Effects of Sex Hormones on Reconstructed Vaginal Epithelial Tissues and the Relevance to Sexually Transmitted Infections

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Abstract

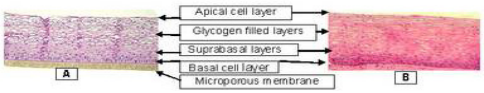
Problem: One of the major barriers to studying sexually transmitted infections (STIs) is the lack of adequate *in vitro* models for the complex tissues that makes up the lower female genital tract. Moreover, little is known about how the sex hormones estradiol-17β (E_2) and progesterone (P_4) influence vaginal barrier and immune function.

Methods of study: MatTek Corp. has developed a vaginal epithelial tissue model (EpivaginalTM), a fully differentiated stratified squamous epithelium derived from primary human vaginal-ectocervical epithelial cells that morphologically resembles normal vaginal tissue. We have conducted an Affimetrix gene array study to describe changes in gene expression in Epivaginal tissue following hormone treatment. Tissues were treated with high dose (100nM) of E_2 or P_4, or low doses (10nM) of E_2+P_4.

Results: Our data confirm the hormonal responsiveness of these tissues, and describe the genes that are up-regulated and down-regulated following exposure to physiologically relevant levels of sex hormones during the differentiation of the tissues. Many affected genes and pathways identified by the Database for Annotation, Visualization and Integrated Discovery (DAVID) reflect classical hormone responses and epithelial differentiation. In addition, a number of pathways that potentially affect STI acquisition were altered including mediators of innate immunity, cell death, and tight junction molecules. For example, E_2-treated tissues showed up-regulation of 30 genes involved in immune responses including *IL-6* and *IL-1α*. On the other hand, E_2-treatment down-regulated the expression of *TLR3* as well as negative regulators of cell proliferation. The E_2+P_4 co-stimulated tissues showed increased expression of pathways that promote wound healing and intercellular junctions, and down-regulation of cytokine *IL-33* and cytokine regulators *BMP3* and *BMP7*.

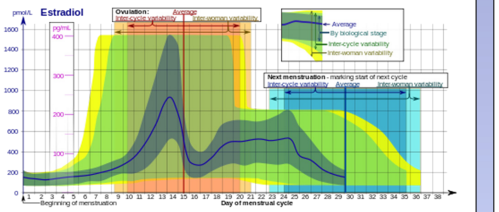
Conclusion: Our studies identify some of the molecular mechanisms underlying endocrine effects on the vaginal epithelial barrier and immune function, and in particular, how they affect the vaginal immune response to sexually transmitted pathogens.

The EpiVaginal™ Tissue Model

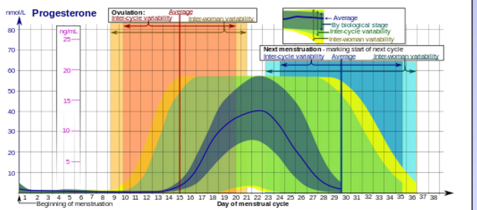


MatTek EpiVaginal™ tissues are produced from primary, human-derived vaginal and ectocervical epithelial cells (VEC) and grown as a stratified squamous epithelial tissue (A) that resembles vaginal explant tissue (B). Partial thickness (PT) tissue contains only VEC, while full thickness (FT) tissue consists of VEC with a fibroblast-containing lamina propria. MatTek is developing an endocervical tissue model from endocervical epithelial cells that is grown as a polarized columnar epithelial layer.

Range of serum estradiol-17β (E_2) and progesterone (P_4)



Mikael Häggström, based on Stricker et al., 2006.



	Serum Hormone	Conc.	Molarity
E_2	Cycle peak	600 pg/mL	2.2 nmol/L
	Pregnancy	40 ng/mL	147nmol/L
P_4	Cycle peak	22 ng/mL	70 nmol/L
	Pregnancy	400 ng/mL	1272 nmol/L

Levels within reproductive tissues are unknown: animal and human studies suggest they could be 2-5x higher (Weems *et al*., 1989 and Cicinelli *et al.,* 1998).

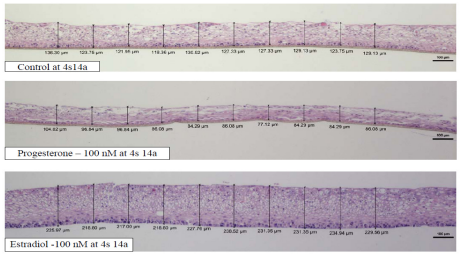
Methods

PT and FT VEC tissues were grown in standard growth medium, with or without the addition of 100 nM E_2 or P_4 or both for 7 days. Total RNA was prepared from quadruplicate wells, pooled and provided to the Boston University Microarray Core for gene expression analysis using the Affymetrix GeneChip® Gene 1.0 ST Array. Genes identified as ≥2-fold up or down regulation in the presence of E_2 or P_4 compared to control wells were determined to be differentially expressed in the presence of hormones.

Table 1: Thickness measurements of hormone treated EpiVaginal™ tissues

Condition	Thickness (uM)	SD (n=10)
Control	126.8	4.82
E_2	227.4	6.89
P_4	88.6	7.62

E_2 and P_4 alter growth of MatTek VEC tissues



H&E stained partial thickness VEC tissues treated with 100 nM E_2 or P_4 for 7 days. Thickness measurements at several locations are shown in the figure and summarized in Table 1.

Genes regulated by E_2 in PT VEC*

Symbol	Gene Name	Fold Change
	Upregulated Genes	
IL1F6	interleukin 1 family, member 6 (epsilon)	12.05
IL13RA2	interleukin 13 receptor, alpha 2	8.98
IFIT1	interferon-induced protein with tetratricopeptide repeats 1	4.58
IFIT3	interferon-induced protein with tetratricopeptide repeats 3	3.41
IL1RL1	interleukin 1 receptor-like 1	3.41
CD22	CD22 molecule	2.97
IL23A	interleukin 23, alpha subunit p19	2.68
IL1A	interleukin 1, alpha	2.63
CD14	CD14 molecule	2.61
IL1R2	interleukin 1 receptor, type II	2.59
IFI44	interferon-induced protein 44	2.48
ESAM	endothelial cell adhesion molecule	2.39
IL1F5	interleukin 1 family, member 5 (delta)	2.37
TNFSF11	tumor necrosis factor (ligand) superfamily, member 11	2.35
CCL20	chemokine (C-C motif) ligand 20	2.33
TNFAIP3	tumor necrosis factor, alpha-induced protein 3	2.31
	Downregulated Genes	
CXCR2	chemokine (C-X-C motif) receptor 2	-2.13
CCL28	chemokine (C-C motif) ligand 28	-2.21
CASP1	caspase 1, (interleukin 1, beta, convertase)	-2.35
IL33	interleukin 33	-2.53
CD36	CD36 molecule (thrombospondin receptor)	-2.88
TLR3	toll-like receptor 3	-5.62
CASP14	caspase 14, apoptosis-related cysteine peptidase	-6.22

* Selected genes

DAVID Pathway analysis of E_2 treatment

In response to 100 nm E_2, many biological processes involving epithelial differentiation and estrogen responses were up-regulated. Additional affected processes included: immune response (p=0.00013), wound healing (p=0.001), regulation of I-kappaB kinase/NF-kappaB cascade (p=0.005), defense response (p=0.01), regulation of cell division (p=0.01), anti-viral response (p=0.02), anti-apoptosis (p=0.02),and immune response-activating cell surface receptor signaling pathway (p=0.04). Molecular functions identified included: IL1R receptor binding (p=0.003), cytokine activity (p=0.01), and growth factor activity (p=0.03). Kegg pathways identified in this list include 6 genes representing FcγR-mediated phagocytosis (p=0.05).

Ontology of genes down regulated in 100 nm E_2 VEC-PT included positive regulation of cell adhesion (p=0.01), negative regulation of cell proliferation (p=0.03), cell projection organization (p=0.04), protein kinase cascade (p=0.04), and other cytokine activity (p=0.04). Kegg pathways identified included leukocyte transendothelial migration (p=0.03) and O-glycan biosynthesis (p=0.04). (Huang, DW *et al* ., 2009)

Genes regulated by E_2+P_4 in PT VEC*

Symbol	Gene Name	Fold Change
	Upregulated Genes	
IL1F6	interleukin 1 family, member 6 (epsilon)	6.59
BPIL2	Bacteriacidal/permeability-increasing protein-like 2	4.58
IL1RL1	interleukin 1 receptor-like 1	2.81
CD14	CD14 molecule	2.62
CDH26	Cadherin 26	2.36
IFRD1	Interferon related developmental factor	2.16
MUC1	mucin 1, cell surface associated	2.02
IL1 F9	Interleukin 1 family, member 9	2.00
	Downregulated Genes	
IL33	interleukin 33	-2.13
CD36	CD36 molecule (thrombospondin receptor)	-2.25
TLR3	toll-like receptor 3	-3.05
BMP7	Bone morrphogenetic protein 7	-3.23
BMP3	Bone morphogenetic protein 3	-3.24
CASP14	caspase 14, apoptosis-related cysteine peptidase	-6.93

* Selected genes

Pathway analysis of E2+P4 treatment

In response to 10nm E_2 + 10nM P_4, VEC-partial thickness (PT) up regulated many biological processes involving epithelial differentiation and response to estrogen. Equally, keratinization (p=0.0001), wound healing (p=0.007), growth regulation (p=0.045).

Down regulated included regulation of cell-substrate adhesion (p=0.01), response to wounding (p=0.01). Cytokine activity (p=0.008) and PPAR signaling (p=0.02) were down regulated.

qPCR Validation primers

Increased:
Keratin 20
Cytochrome P450, family 2, subfamily B, polypeptide 6
Trefoil factor 1
Glycogen synthase 2
Decreased:
Mucin1

Conclusions

- High levels of E_2 alter numerous biological processes in both PT and FT stratified squamous vaginal epithelium, including immunologic functions.
- By comparison, high levels of P_4 alone had very few effects on gene expression. However, P_4 receptors may require E_2 priming.
- Data must be validated by RT-PCR. Pathways implicated will be confirmed using tissue from additional donors.
- Future studies will utilize more physiologic levels of E_2 and P_4 associated with the menstrual cycle and pregnancy, and E_2/ P_4 combinations. Hormone effects on STI infection will also be examined.

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