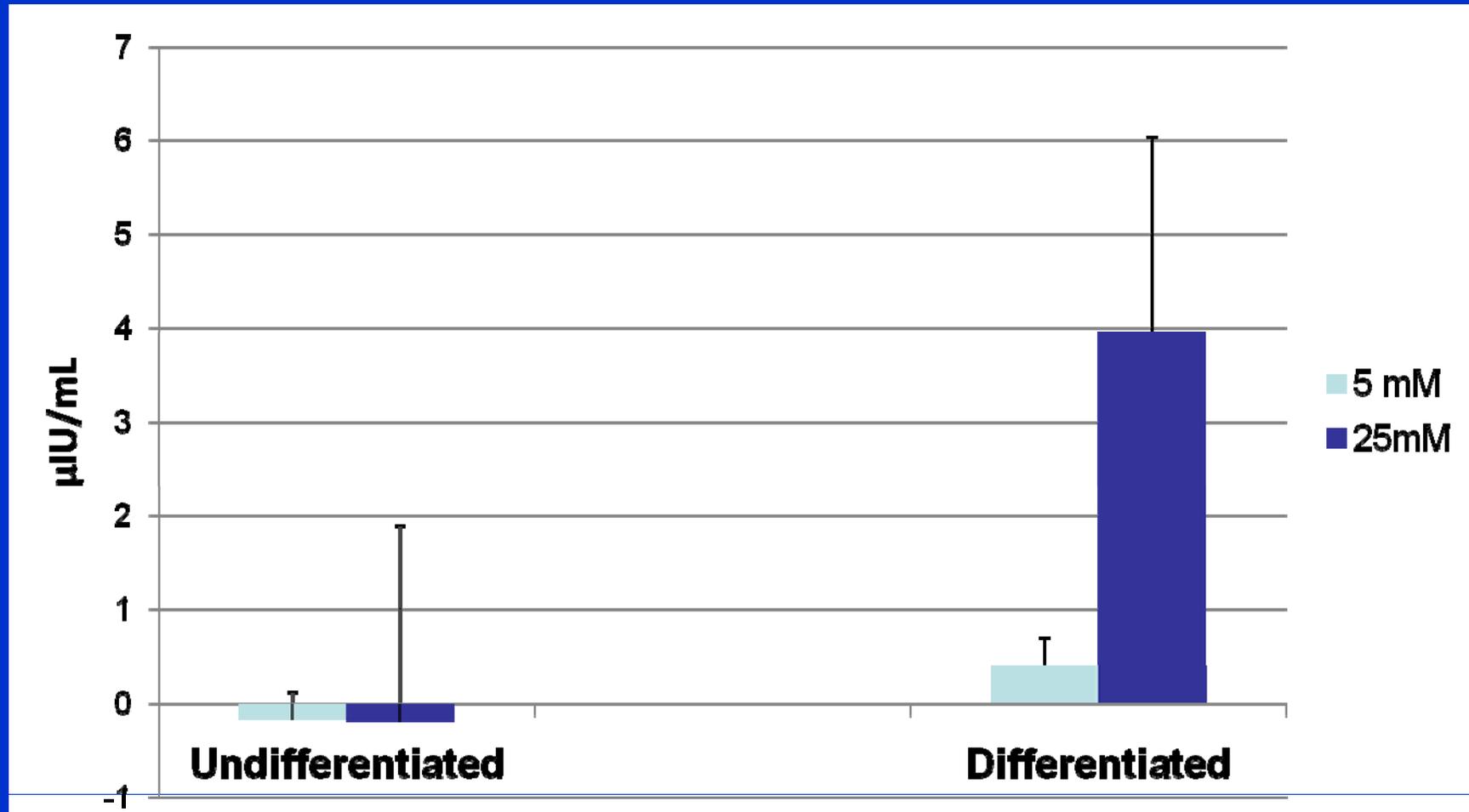


Undifferentiated

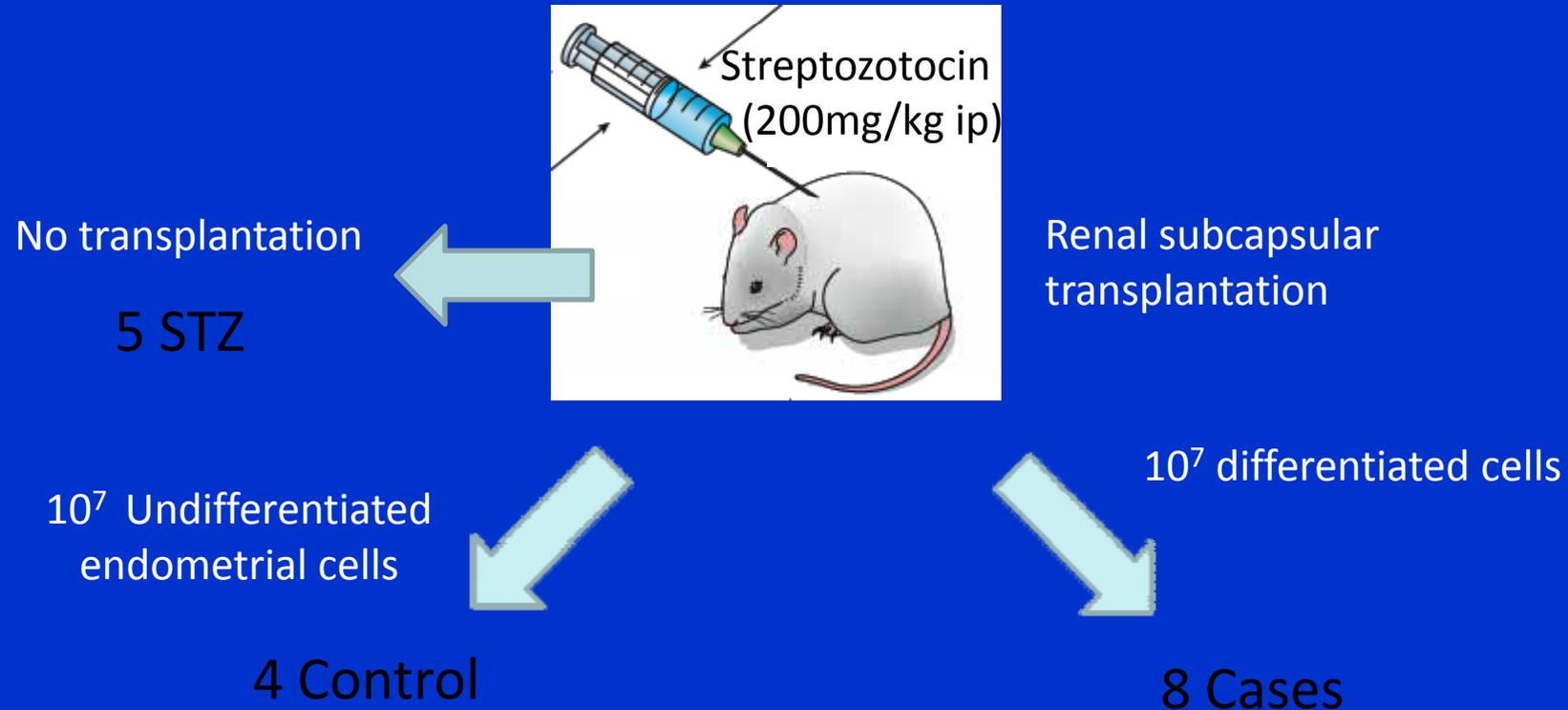


Differentiated

Insulin Secretion (ELISA)



Diabetes Animal Model



SCID Female Mice



Control (undifferentiated tx)

Treatment (differentiated cell Tx)

Endometrial Stem Cells

- Potential source of HLA identical stem cells in women
- Easily Obtainable
- Renewable
- Can treat animal models of Parkinson's disease and diabetes.

Conclusions:

- Cell trafficking into the uterus likely contributes to uterine tissue regeneration and repair.
- Stem cells are a novel etiology of endometriosis
- The endometrium is a source of multipotent mesenchymal stem cells.
- These cells have a vast capacity for differentiation into multiple cell types useful in tissue repair and regenerative medicine.



Acknowledgements

Stem Cell Group:

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- Tamas Horvath

