The early life of endometriosis: lessons learned from the baboon

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What is Endometriosis?

- The presence of hormone-responsive endometrial tissue at ectopic sites beyond the uterine cavity
- Associated with chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility
- Affects 1 in 10 women of reproductive age
- Average time to diagnosis is 8-11 years [1]
- Estimated economic burden of ~$22 billion (in 2002) [2]

1. Sinaii *et al.*, 2008. Fertil Steril;89:538-545
2. Simoens *et al.*, 2007. Hum Reprod Update; 13:395-404

How does Endometriosis Develop?

- Halban's theory of hematogenic or lymphogenic spread of viable endometrial cells
- Meyer's theory of coelomic metaplasia of peritoneal mesothelial lining
- Sampson's theory of retrograde menstruation[1]

1. Sampson, 1927. Am J Obstet Gynecol;14:422-469

Limitations in Understanding the Pathophysiology of Endometriosis

- 90% of women experience retrograde menstruation
- Only 10% of women develop endometriosis
- 30-50% of women with endometriosis are infertile

What is the pathophysiology of endometriosis-associated infertility?

Is Endometriosis Associated with Infertility?

TABLE 3

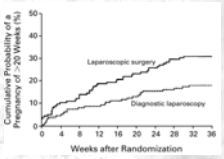
Results of bivariate analysis and multiple logistic regression analyses comparing endometriosis patients with controls.

Outcome	Endometriosis	Control	Crude OR (95% CI)	Adjusted OR* (95% CI)
All patients[a] (n = 2,909)				
Pregnancy rate	25.42	29.52	0.81 (0.72–0.91)	0.63 (0.51–0.77)
Fertilization rate	59.69	65.94	0.95 (0.94–0.95)	0.87 (0.85–0.88)
Implantation rate	12.72	18.08	0.86 (0.85–0.87)	0.86† (0.85–0.88)
Mean no. of oocytes	7.81	7.30	1.06 (1.04–1.08)	0.92 (0.85–0.99)
Peak E_2	3545.04	4399.93	N/A	N/A
Endometriosis only vs. tubal factor only[b] (n = 2,893)				
Pregnancy rate	25.38	27.71	0.88 (0.79–1.00)	0.56 (0.44–0.70)
Fertilization rate	59.50	66.09	0.95 (0.94–0.95)	0.81 (0.79–0.83)
Implantation rate	12.72	18.08	0.86 (0.85–0.88)	0.86† (0.85–0.88)
Mean no. of oocytes	7.79	7.30	1.06 (1.04–1.08)	0.82 (0.75–0.90)
Peak E_2	3545.04	4399.93	N/A	N/A
Stage I-II[c] (n = 2,602)				
Pregnancy rate	21.11	27.71	0.70 (0.56–0.87)	0.79 (0.60–1.03)
Fertilization rate	58.38	66.09	0.93 (0.92–0.94)	0.94 (0.93–0.96)
Implantation rate	11.31	18.08	0.80 (0.78–0.82)	0.88 (0.85–0.90)
Mean no. of oocytes	8.19	7.30	1.11 (1.06–1.14)	0.56 (0.49–0.65)
Peak E_2	5813.38	4399.93	N/A	N/A
Stage III-IV[c] (n = 2,575)				
Pregnancy rate	13.84	27.71	0.42 (0.31–0.57)	0.46 (0.28–0.74)
Fertilization rate	74.47	66.09	1.08 (1.06–1.10)	1.54 (1.39–1.70)
Implantation rate	10.23	18.08	0.75 (0.72–0.79)	not interpretable
Mean no. of oocytes	6.70	7.30	0.94 (0.91–0.98)	not interpretable
Peak E_2	1447.74	4399.93	N/A	N/A

Note: $P<.001$ comparing endometriosis with control group in every outcome category. N/A = not applicable.

Barnhart *et al*, 2002. Fertil Steril; 77:1148-1155

Does Lesion Ablation Improve Fertility?



Marcoux *et al.*, 1997. N Engl J Med; 337:217-222

Table II. Pregnancies and births according to treatment allocation. Italy

	Resection or ablation of visible endometriosis			
	Yes		No	
	No.	(%)	No.	(%)
Pregnancy				
No	39	(76.5)	32	(71.1)
Yes	12	(23.5)	13	(28.9)
Outcome of pregnancy				
Abortion	2	(16.7)	3	(23.1)
Term delivery	10	(83.3)	10	(76.9)

Parazzini, 1999. Hum Reprod; 14:1332-1334

Limitations in Understanding the Pathophysiology of Endometriosis

Does the presence of endometriotic lesions affect reproductive/endometrial function?

OR

Does an inherently defective endometrium affect reproductive function and the growth of endometriotic lesions?

Limitations in Understanding the Pathophysiology of Endometriosis

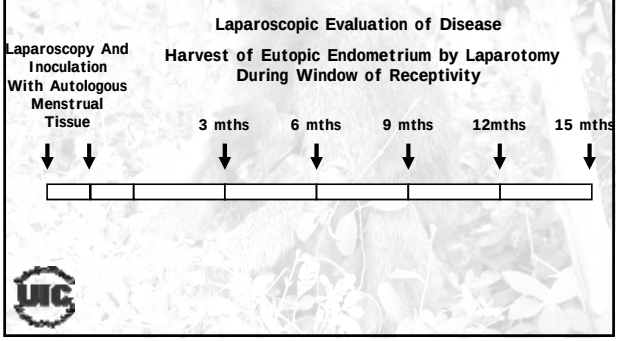
- **Disease has been present for an extended period at the time of diagnosis**
- **Availability of endometrial tissue during the receptive phase is limited**
- **Clinical studies with appropriate controls are not feasible**

Experimental Models of Endometriosis in the Non-human Primate

- Spontaneous endometriosis reported in the macaque and baboon families of monkeys[1-4]
- Surgical diversion of the cervix into the abdomen resulted in endometriosis[5-7]
- Intra-peritoneal seeding with autologous menstrual endometrium in baboons results in endometriosis[8]
- Fecundity was reduced in baboons with advanced spontaneous and experimental endometriosis[9]

1. McClure *et al.*, 1971. J Med Assoc Ga;60:11-13
2. Fanton *et al.*, 1983. Lab Anim Sci;33:597-599
3. Digiacomo *et al.*, 1977. J Am Vet Med Assoc;171:859-861
4. Merrill, 1968. Am J Obstet Gynecol;101:569-570
5. Te Linde and Scot, 1950. Am J Obstet Gynecol;60:1147-1173.
6. Allen *et al.*, 1954. Am J Obstet Gynecol;68:356-375
7. D'Hooghe *et al.*, 1994. Fertil Steril;62:635-638
8. D'Hooghe *et al.*, 1995. Am J Obstet Gynecol;173:125-134
9. D'Hooghe *et al.*, 2006. Fertil Steril; 66:809-813

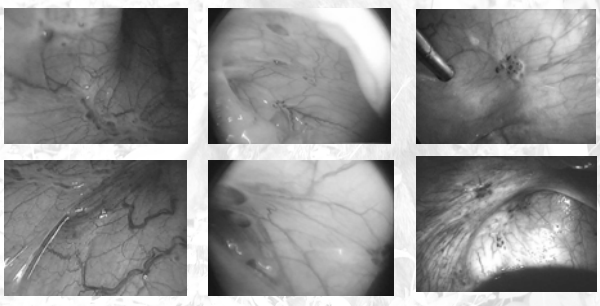
Experimental Induction of Endometriosis in the Baboon



Experimental Endometriosis Recapitulates Spontaneous Disease

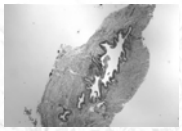
Induced Disease in the Baboon

Spontaneous Human Disease

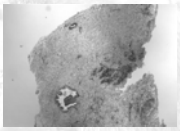


Pathological Diagnosis of Endometriosis

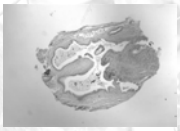
Chocolate Lesion on the uterus



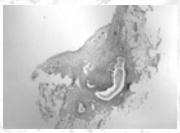
Red/Blue Lesion on the uterus



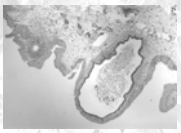
Blue Lesion on the uterus



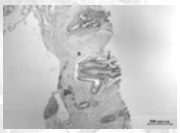
Blue Lesion in peritoneal fat



Chocolate Lesion on the bladder

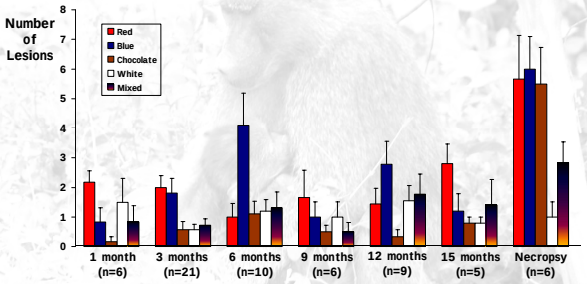


Red Lesion on the body wall



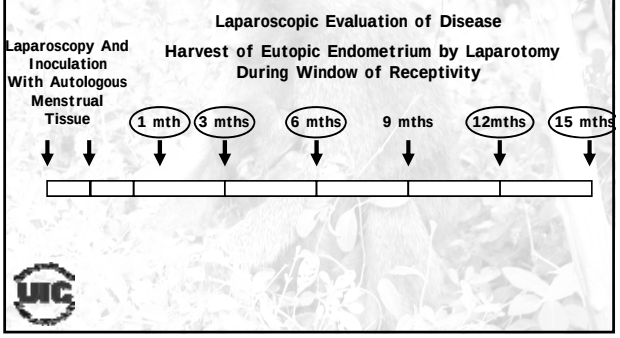
Hastings and Fazleabas 2006. Reprod Biol Endocrinol;4 (Suppl):S7

Characterization of Lesions Visualized by Laparoscope in Baboons Experimentally Induced with Endometriosis

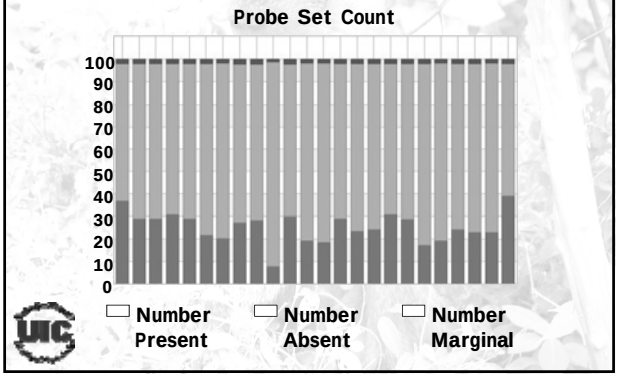


Is endometriosis-associated infertility mediated, in part, by a defective endometrium?

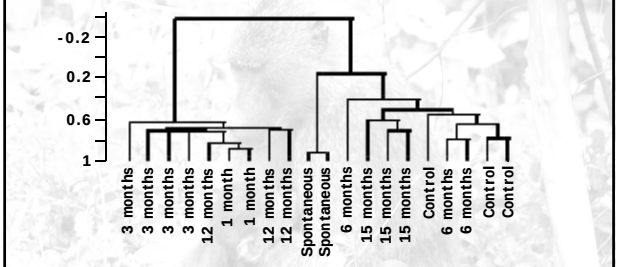
Affymetrix Microarray Analysis in Baboons with Endometriosis



Hybridisation Efficiency of Baboon cDNA to HG U133 Array



Filtered Hierarchical Clustering*



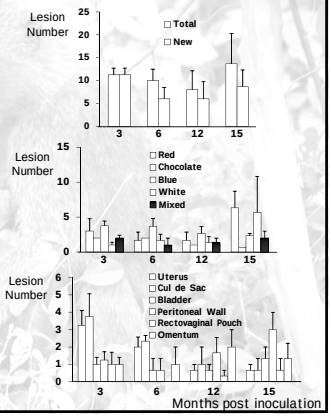
*Probe sets (11687) that are differentially expressed between controls and 1, 3, 6, 12, and 15 months of experimental disease or spontaneous disease. (Biological and hybridization outliers are excluded.)

Time Course of Gene Dysregulation

	Up-regulated	Down-regulated
1 Month	2403	2003
3 Months	746	826
6 Months	51	64
12 Months	767	735
15 Months	102	50
Spontaneous	2464	2811

Time Course of Gene Dysregulation

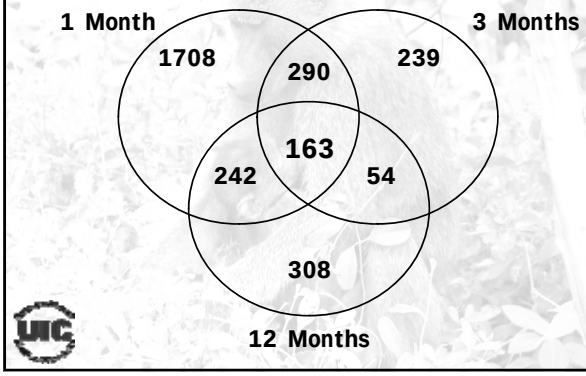
- No correlation with pain
- No correlation with total lesion or new lesion load
- No correlation with lesion type
- No correlation with lesion position



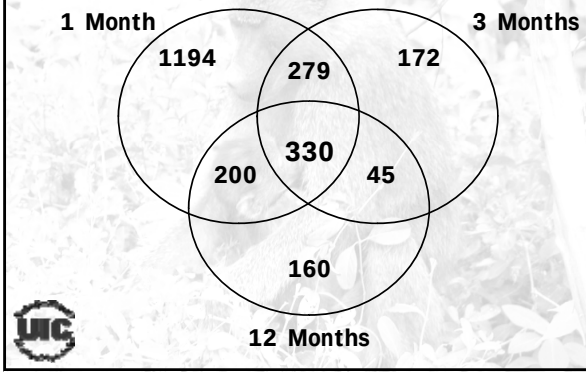
Comparison of Gene Dysregulation

		Up-regulated		Down-regulated	
1 month	3 months	453	(19%)	609	(30%)
	6 months	4	(0.2%)	16	(0.8%)
	12 months	405	(17%)	530	(26%)
	15 months	5	(0.2%)	24	(1%)
	Spontaneous	289	(12%)	145	(7%)
3 months	6 months	6	(0.8%)	11	(1.3%)
	12 months	217	(29%)	375	(45%)
	15 months	1	(0.1%)	13	(2%)
	Spontaneous	109	(15%)	67	(8%)
6 months	12 months	2	(4%)	8	(13%)
	15 months	2	(4%)	0	(0%)
	Spontaneous	15	(29%)	1	(2%)
12 months	15 months	6	(0.7%)	12	(2%)
	Spontaneous	121	(6%)	64	(9%)

Common Up-regulated Genes at 1, 3, & 12 Months of Disease



Common Down-regulated Genes at 1, 3, & 12 Months of Disease



Gene Ontology Classification

Selected Pathways Regulated by Endometriosis

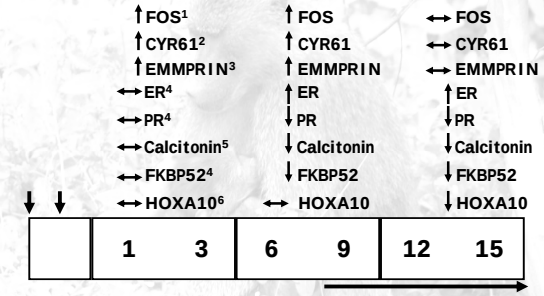
- EGF-R signaling pathway
- TGFβ-R signaling pathway
- WNT signaling pathway
- Olfactory receptor activity
- Rho protein signal transduction
- Ras GTPase activity/signal transduction
- Angiogenesis/vasculogenesis
- Chemokine/cytokine activity
- Immune response/inflammatory response
- Chromatin assembly/disassembly
- Methyltransferase/acetyltransferase activity
- Chromatin binding
- Microtubule cytoskeleton organization and biogenesis
- Cell adhesion/cell matrix/extracellular matrix
- Regulation of cell growth/proliferation
- Nervous system development
- GTPase signal transduction

Commonly Dysregulated Genes Induced vs. Human Endometriosis

1 Month ↑	1 Month ↓		3 Months ↑	3 Months ↓		12 Months ↑	12 Months ↓	
ABCB6	KCNJ4	HIPK3	CDKN2B	ALDH6A1		CARD10	BBP	
AGRN	MEG3	JAG1	CRNN3	BRG1		CDKN2B	JAG1	
CARD10	MGAT3	SUCLG2	MGAT3	SUCLG2		MGAT3	SUCLG2	
CD7	MUC3B		MUC3B	HIPK3		MUC3B		
CDKN2B	PDE4A		RCN3	JAG1		RGR		
CENTB1	RGR		MGAT3	SUCLG2		RIN3		
CHRNE	RIN3		MUC3B			TMEPAI		
COL1A1	SFTPC		RIN3					
DGCR8	SHANK1		TCF3					
EDIL3	TCF15		ZNF205					
GSK3A	TCF3							
HRK	TPARL							
IGFALS	ZNF205							
IGLJ3								

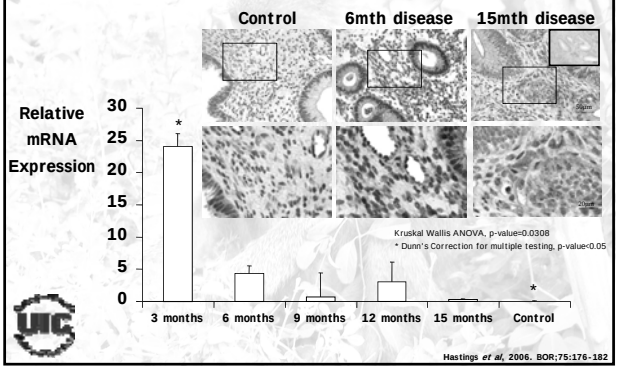
Burney *et al*, 2007. Endocrinol;148:3814-3826

Coordinated Endometrial Changes Associated with Endometriosis



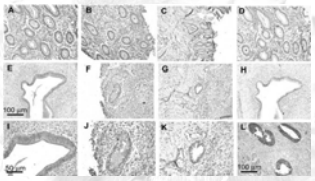
1. Hastings *et al*, 2006. BOR;75:176-182
2. Gashaw *et al*, 2006. BOR;74:1060-1066
3. Braundmeier *et al*. SSR 2006; Abstract #217
4. Jackson *et al*, 2007. Reprod Sci; 14:137-150
5. Jackson *et al*, 2007. Reprod Sci; 14 (Suppl1):A62
6. Kim *et al*., 2007. Mol Hum Reprod;13:323-332

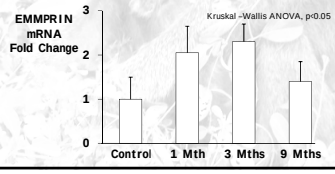
FOS is Transiently Increased in Baboons with Endometriosis



Hastings *et al*, 2006. BOR;75:176-182

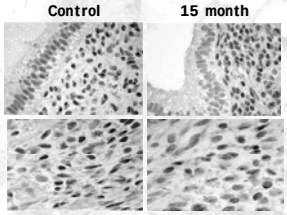
EMMPRIN is Transiently Increased in Baboons with Endometriosis

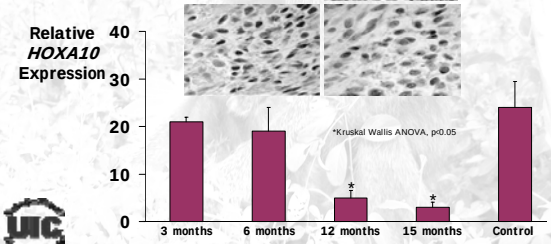




Braundmeier, unpublished

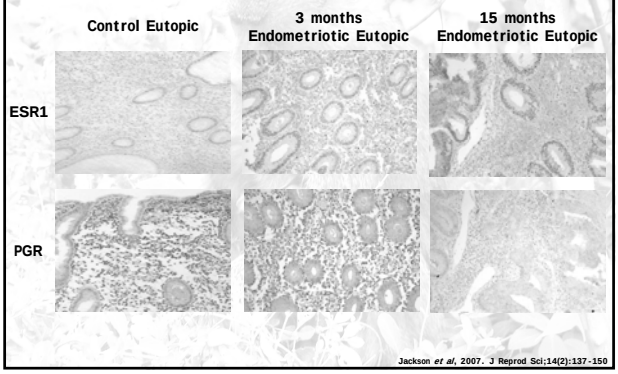
HOXA10 is Decreased in Baboons with Endometriosis





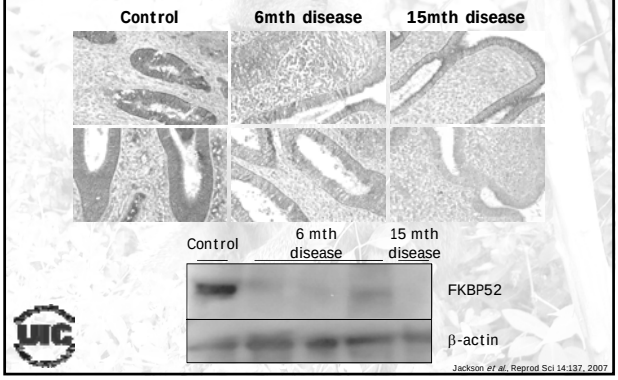
Kim *et al*, 2007 Mol Hum Reprod;13:323-332

SHRs are Altered in Baboons with Endometriosis



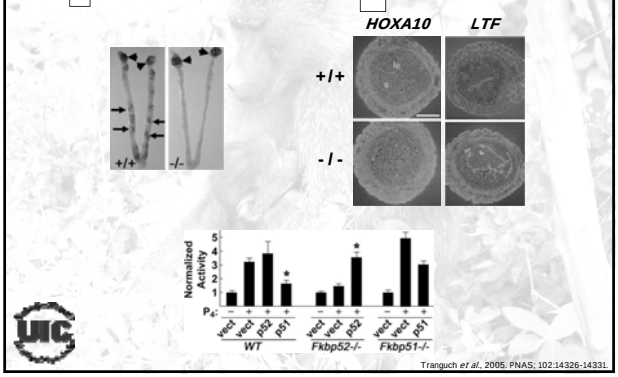
Jackson *et al*, 2007. J Reprod Sci;14(2):137-150

FKBP52 is Decreased in Baboons with Endometriosis



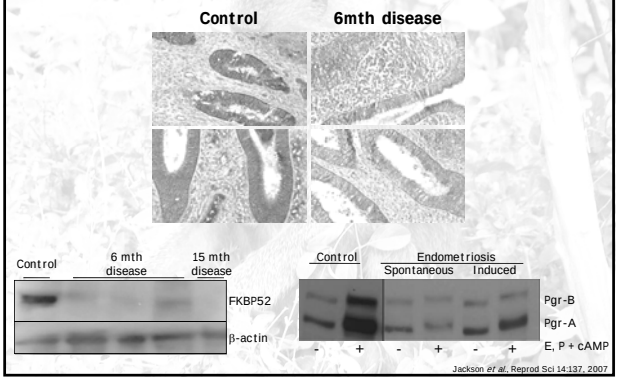
Jackson *et al.*, Reprod Sci 14:137, 2007

FKBP52 is Critical for Uterine Receptivity



Tranguch *et al.*, 2005. PNAS; 102:14326-14331

FKBP52 is Decreased in Baboons with Endometriosis



Jackson *et al.*, Reprod Sci 14:137, 2007

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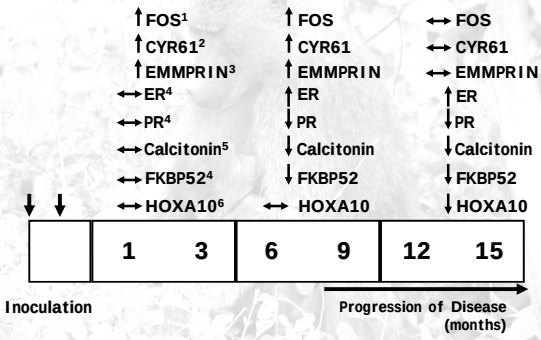

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4. Jackson *et al*, 2007. Reprod Sci; 14:137-150
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6. Kim *et al.*, 2007. Mol Hum Reprod;13:323-332

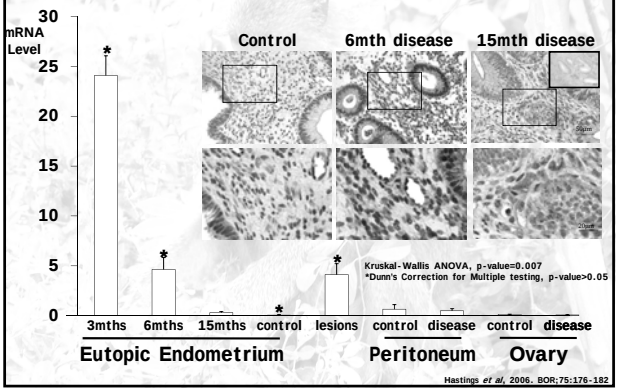
Summary

- Experimental endometriosis in the baboon closely recapitulates the human disease
- Induction of disease has an immediate effect on the transcriptome of the eutopic endometrium
- Endometriosis temporally regulates gene expression

The presence of peritoneal endometriotic lesions impact the transcriptome of the eutopic endometrium

How does endometriosis mediate this massive gene dysregulation?

FOS is Increased in Baboons with Endometriosis



Hastings *et al*, 2006. BOR;75:176-182

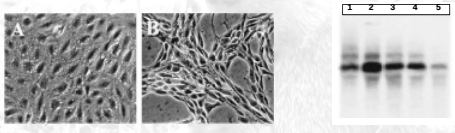
The Role of *FOS* in the Endometrium

- Murine uterine mRNA specifically induced by E2[1,2]
- Mediates murine estrogenic proliferative response[1,2,3]
- FOS protein and mRNA levels are maximal in human proliferative endometrium[4]
- FOS protein and mRNA levels are completely suppressed by MPA *in vivo*[5]
- FOS inhibits the transcriptional activity of PR[6]
- GnRH decreased *c-fos* mRNA in endometrial cells *in vitro*[7]

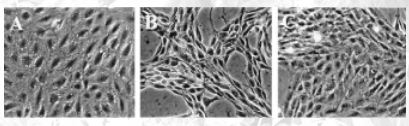
1. Nephew *et al.*, 1993. J Steroid Biochem Mol Biol;46:281-287
2. Cicatiello *et al.*, 1993. Receptor;2:17-30
3. Mendoza-Rodriguez *et al.*, Mol Reprod Dev;64:379-388
4. Reis *et al*, 1999. Fertil Steril;71:1125-1132
5. Kirkland *et al*, 1992. Endocrinol;130:3223-3230
6. Shemshedini *et al*, 1991. EMBO J;10:3839-3949
7. Luo *et al*, 2004. Am J Physiol Endocrinol Met; 287: E991-1001

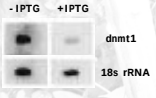
FOS Regulates Methylation

- *FOS* induced transformation of fibroblast mediated by DNA methyltransferase 1 (DNMT1)



- inhibition of *FOS* partially reverses transformation

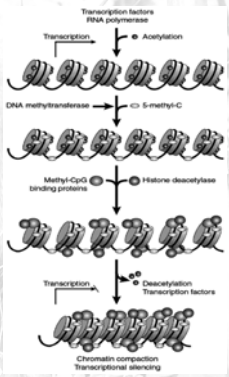




Bakin and Curran, 1999. Science;283:387-390

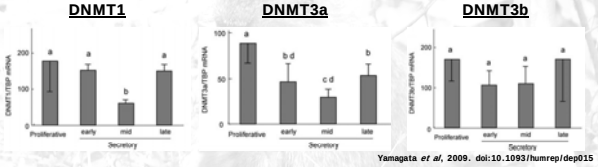
What is Methylation?

- Epigenetic modification of DNA (genomic imprinting)
- Cytosine in CpG dinucleotides
- DNA methyltransferases (DNMT) establish/maintain pattern of methylation
- Histone deacetylase (HDAC) mediates chromatin compaction
- Transcriptional silencing

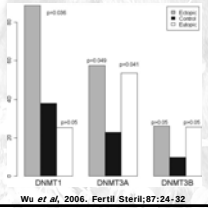


DNMT Enzymes in the Endometrium

DNMT1 DNMT3a DNMT3b



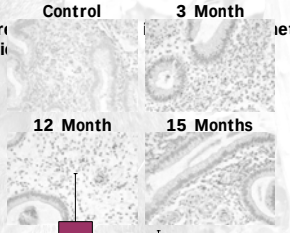
Yamagata *et al*, 2009. doi:10.1093/humrep/dep015

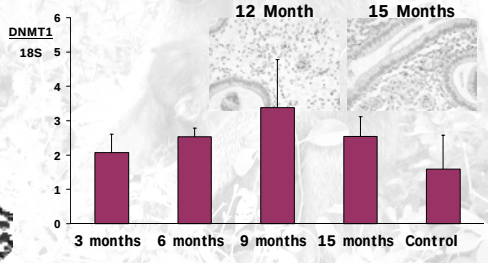


Wu *et al*, 2006. Fertil Steril;87:24-32

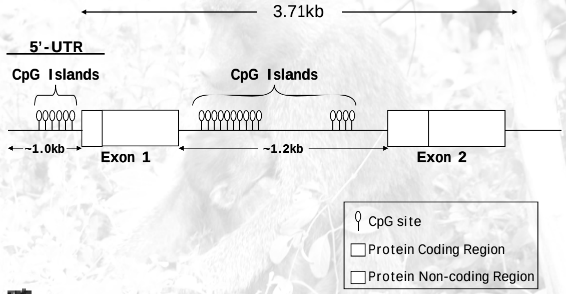
DNMT1 Is Increased in Baboons with Endometriosis

- DNMT3a and DNMT3b were [illegible] (normal or endometri[illegible])etrium





CpG Islands in the HOXA10 Gene



The *HOXA10* Gene is Methylated in Baboons With Endometriosis

CpG	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Disease	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**2**	**1**	0	0	**2**	0
Disease	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Disease	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	**1**	0
Disease	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	**1**	0	0	0	**1**	0
Disease	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Disease	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	**2**	**1**	0	0

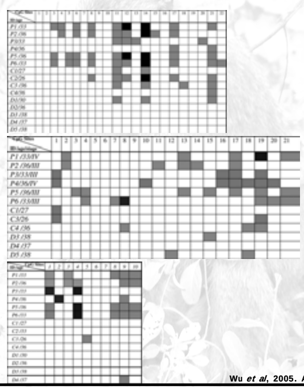
Kim *et al*, 2007 Mol Hum Reprod;13:323-332

The *HOXA10* Gene is Methylated in Women With Endometriosis

F1 Fragment

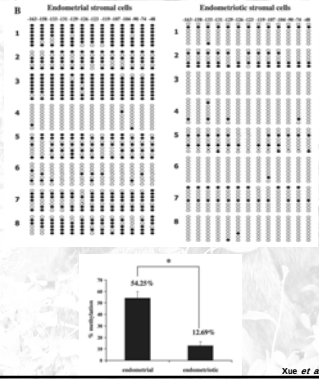
F2 Fragment

F3 Fragment



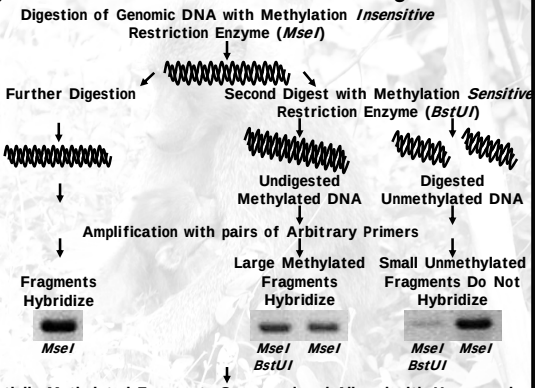
Wu *et al*, 2005. Am J Obstet Gynecol; 193:371-380

ESR1 is Hypomethylated in Human Endometrioma



Xue *et al*, 2007. Biol Reprod: 77:681-687

Methylation Sensitive Restriction Fragmentation



Differentially Methylated Fragments in Baboon Endometriotic Endometrium

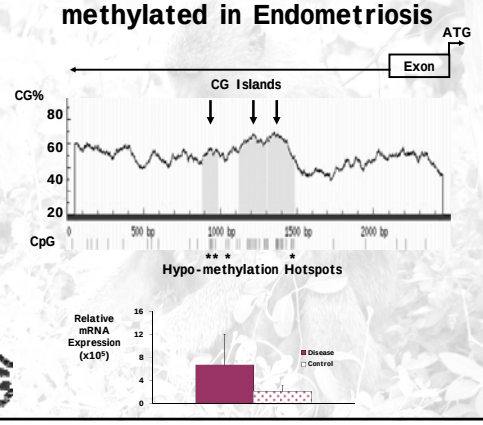
Hypo-methylated

Fragment Size	Gene Homology	Location of Fragment	Gene Function or Description
143bp	WNT2B	5'-UTR	Growth & development
515bp	TMEM97	5'-UTR	Regulation of cell death
548bp	OR4D10	5'-UTR	Olfactory receptor
1036bp	PLEKHG2	5'-UTR	Rho signal transduction
1077bp	SHOX	Intron/Exon	Growth & development

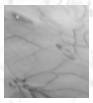
Hyper-methylated

Fragment Size	Gene Homology	Location of Fragment	Gene Function or Description
209bp	LGALS9	5'-UTR	Immune cell apoptosis

The Human WNT2B Gene is Hypo-methylated in Endometriosis



Endometriosis-Associated Infertility – a working hypothesis



Aberrant levels of enzymes involved in methylation → Epigenetic modification of endometrial genome

Altered SHR Profile → Aberrant gene expression

Cell transformation

Non-responsive to the embryonic signal → IMPLANTATION FAILURE