Endometriosis

Epigenetics and Stem Cells

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What is the Pathogenesis of Endometriosis?

Theories for the Origin of Endometriosis

- Retrograde menstruation
- Embryonic rests
- Colemic metaplasia
- Immune
- Genetic
- Stem cells

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 menarche.
- Animal models involving peritoneal transplant

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

The Stem Cell Theory of Endometriosis

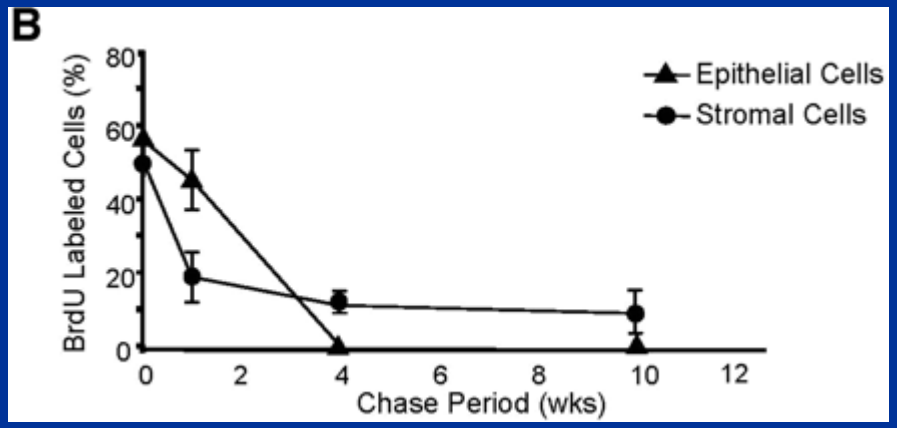
Adult Stem Cells

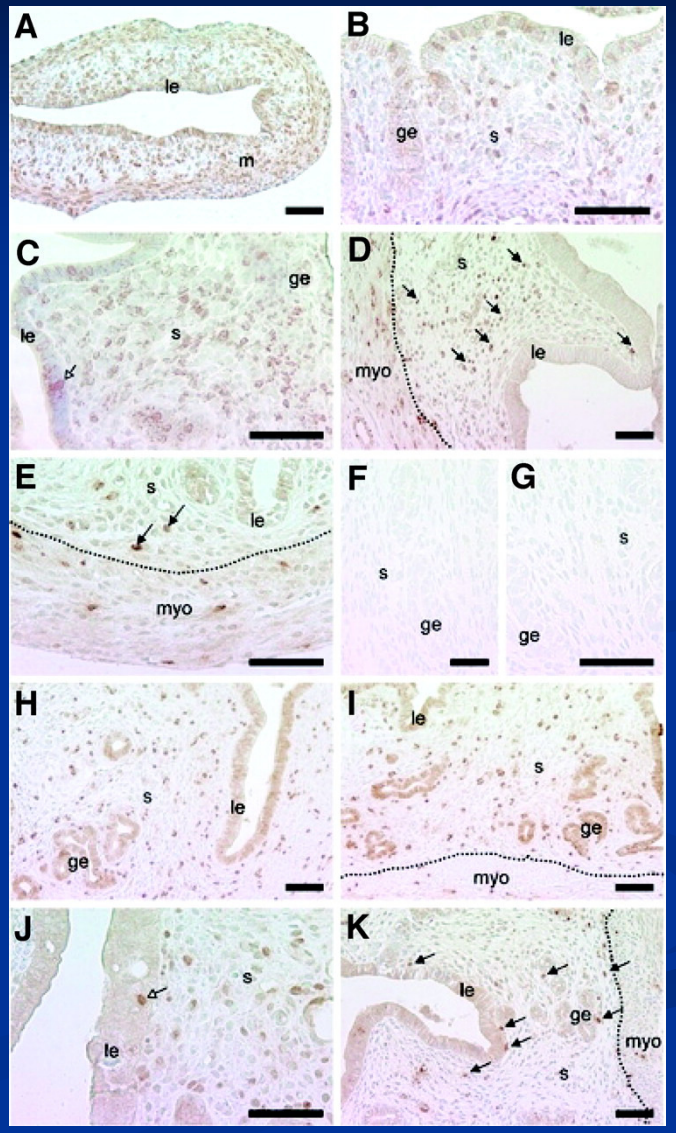
- Adult stem cells are cells that have traveled to tissue niches early in an organisms development to reside there in a relatively undifferentiated state.

- These adult stem cells, also called progenitor stem cells (PSC) can **renew themselves and differentiate** into most of the specialized cell types located in the surrounding tissue.

Localization of label-retaining cells (LRCs) in postnatal and prepubertal-labeled mice

Stromal BrdU retaing cells

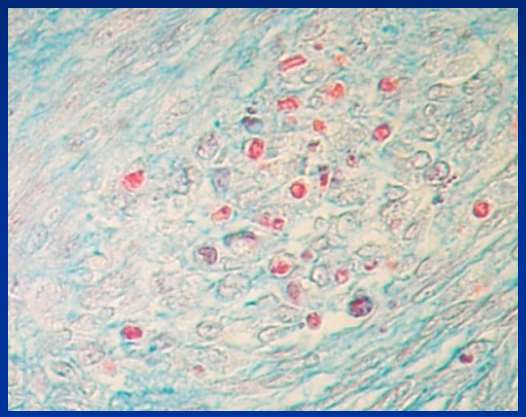




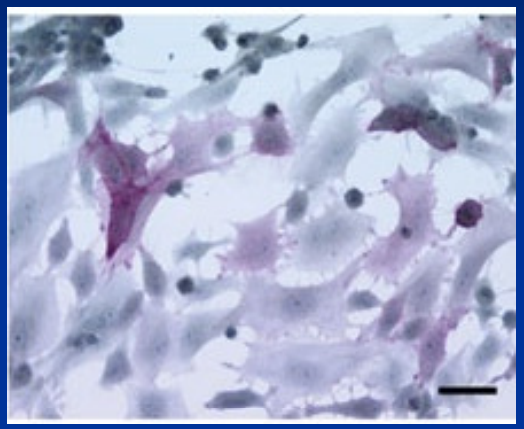
Endometriosis

It is likely that endometriosis that arises via retrograde menstruation is derived from shed progenitor stem cells.

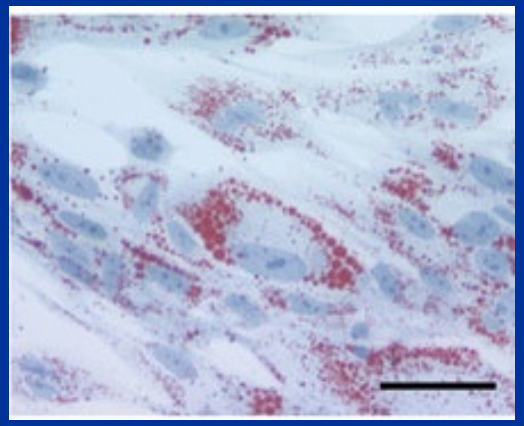
Multipotent Endometrial Stromal Cells



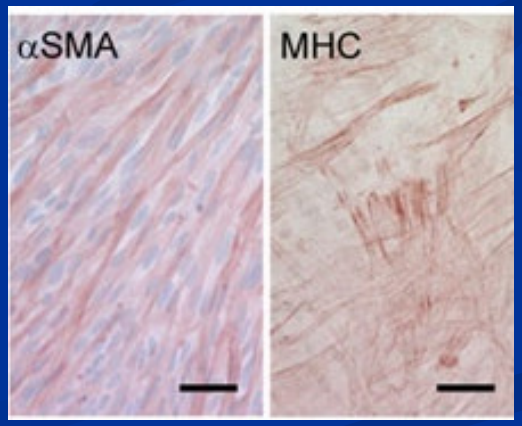
Chondrocytes



Osteocytes



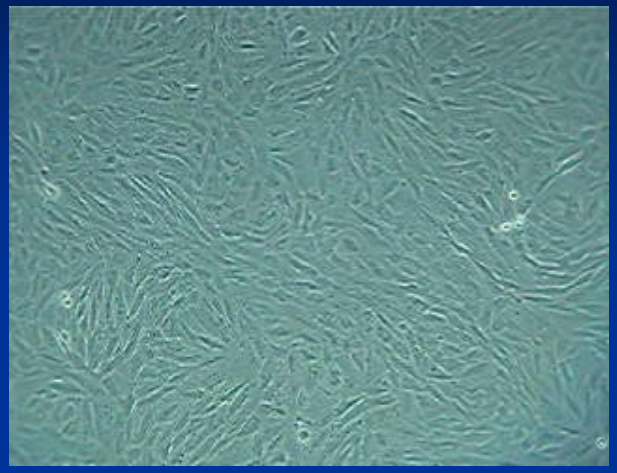
Adipocytes



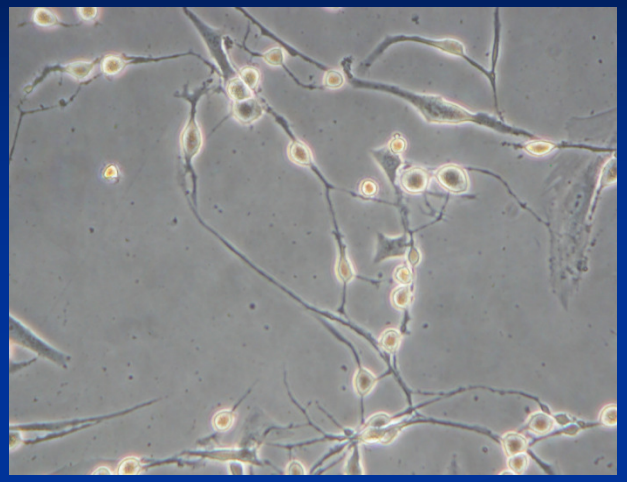
Myocytes

Wolff 2007; Schwab 2007; Dimitrov 2008

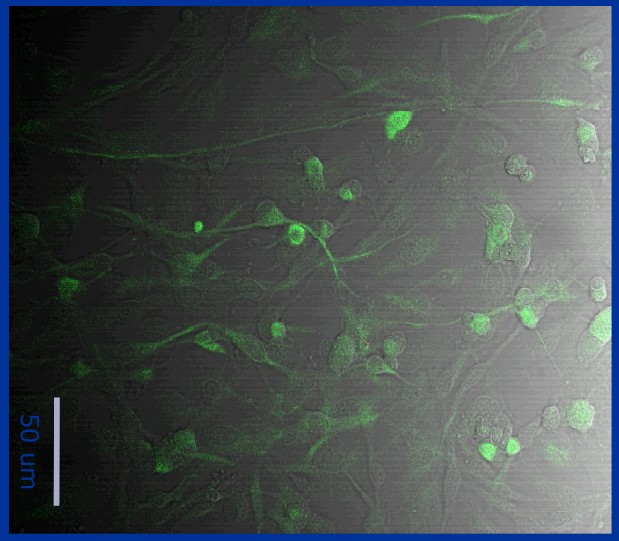
in vitro Differentiation



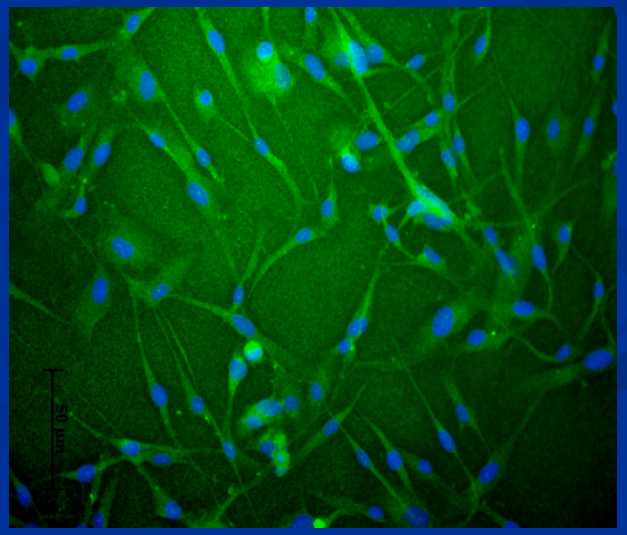
Control Endometrial Stromal Cells



Neurogenic Differentiated

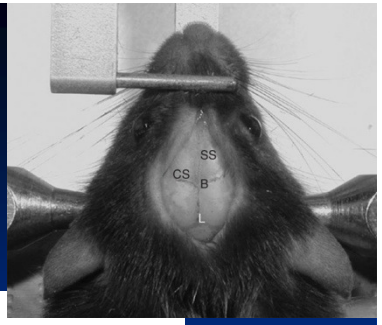


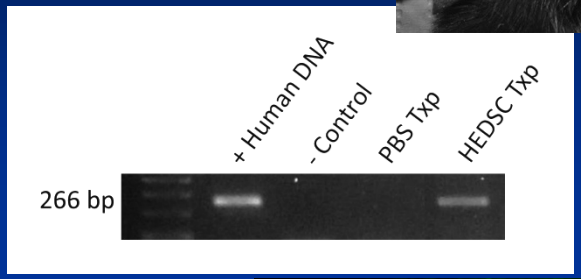
Nestin



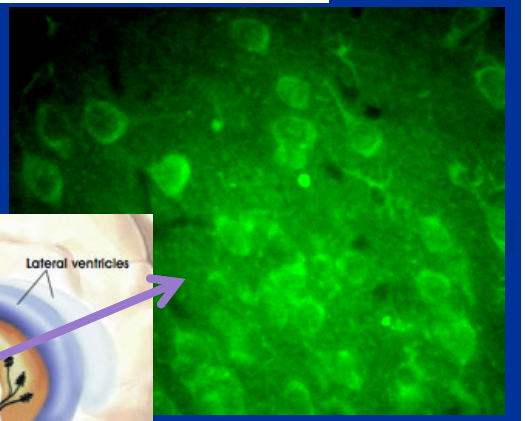
Tyrosine Hydroxylase

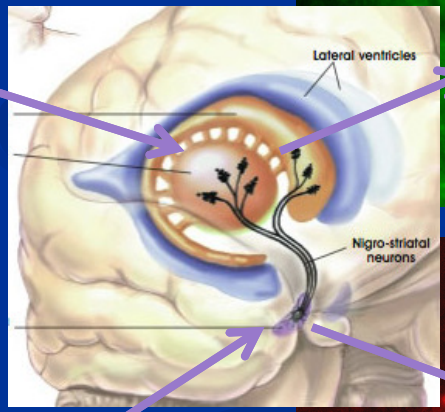
ESC Neurotransplantation



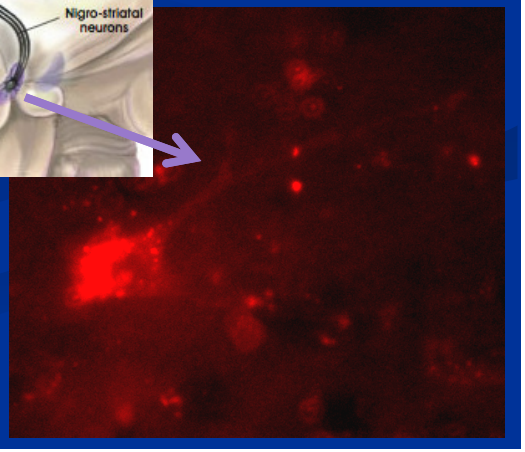


- Engraft in mice brains
  - PCR detects human DNA
- Engraft in mice brains
  - Striatum (transplant site)

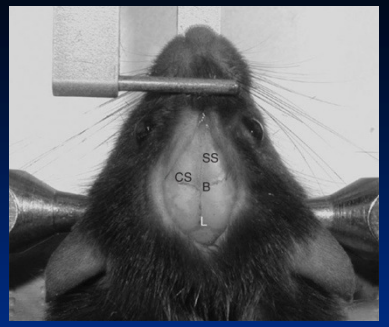




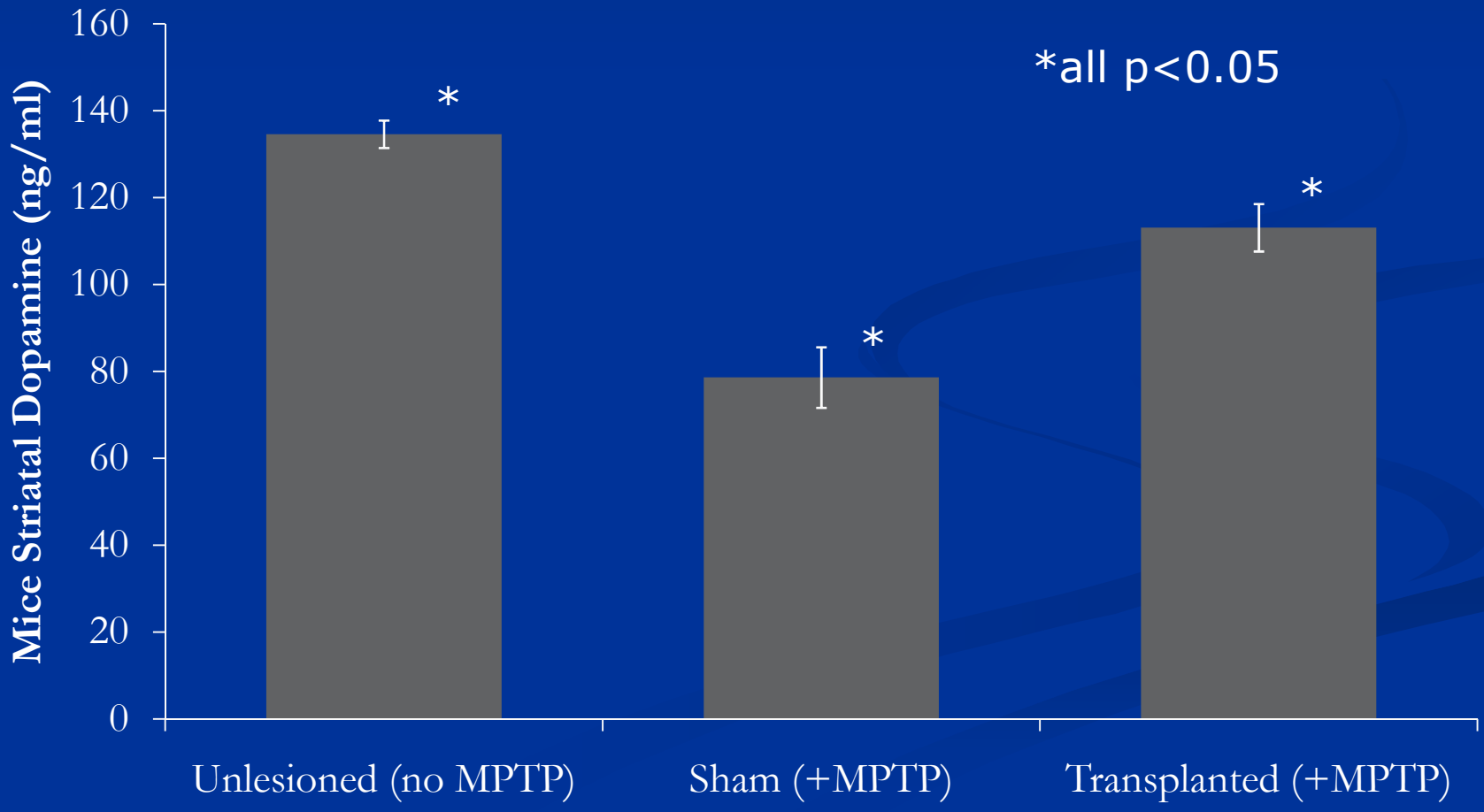
- Migrate & Differentiate morphologically *in vivo*
  - Substantia nigra (lesion site)



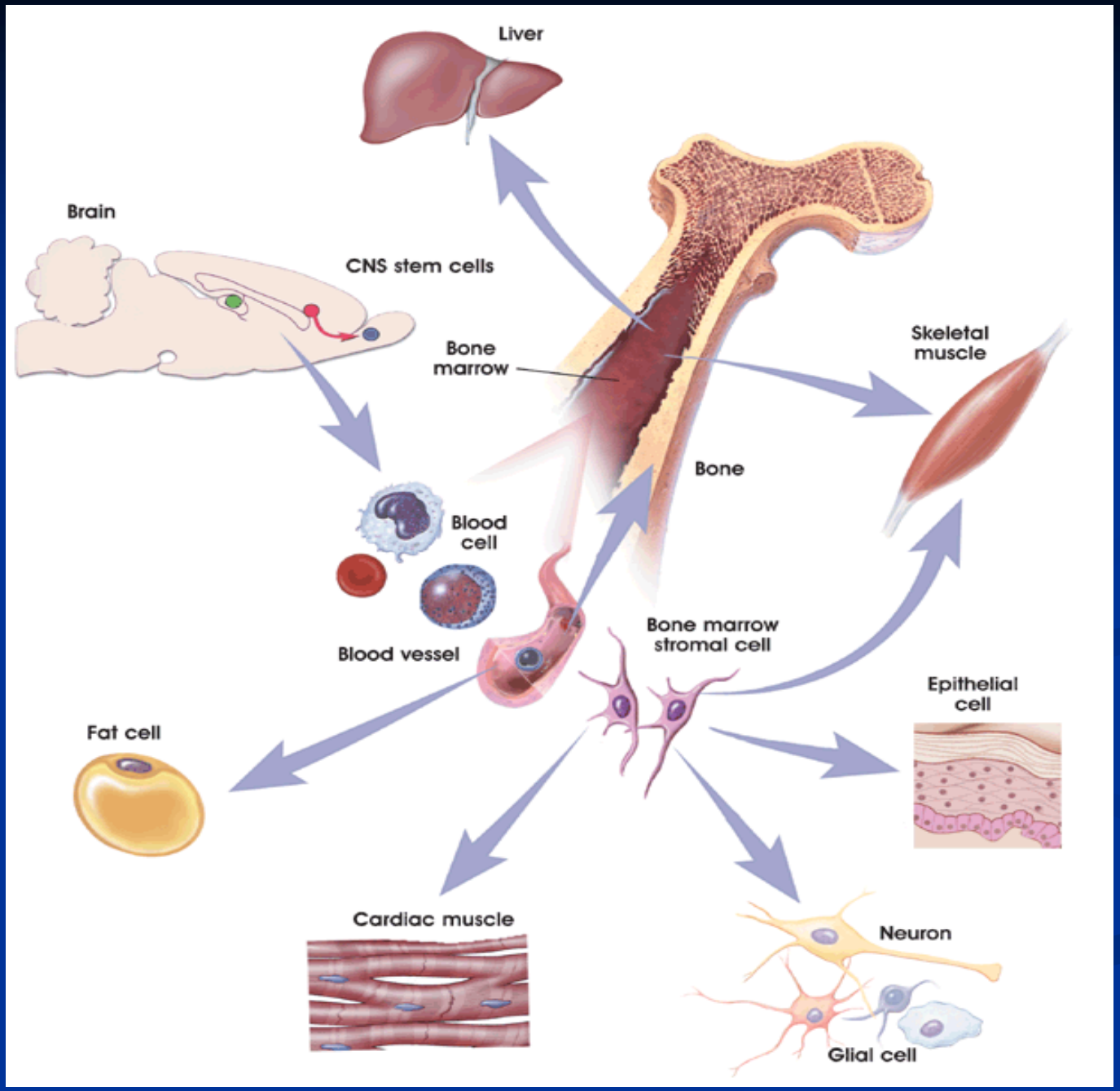
ESC Neurotransplantation



- Rescues dopamine concentrations



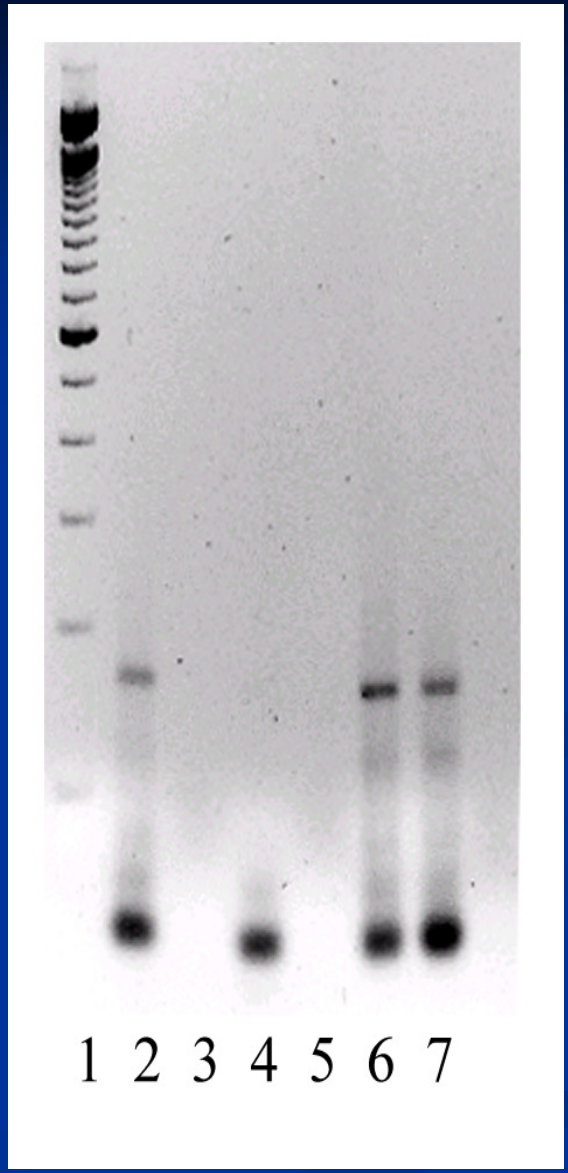
Can Bone Marrow Derived Stem Cells Differentiate Into Endometrium?

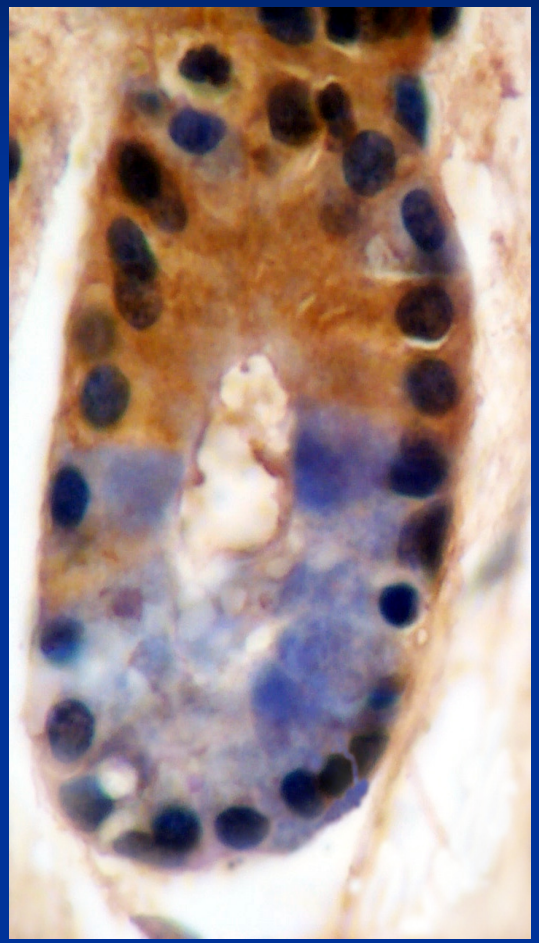

Liver
Brain
CNS stem cells
Bone marrow
Skeletal muscle
Bone
Blood cell
Blood vessel
Bone marrow stromal cell
Epithelial cell
Fat cell
Cardiac muscle
Neuron
Glial cell

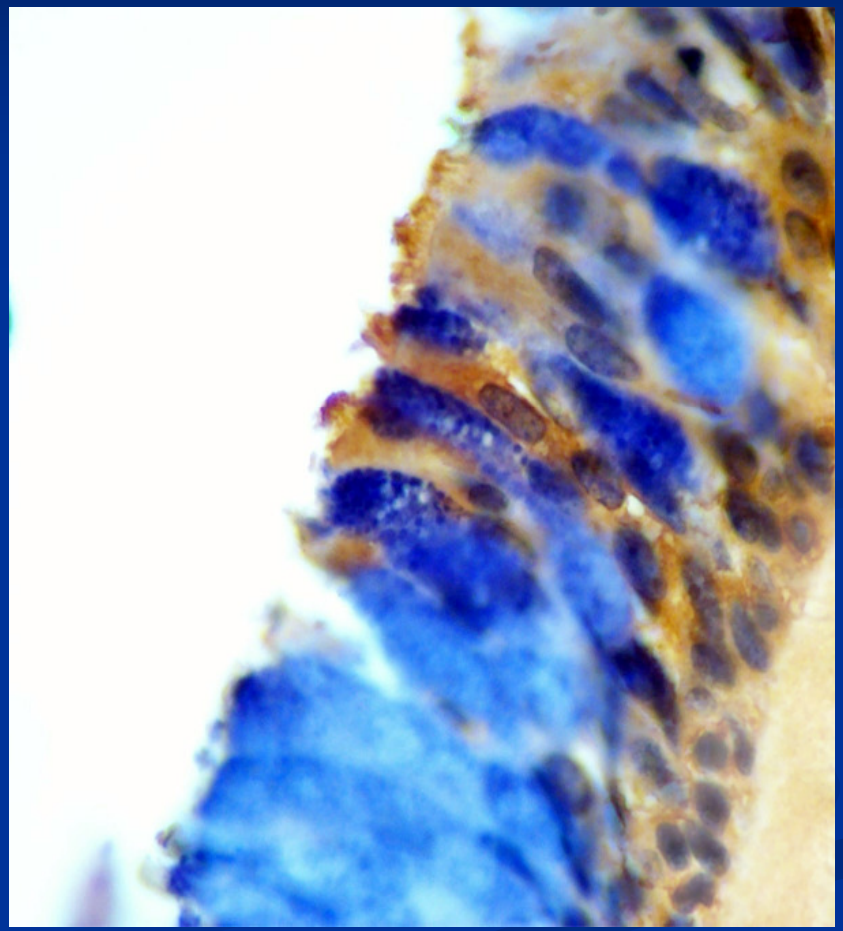
Patients

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

RT-PCR amplifying HLA A11

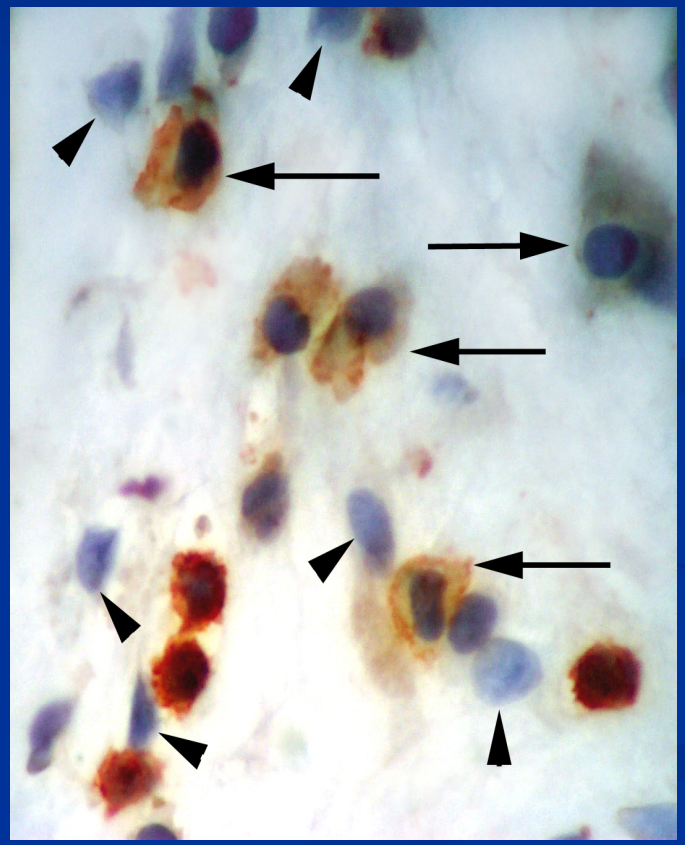






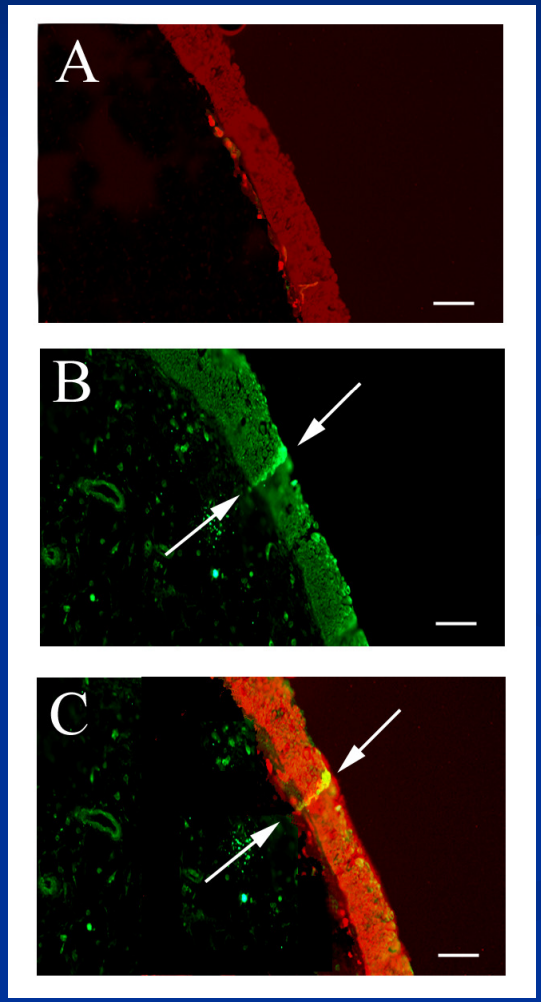
Taylor HS, JAMA. 2004;292(1):81-5

Bone Marrow Stem Cell-Derived Endometrium



Taylor HS. *JAMA*. 2004;292(1):81-85.

Marker of Differentiation



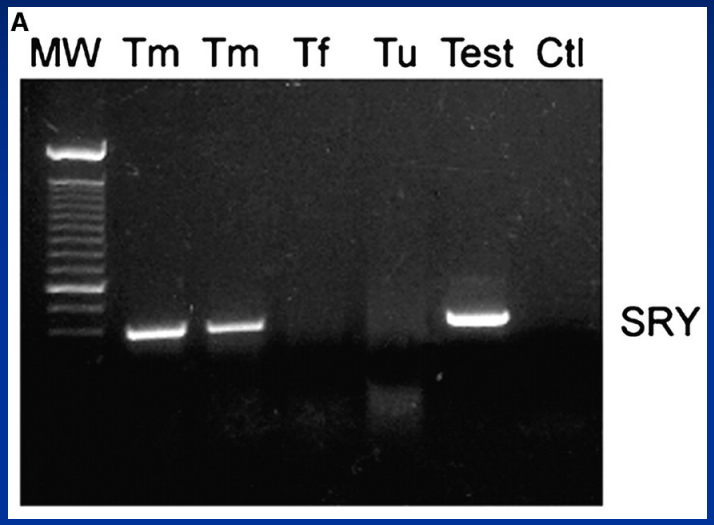
Calcitonin

HLA

Merge

Do Stem Cells Contribute to Endometrium in a murine model?

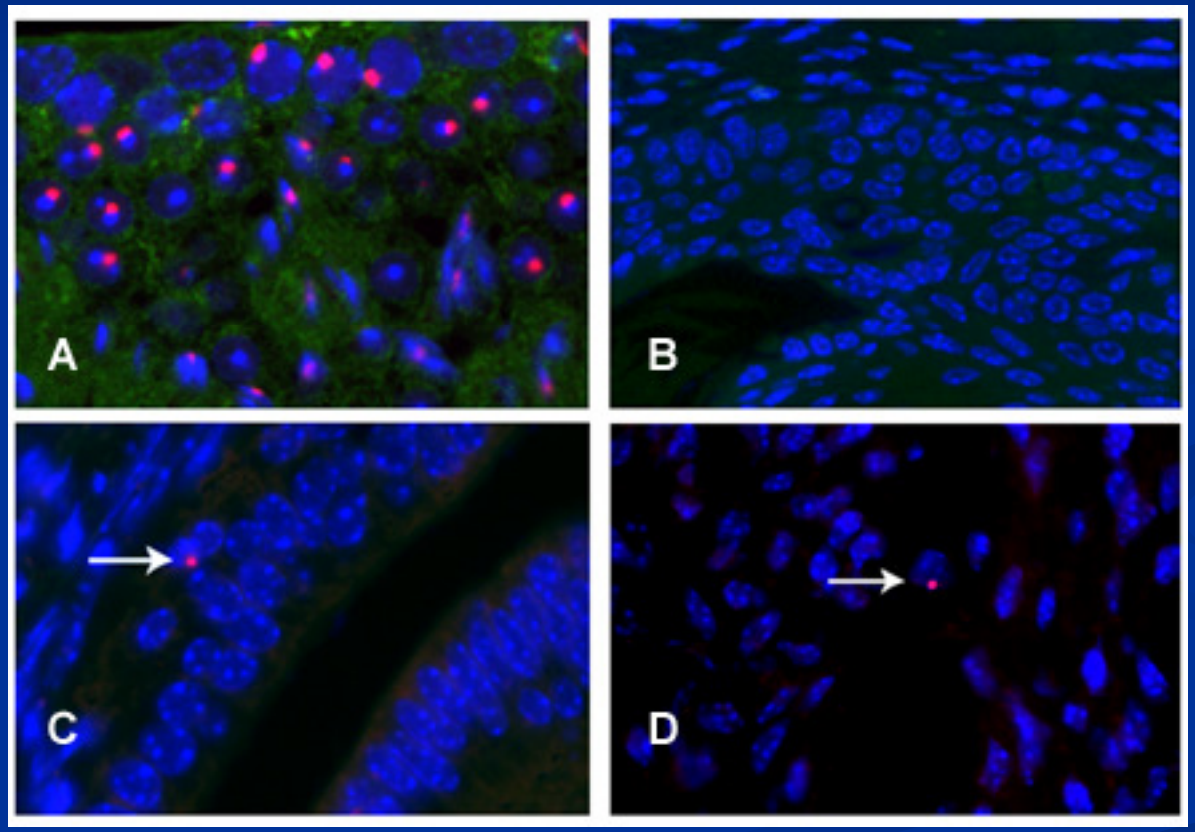
Identification of bone marrow-derived cells in murine-endometrium



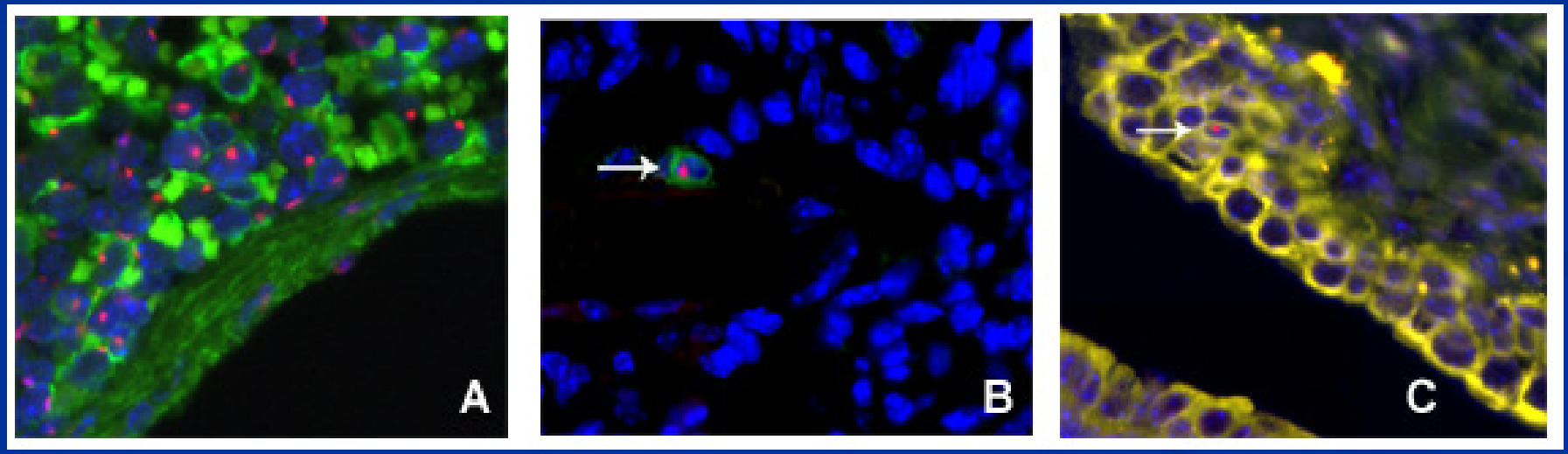
Transplant of Male bone marrow into female mice. Assessment of SRY gene expression and Y chromosome by FISH

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

Stem cell origin of endometrium in mouse



Stem Cell Origin of Endometrium in a Mouse Model



Male WBC Cytokeritin

Du H, Taylor H. *Stem Cells*. 2007;25(8):2082-2086.

Stem cells are recruited to the uterus for repair and tissue regeneration.

Stem Cells and Disease

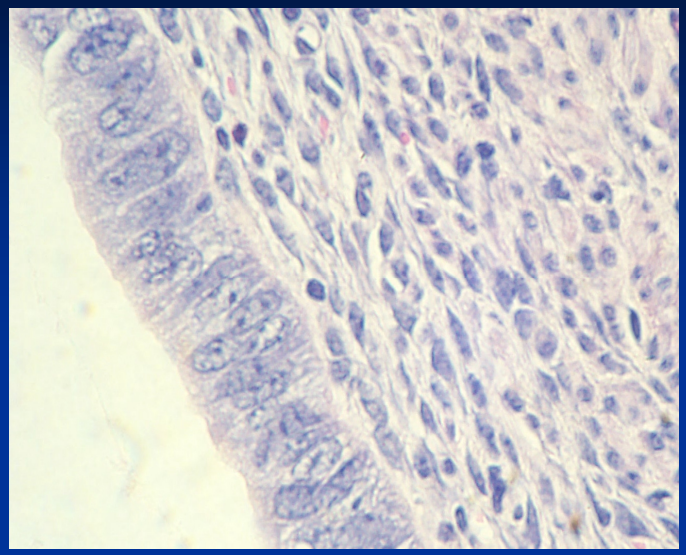
Can Stem Cells Contribute

to Endometriosis ?

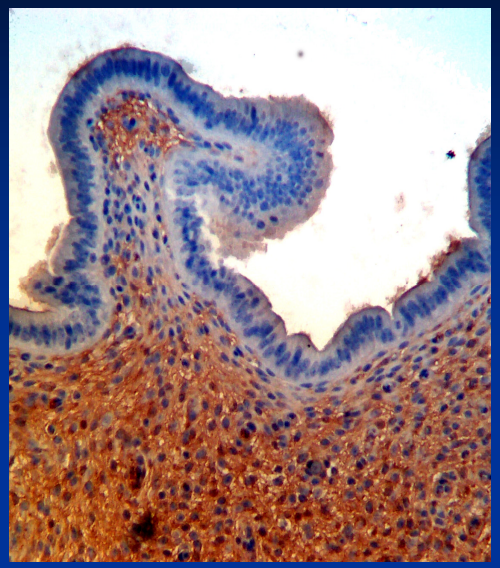
Methods

- Wild Type and LacZ transgenic mice
- Hysterectomy and ectopic uterine transplant
- Beta-Galactosidase activity and expression

IHC using anti Beta-galacotosidase antibody

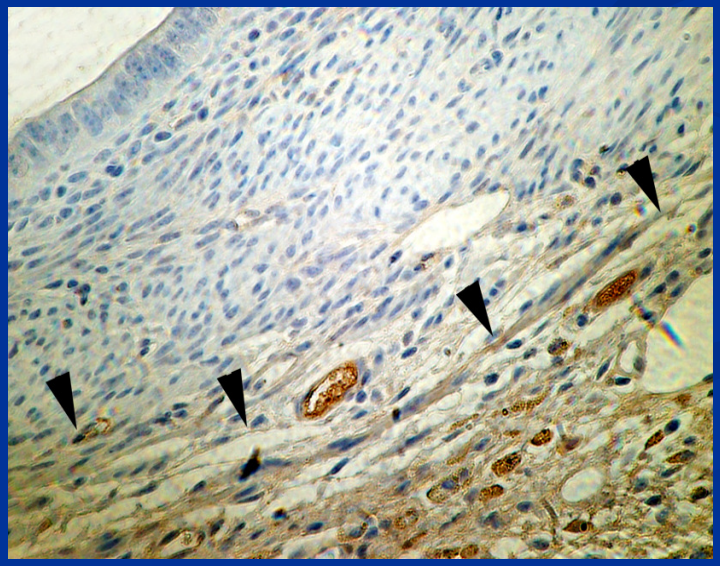


Wt control

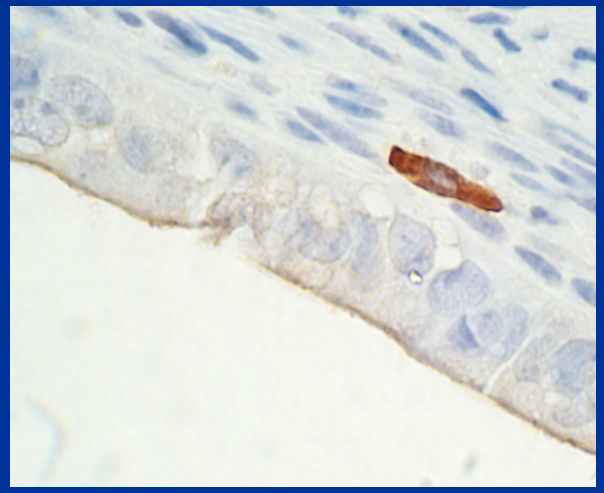


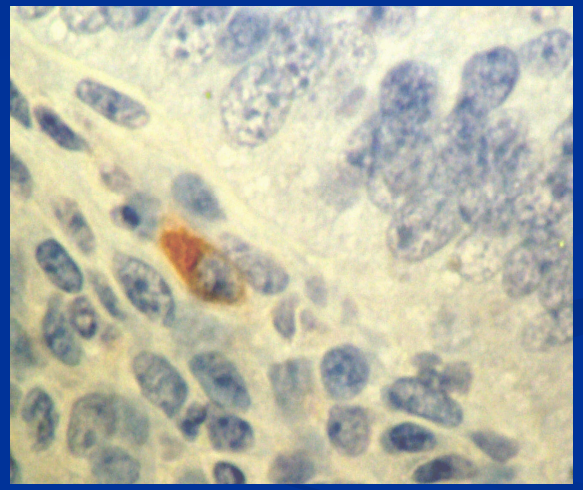
LacZ transgenic

Wt transplanted
to LacZ transgenic



IHC using anti Beta-galactosidase

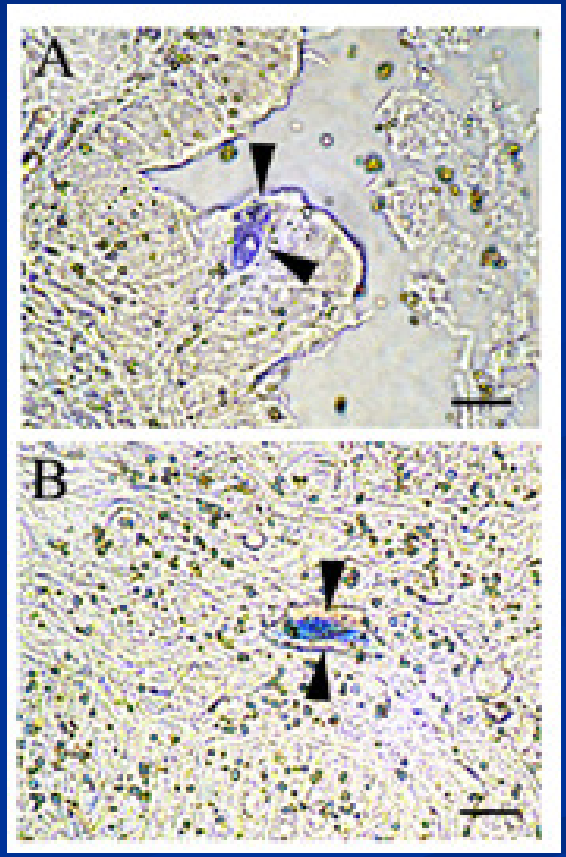




X-GAL staining of Beta-Galactosidase activity

Glandular cell

Stromal Cell



A Novel Origin of Endometriosis

- Stem cells contribute to murine endometriosis

Endometriosis Is Not One Disease!

- Retrograde menstruation leads to peritoneal disease
- Metaplasia leads to endometriomas
- Stem cells lead to lung and brain endometriosis as well as contribute to endometiosis in the peritoneal cavity

Novel Treatments

PATHOGENESIS

Genetic predisposition

↓

Retrograde menstruation

↓

Peritoneal invasion

↓

Dysfunctional immune response

↓

Angiogenesis & increased local estrogen

↓

Endometriosis

Novel Treatments

PATHOGENESIS

Genetic predisposition

↓

Retrograde menstruation and /or stem cell recruitment

↓

Peritoneal invasion

↓

Dysfunctional immune response

↓

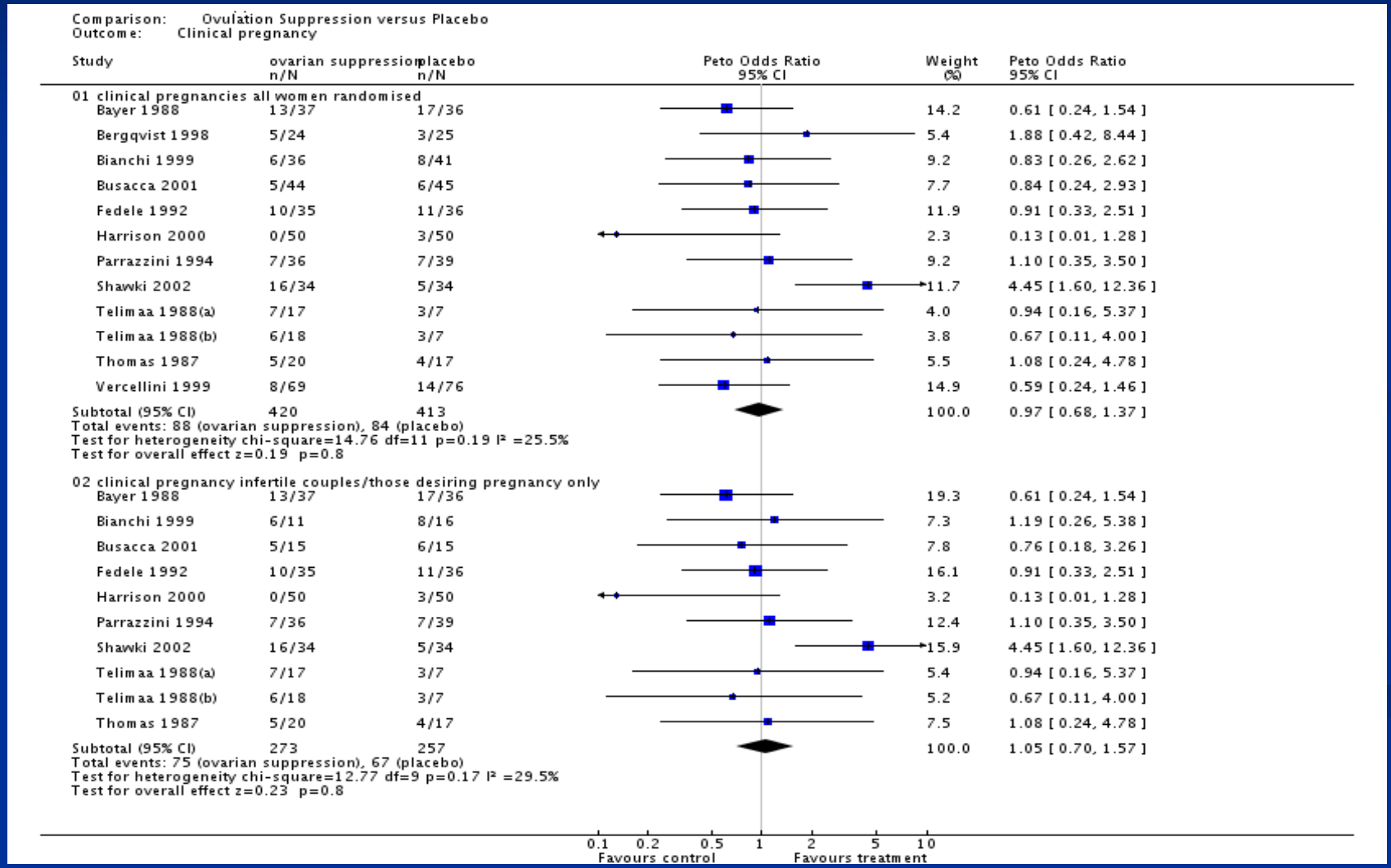
Angiogenesis & increased local estrogen

↓

Endometriosis

How does endometriosis lead to infertility?

Infertility Treatment: No Role for Medical Suppression



Adapted from Yap C et al. *Cochrane Database Syst Rev*. 2004;(3):CD003678.

Surgical Intervention: Fecundity Rate

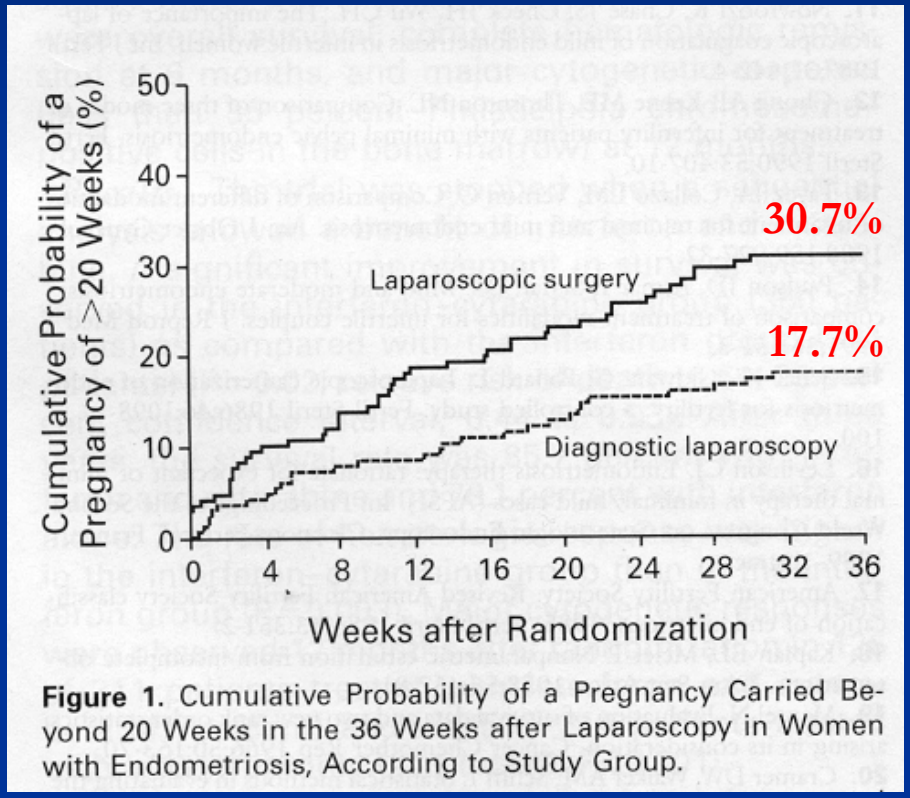


Figure 1. Cumulative Probability of a Pregnancy Carried Beyond 20 Weeks in the 36 Weeks after Laparoscopy in Women with Endometriosis, According to Study Group.

- Control group
  2.4 %
- Intervention group
  4.7 %

Marcoux S et al. *N Engl J Med.* 1997;337(4):217-222.

Why do our treatments fail?

How does endometriosis effect fertility?

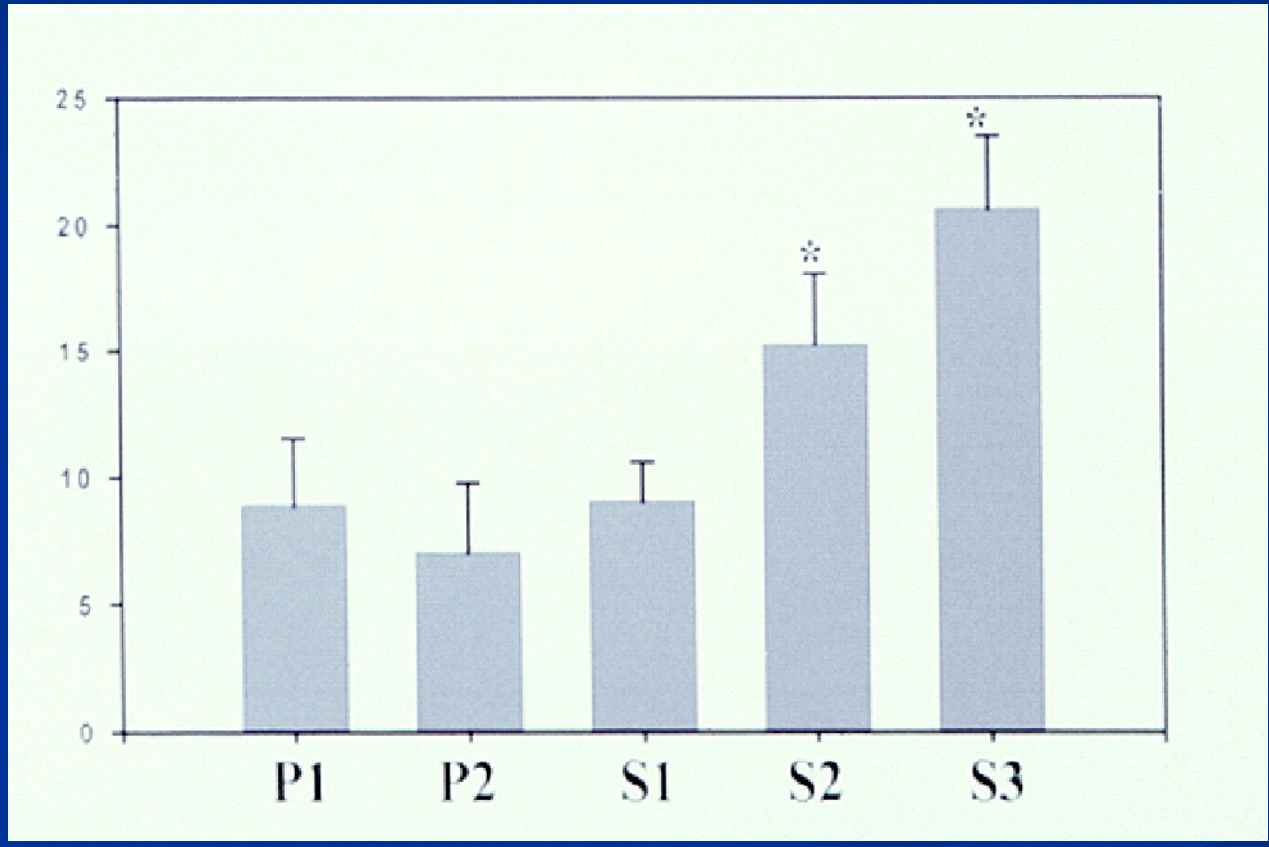
HOXA10 in the Human Endometrium

- HOXA10 is expressed in the endometrium where it is necessary for implantation.
- HOXA10 expression varies with menstrual cycle; epithelial expression dramatically rises at the time of implantation
- Estrogen and Progesterone regulate HOXA10

Taylor et al, J Clin Invest 1998; 101:1379

HOXA10 Expression

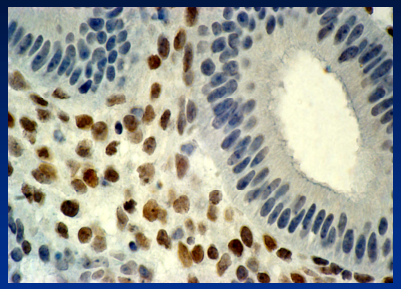
human endometrium



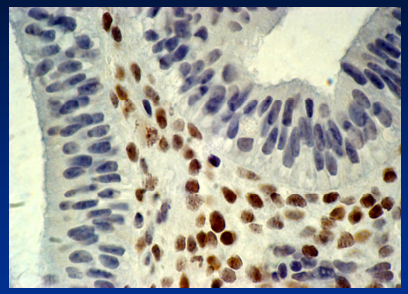
Taylor et al, J Clin Invest 1998; 101:1379-1384

HOXA10 Expression

Implantation Window



d14



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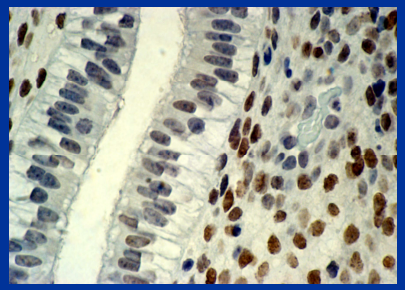

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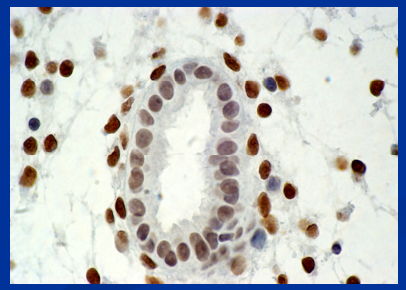

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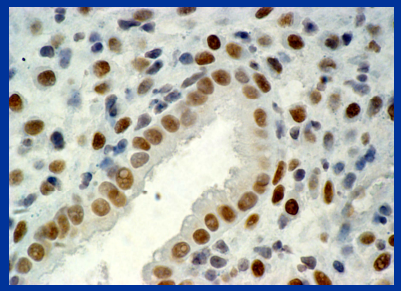

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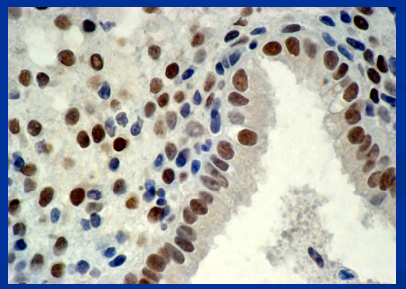
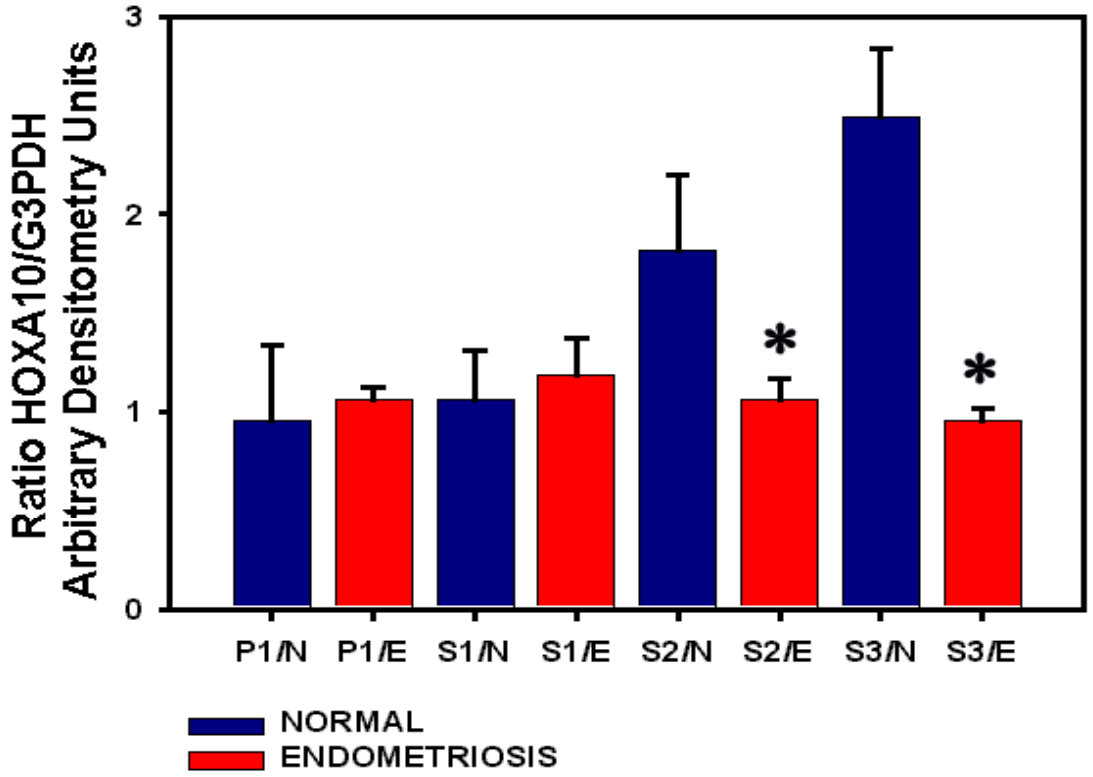


d27

Taylor et al J Clin Invest 1998, 101:1379-1384, Sarno and Taylor JCEM 2005, 90: 533-528.



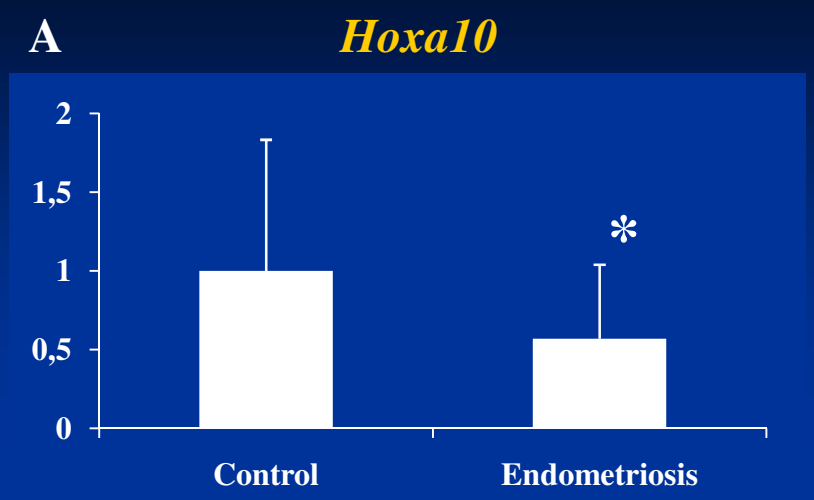
Taylor et al, Hum Reprod 1999; 14:1328

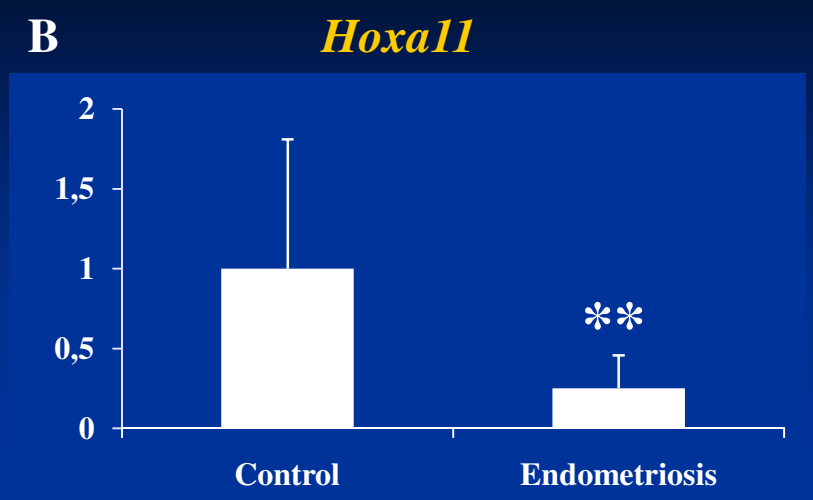
Animal Models of Endometriosis

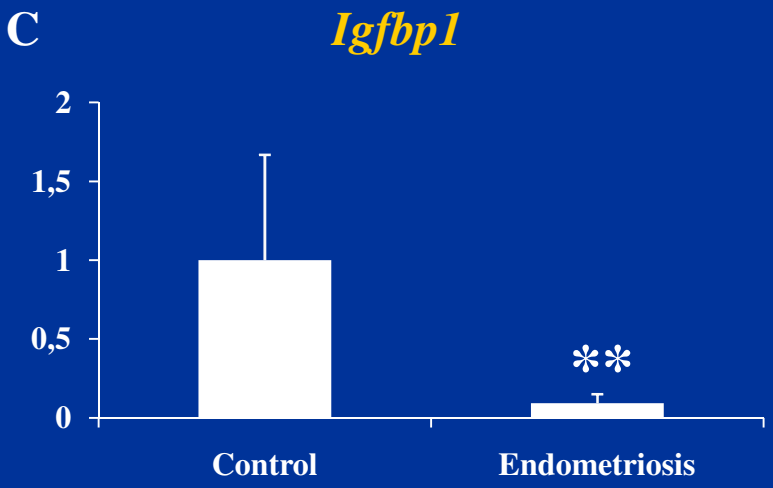
Allows determination of cause and effect

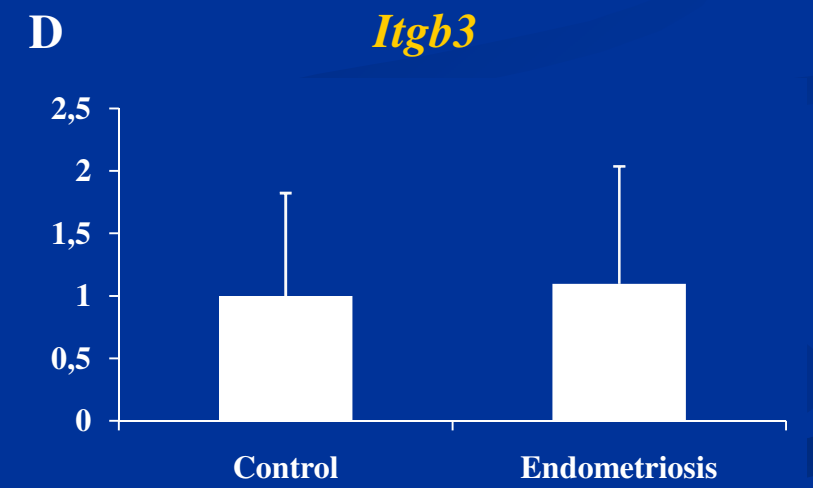
- Mouse
- Non-Human Primate

Murine Experimental Endometriosis



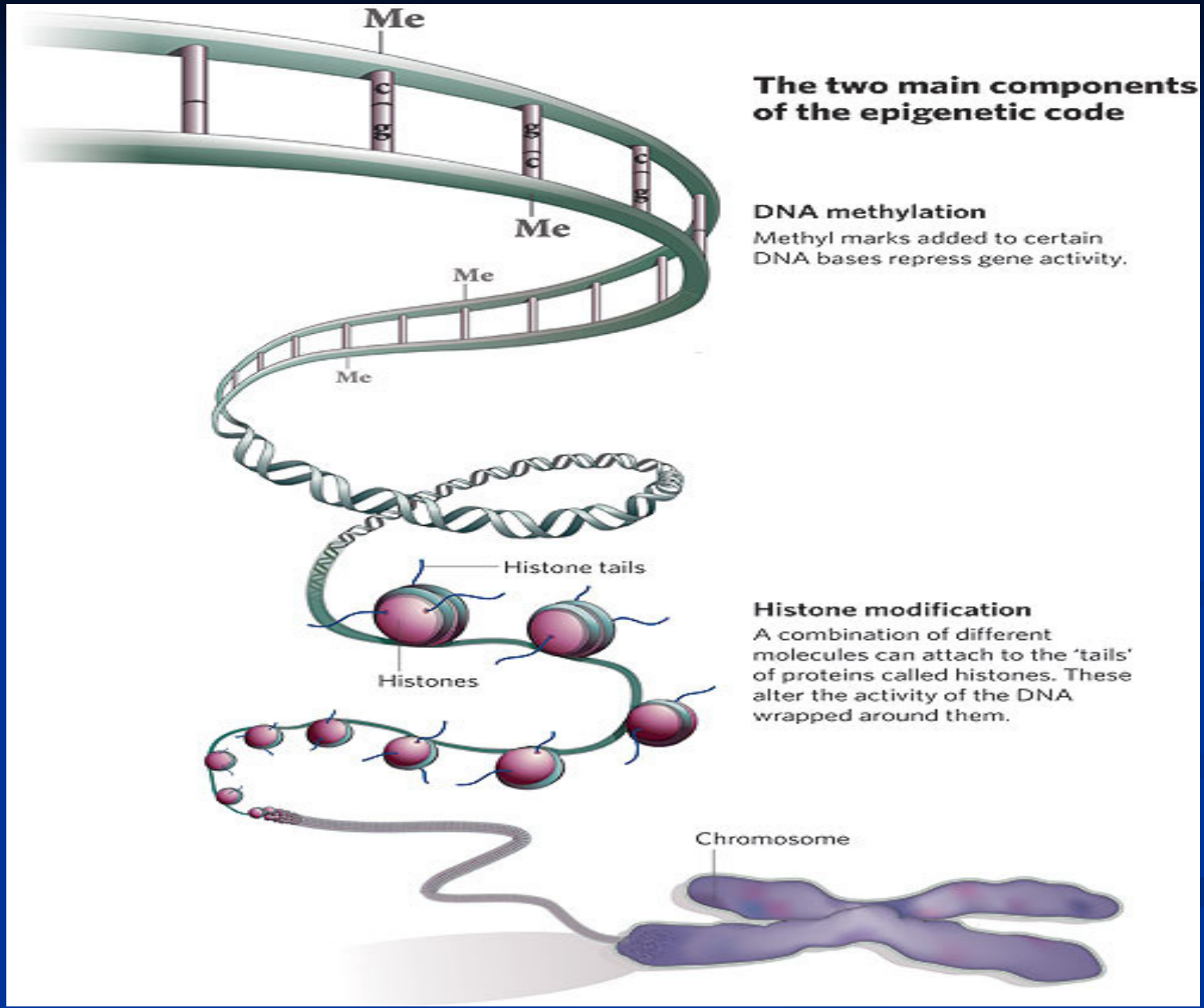




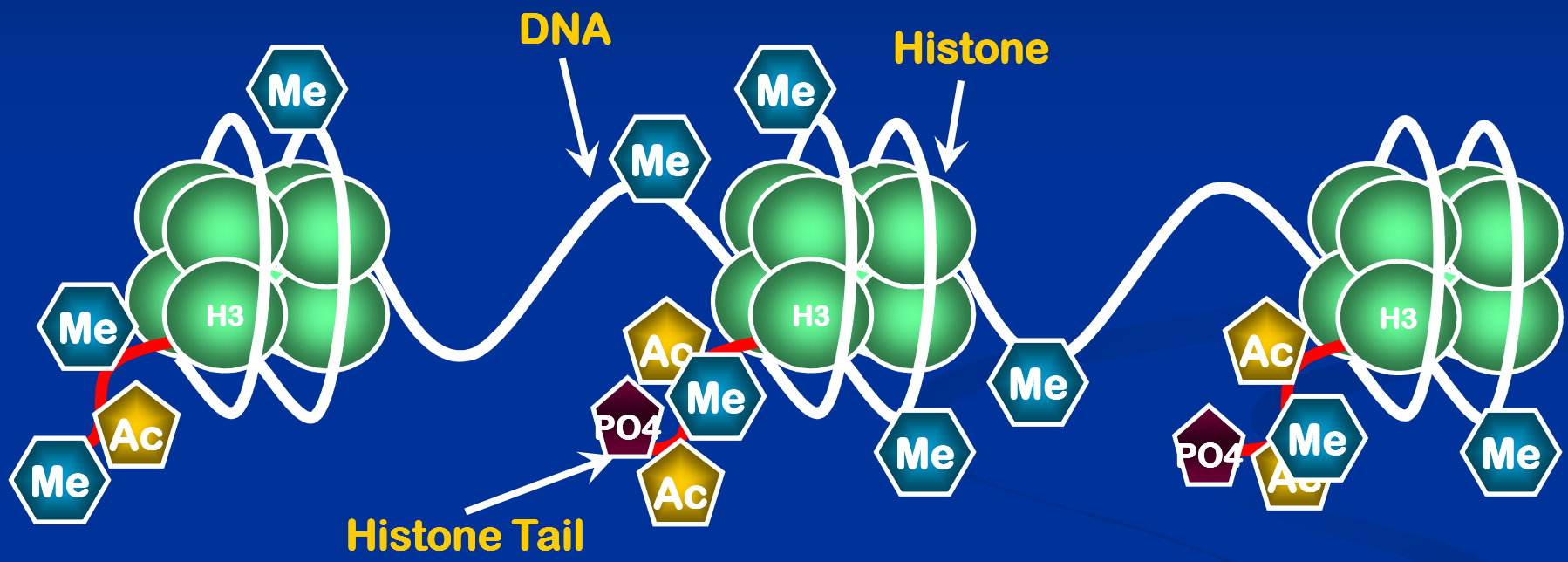


Lee and Tayor, BOR 2009

Epigenetic Alterations


Me
Me
Me
Me
Histone tails
Histones
Chromosome
The two main components of the epigenetic code
DNA methylation
Methyl marks added to certain DNA bases repress gene activity.
Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

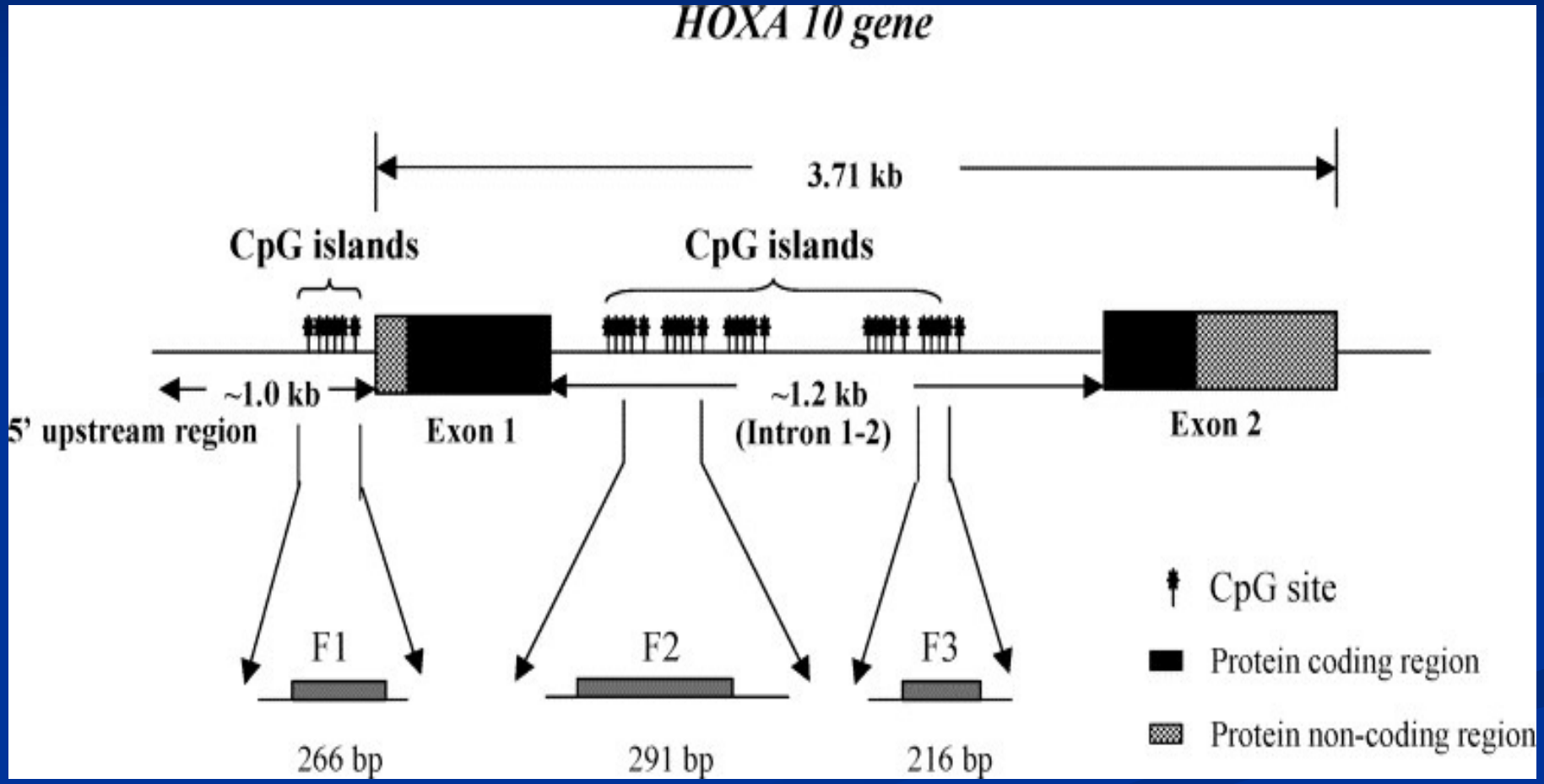
Chromatin Modifications



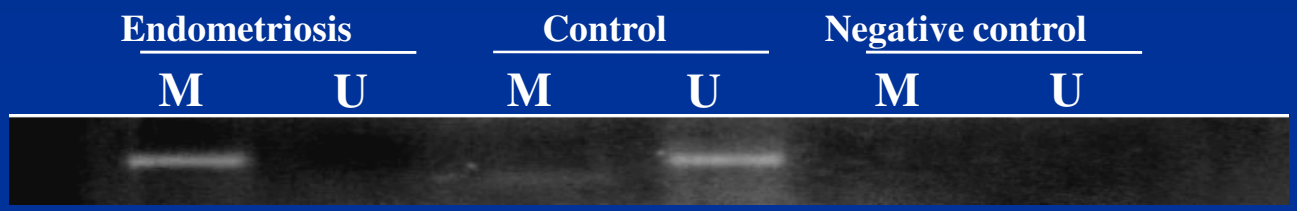
Euchromatin: Gene Activation

Heterochromatin: Gene Silencing

The HOXA10 Gene



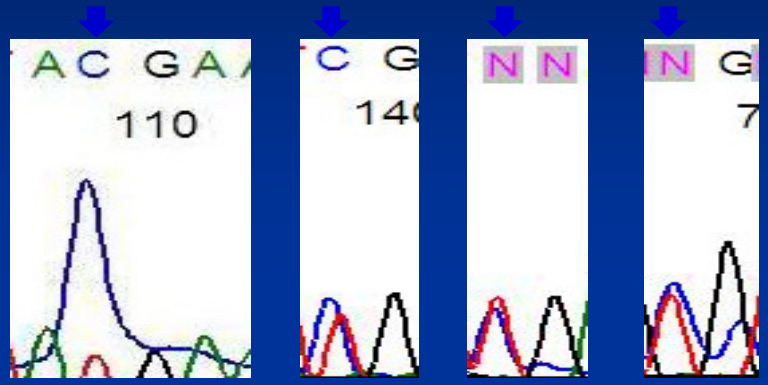
Hoxa10 DNA Methylation



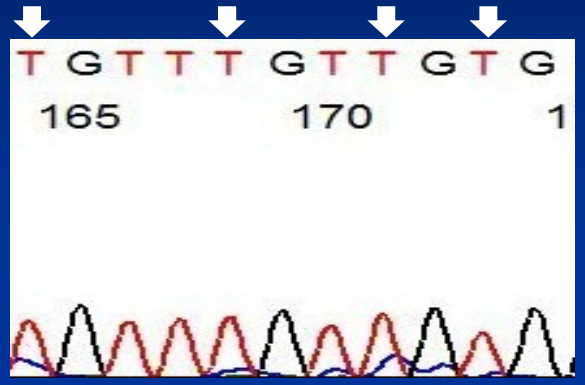
Lee and Taylor, Biol Reprod 2009

Hoxa10 DNA Methylation

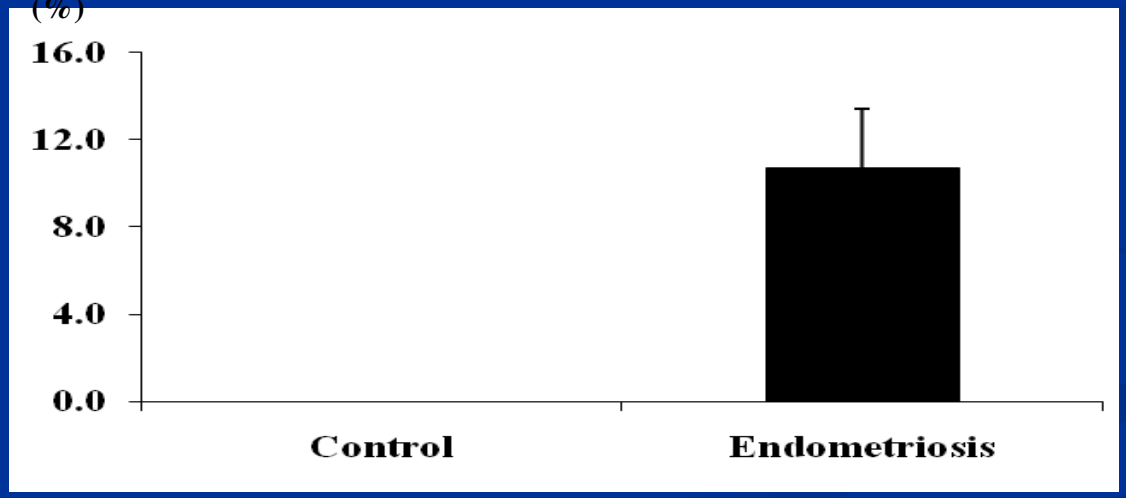
A



B



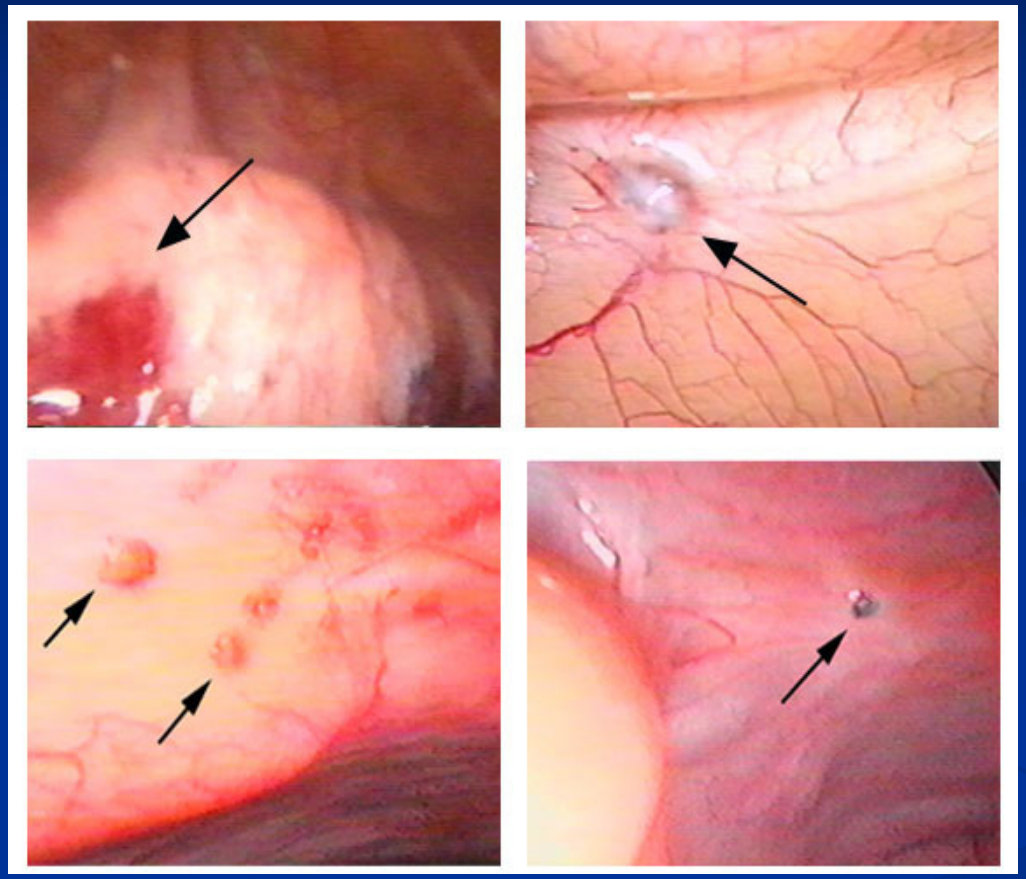
C



Baboons with experimentally induced endometriosis

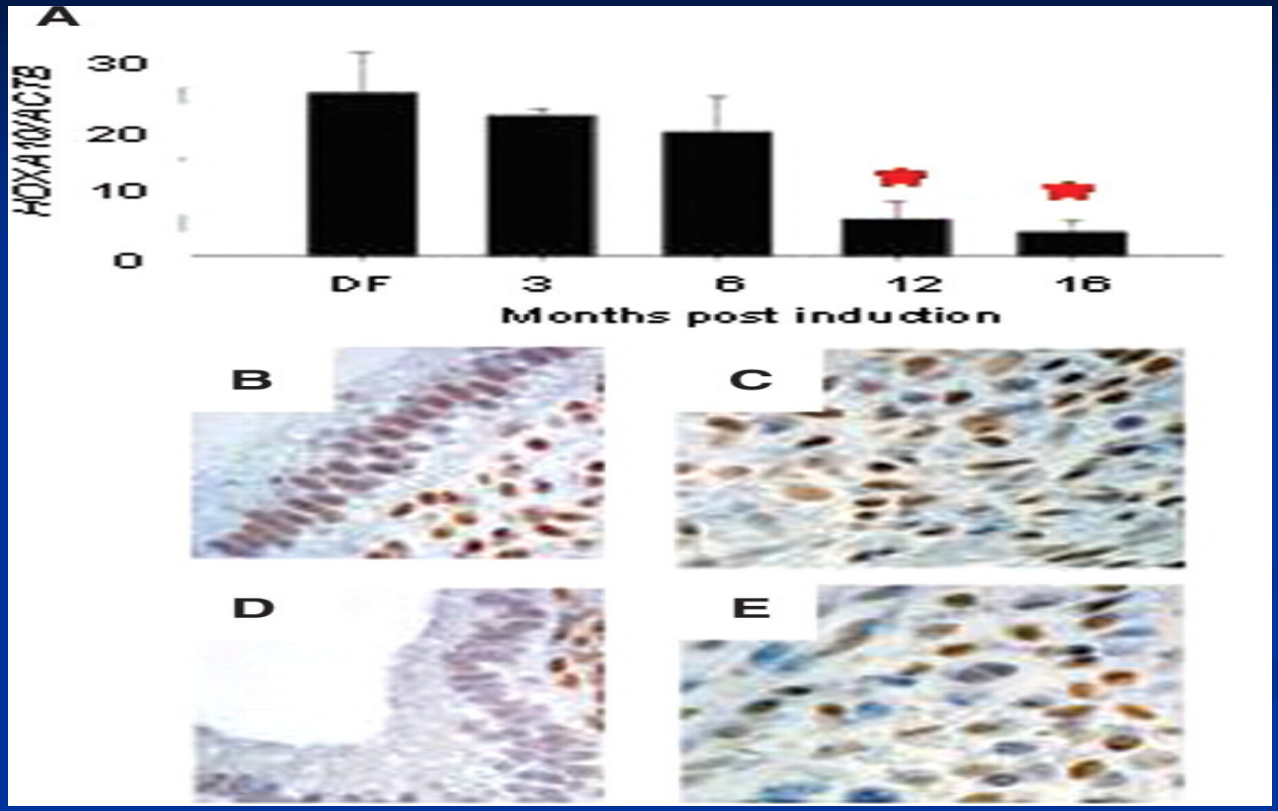
LAPROSCOPIC AND HISTOLOGICAL EVALUATION OF LESIONS

Uterus Peritoneum



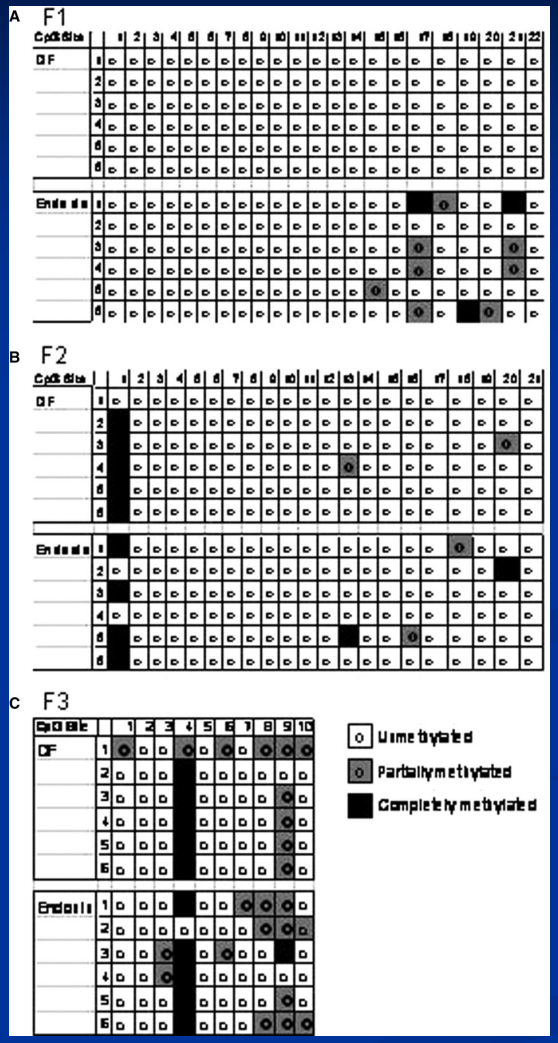
Upper Panel – *One Month*
Lower Panel – *Four Months*

Expression of HOXA10 in the eutopic endometrium of baboons with endometriosis



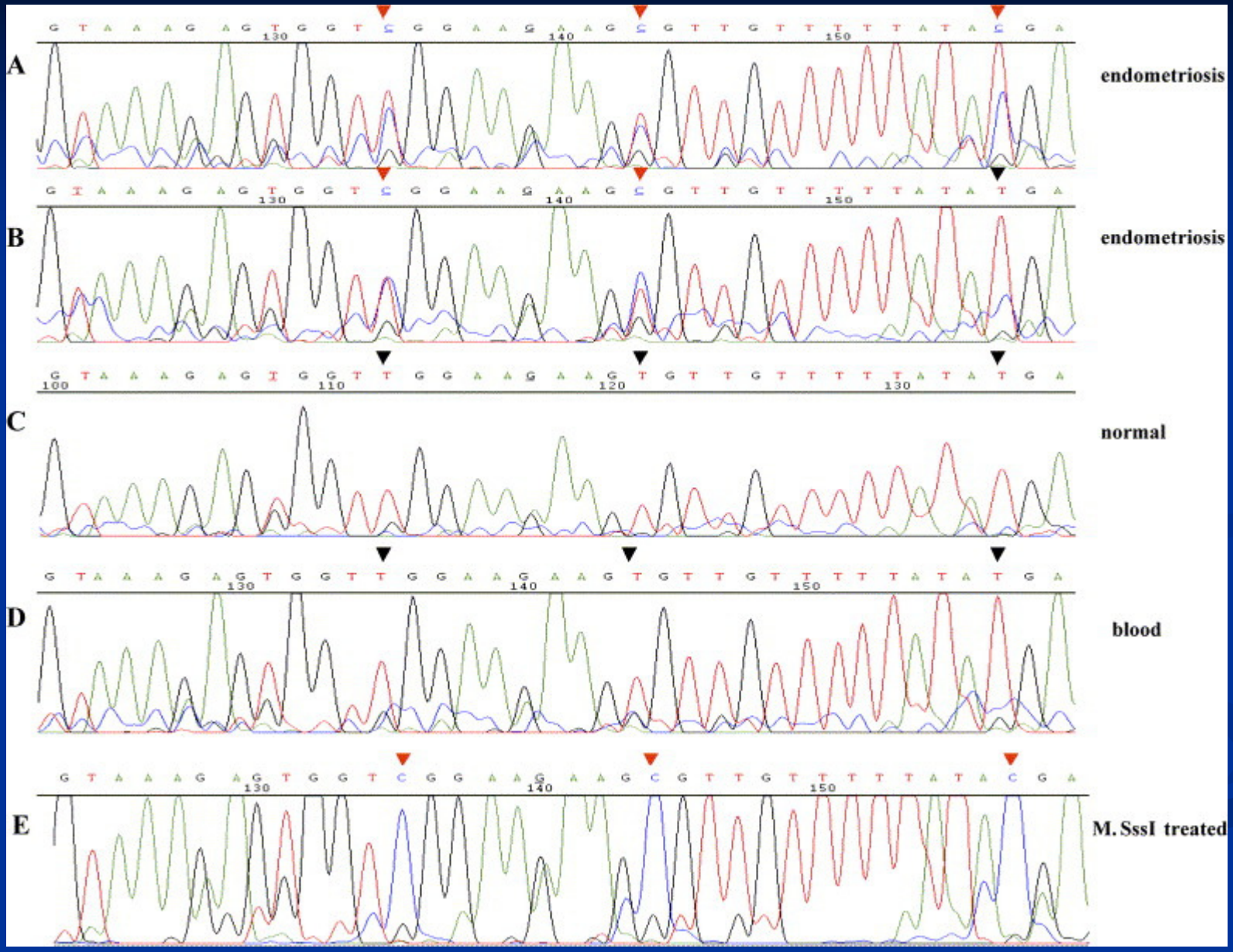
Kim, J.J. et al. Mol. Hum. Reprod. 2007 13:323-332

Methylation of the HOXA10 gene



Kim, J.J. et al. Mol. Hum. Reprod. 2007 13:323-332

Epigenetic changes in HOXA10 in women with endometriosis



Wu et al, Am J Obstet Gynecol. 2005;193(2):371-80.

Surgical Intervention: Fecundity Rate

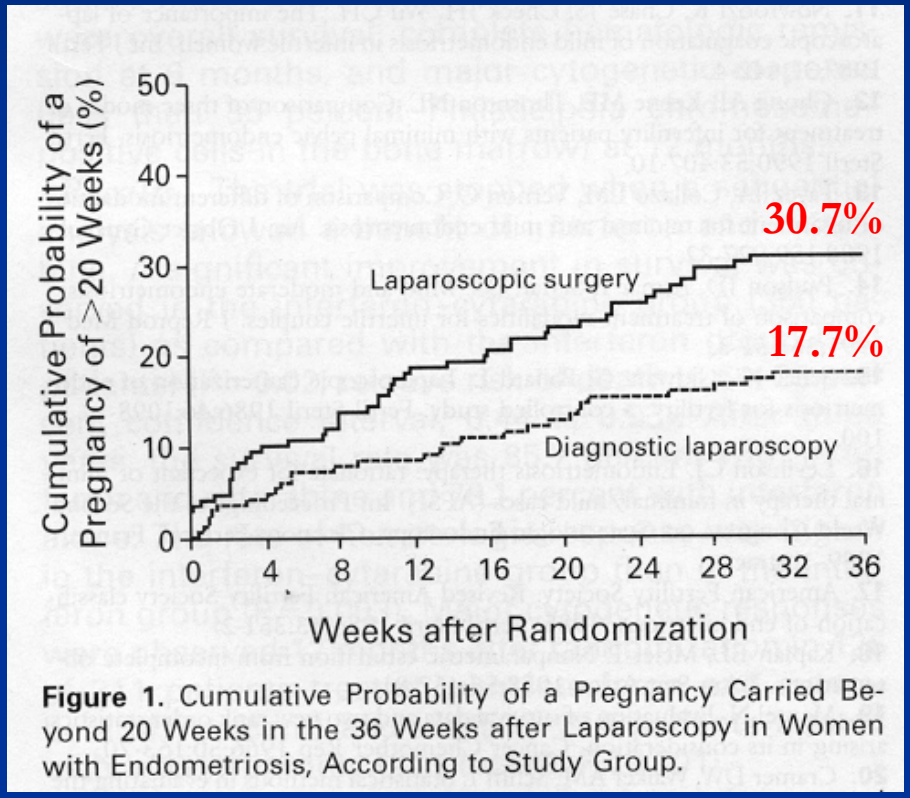


Figure 1. Cumulative Probability of a Pregnancy Carried Beyond 20 Weeks in the 36 Weeks after Laparoscopy in Women with Endometriosis, According to Study Group.

- Control group
  2.4 %
- Intervention group
  4.7 %

Marcoux S et al. *N Engl J Med.* 1997;337(4):217-222.

Conclusion

- **Stem Cells contribute to endometrium and endometriosis**

- **Endometriosis induces irreversible epigenetic changes in the endometrium**
  - Disease may be chronic and not curable

Mobilized stem cells may be capable of replacing endometrial cells that were epigenetically altered by endometriosis.

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