

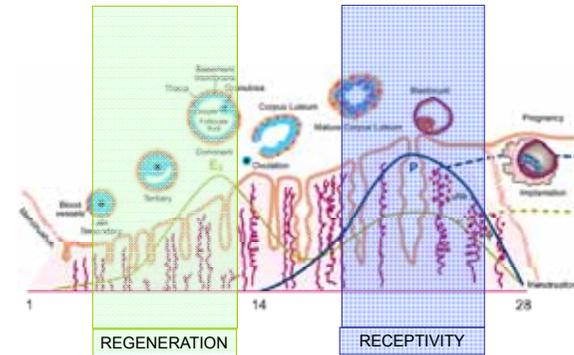


Sex hormone receptor regulation of pathways and players in implantation

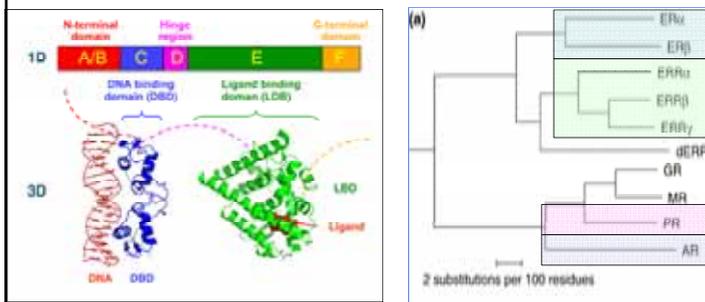
Philippa Saunders  
Centre for Reproductive Health  
Edinburgh



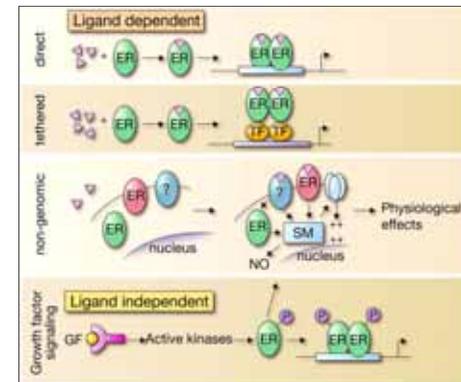
Human endometrium: a sex steroid target tissue



Steroid receptor super-family



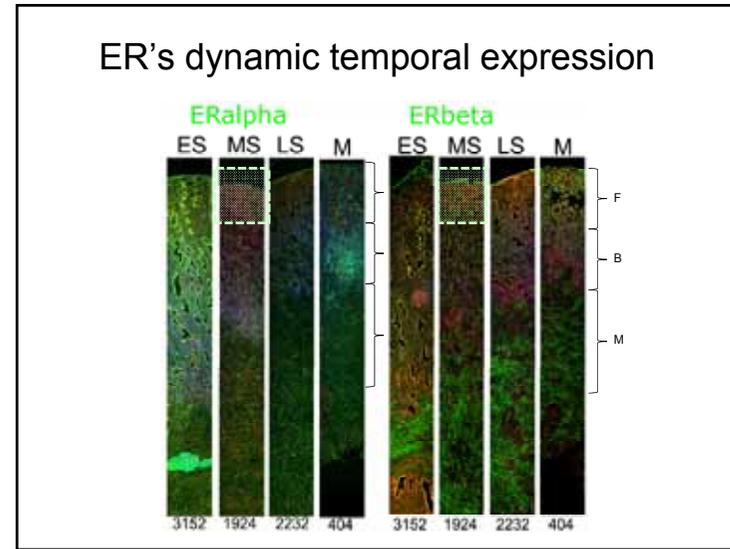
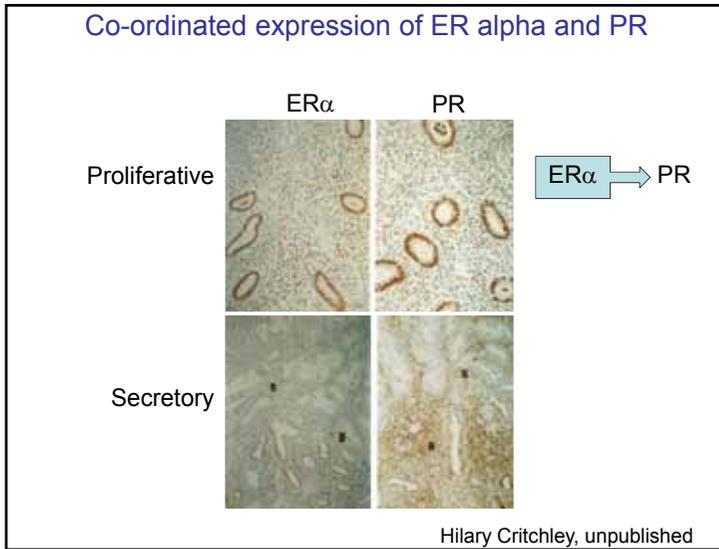
Steroid receptors and gene regulation



Heldring, N. et al. Physiol. Rev. 87: 905-931 2007

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Physiological Reviews



### Steroid regulation of implantation

**Strong evidence**

- Brief receptive window
- Progesterone plays a key role
- ERα-PR co-regulation
- Key processes include
  - stromal cell decidualisation
  - inflammation
  - vessel maturation
- PR antagonists increase expression of AR

**Emerging evidence**

- Local biosynthesis of steroids is important
- Oestrogens play a role in inflammatory processes
- AR dependent gene expression may alter receptivity
- ERRs mediate stromal cell function

### Endometrial 'receptivity'

Vascular maturation

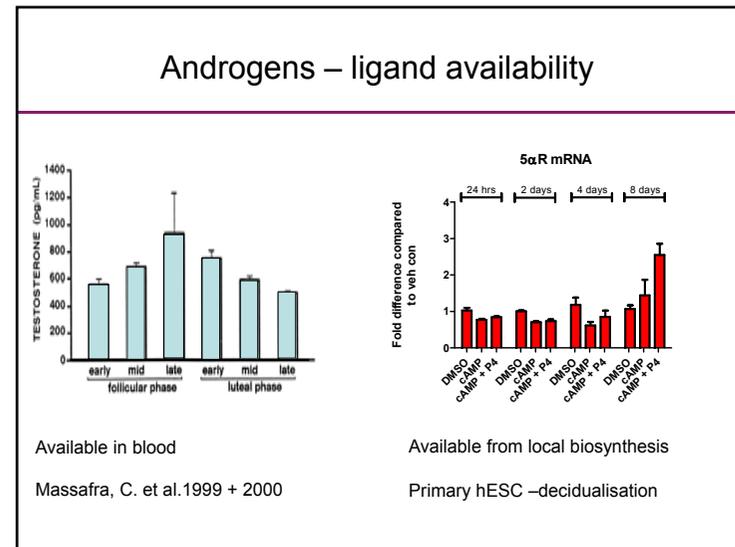
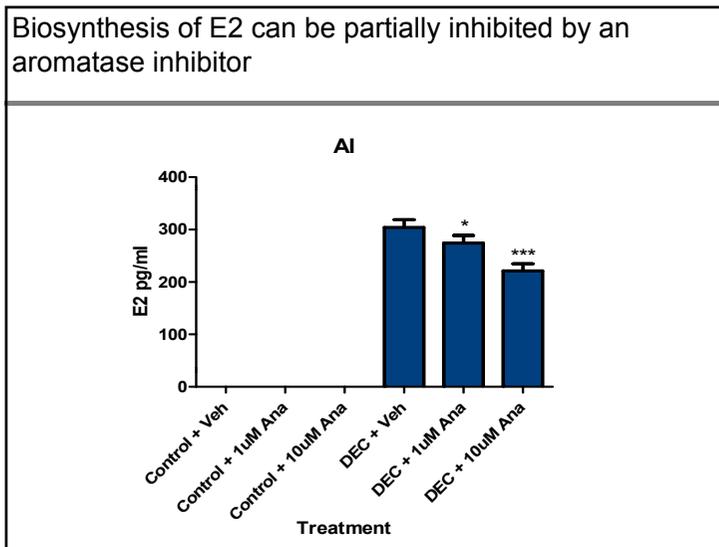
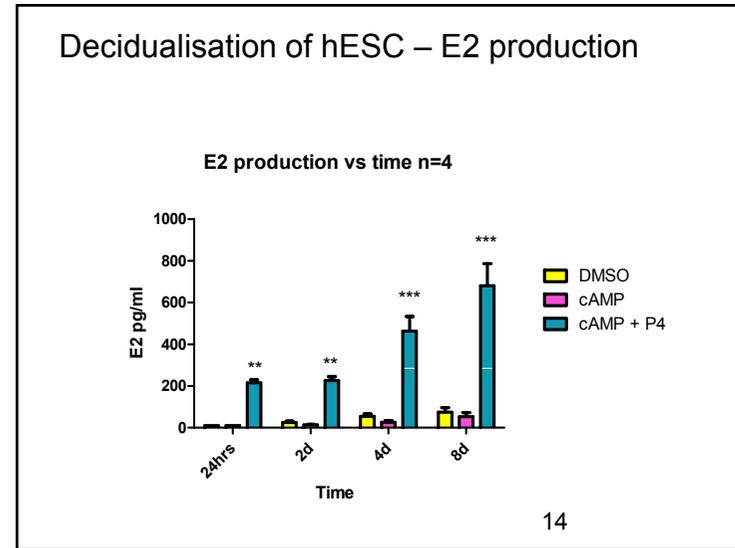
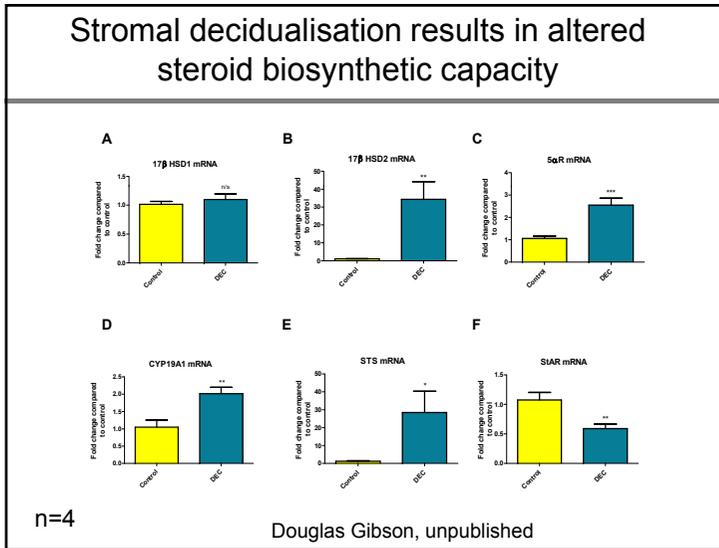
Decidualisation

Inflammation

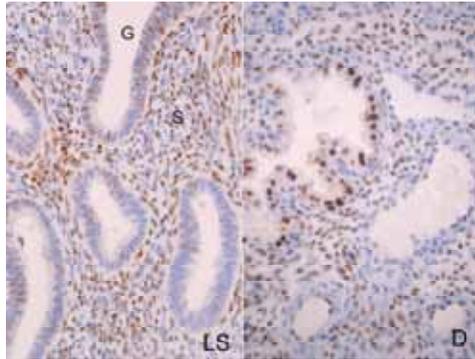
uNK

Junqueira and Carneiro, Basic Histology





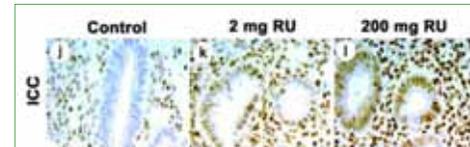
### Temporal differences in receptor expression



Endothelial and immune cells are immuno-negative

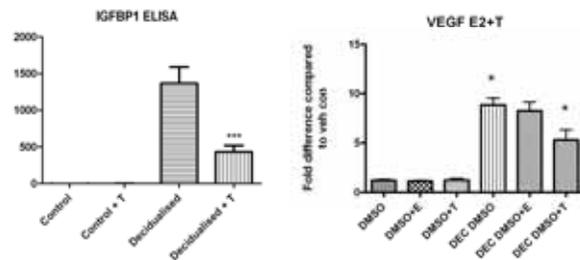
### A role for androgens in endometrial function?

- Administration of androgens results in epithelial atrophy
- Treatment with PR antagonists up-regulates expression of AR
- Women with PCOS have increased risk miscarriage and EndoCa



Macaque model  
Brenner group  
+RU486  
AR immunos

### Androgens have a negative impact on decidualisation



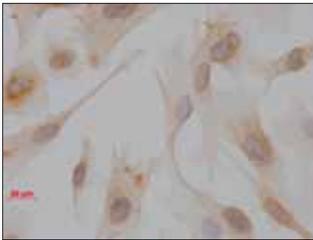
hESC+P4, cAMP 8 days; Douglas Gibson, unpublished

### Targets of androgen action?

- **Resources**
  - Tissue - RNA, total protein, immunos
  - Primary human stromal cells
  - TERT immortalised stromal and epithelial cell lines (3)
  - Ishikawa cells (AR+)
  - Bioinformatics
- **Strategies**
  - Cell based studies - +/- T or DHT, decidualisation, candidates from studies on male reproduction
  - Data mining and validation of targets
  - Arrays using primary stromal cells
    - DHT 2h, 8h
    - Illumina platform

### Primary hESC protein expression

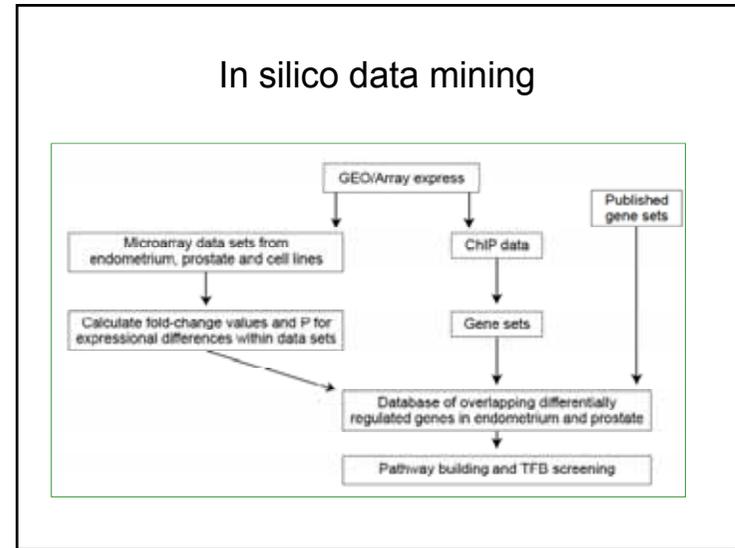
CD10 - stromal cell marker



Androgen receptor



Jacqui Lowrey unpublished. Primary hESC proliferative phase without T



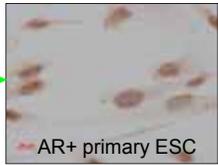
### In silico data mining

- 15 genes 'androgen target gene set
- 14/15 expression in endometrial tissue extracts
- 12/15 androgen regulation in hESC confirmed
- 3 novel
- Pathway/process analysis

### hESC array study

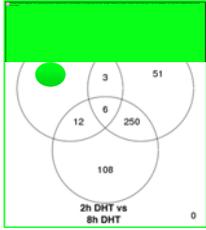
Proliferative phase





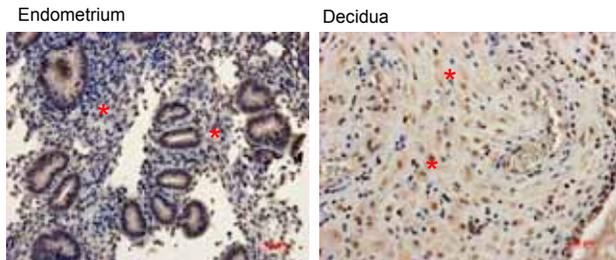
AR+ primary ESC

+/- DHT  
2 or 8h  
Illumina  
Arrays  
n=5



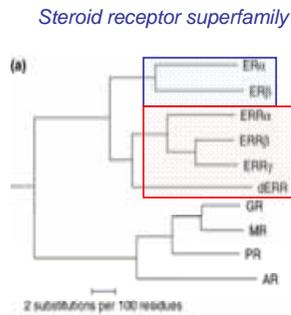
Selected Androgen Regulated Pathways	pValue
Cell adhesion_Cadherin-mediated cell adhesion	0.00006565
Development_Regulation of epithelial-to-mesenchymal transition (EMT)	0.0002119
Development_Slit-Robo signaling	0.002109
Cytoskeleton remodeling_Neurofilaments	1.222E-06
Transcription_Role of heterochromatin protein 1 (HP1) family in transcriptional silencing	0.0005615
Apoptosis and survival_Role of IAP-proteins in apoptosis	0.0003382
Apoptosis and survival_Anti-apoptotic TNFs/NF-kB/IAP pathway	0.006355

### AR-regulated candidate: Prune 2



Antigen retrieval, 1 in 50, Sheila Macpherson unpublished

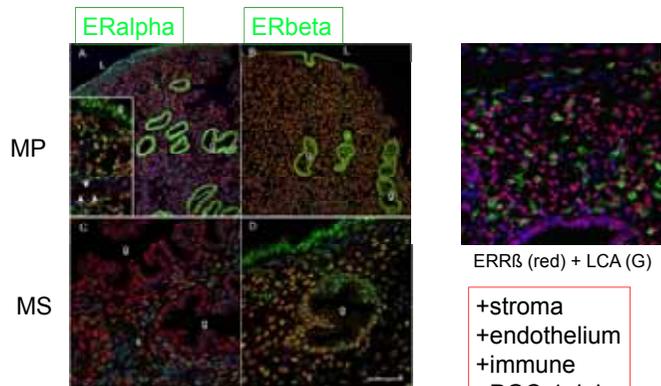
### Estrogen-related receptors: ERR $\alpha$ , ERR $\beta$ and ERR $\gamma$



#### 'Orphan' receptors

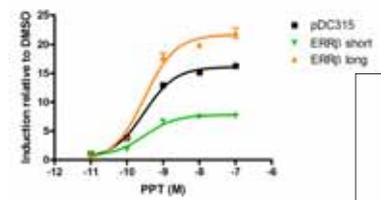
- Role in modulating ER-dependent gene expression?
  - Activity of ERRs altered by tamoxifen
  - Influence expression of E-induced genes (pS2)
- Roles in, germ cell function (KO), placenta (KO) and breast cancer all reported

### ERR $\beta$ protein is expressed in human endometrium

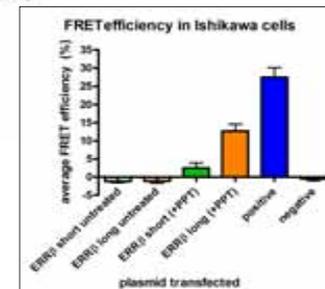


- +stroma
- +endothelium
- +immune
- +PGC-1alpha

### Impact of ERR $\beta$ on cell function?

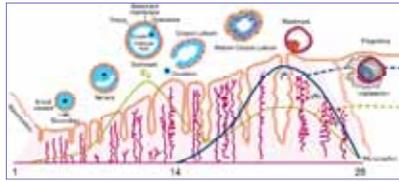


- ERE reporter assay
- ER $\alpha$  positive cells
- ERR transient transfection



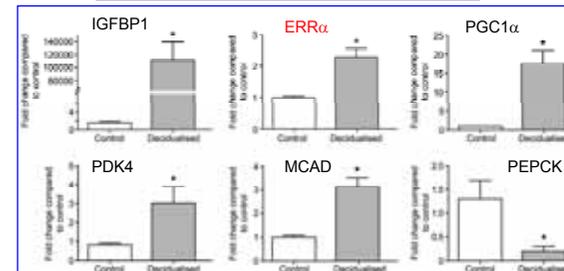
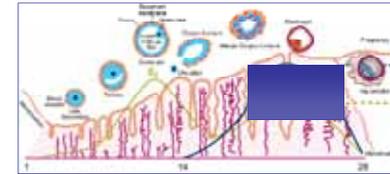
Hetero-dimers-ER $\alpha$ +ERR $\beta$ ?

### A role for ERR $\alpha$ in decidualisation?

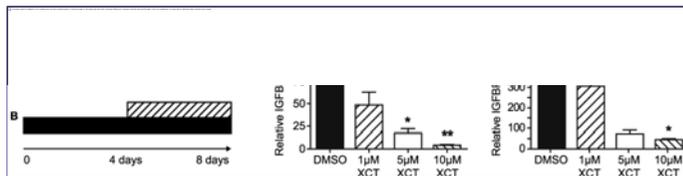
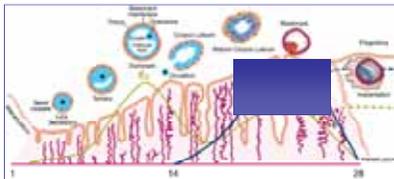


- **Old literature**
  - mid secretory phase = glycogen accumulation, giant mitochondria, changes in lipid content
- **New literature**
  - ERR $\alpha$  involved in regulation of metabolic processes including mitochondrial genesis and fatty acid oxidation
  - ERR $\alpha$  KO mice - deficits in the function of mitochondria and macrophages, resistance to high fat diet induced obesity

### Expression of ERR $\alpha$ and decidualisation

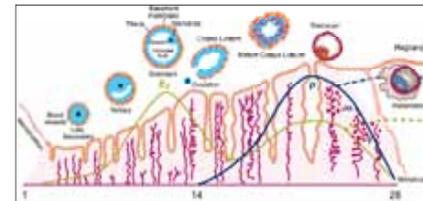


### Expression of ERR $\alpha$ and decidualisation



Bombail et al 2010 JCEM [in press]

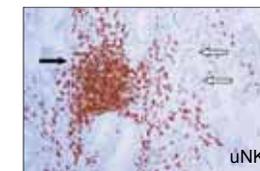
### Endometrial 'receptivity'

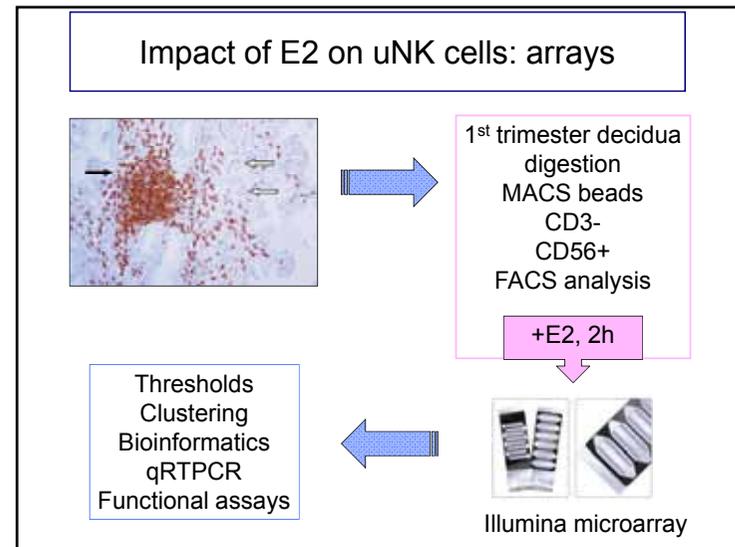
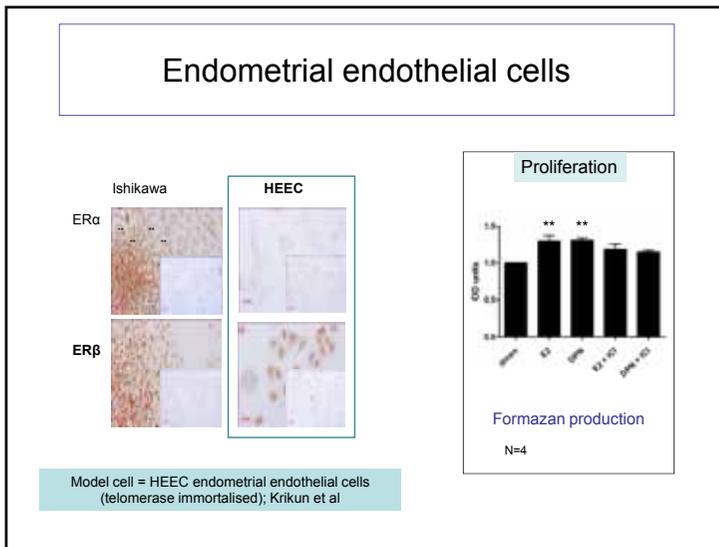
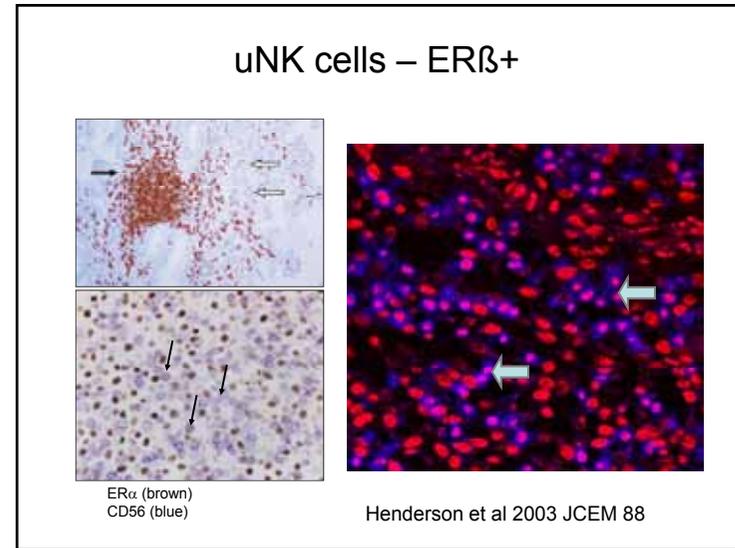
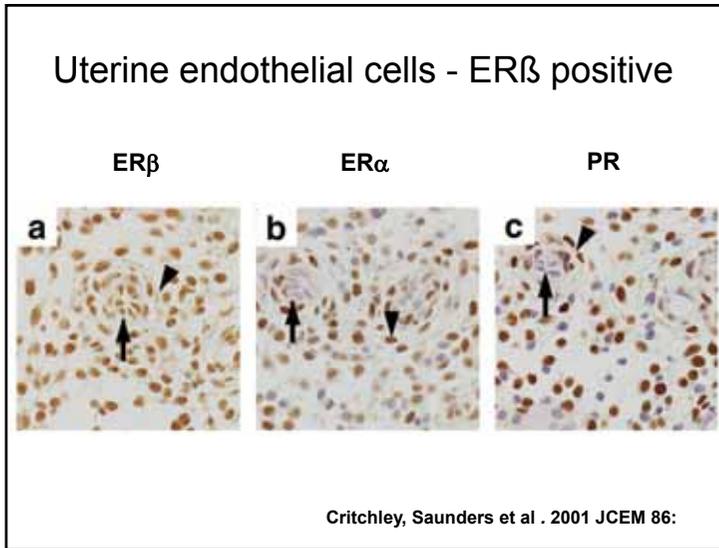


Decidualisation



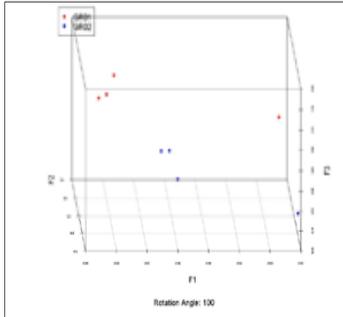
Inflammation



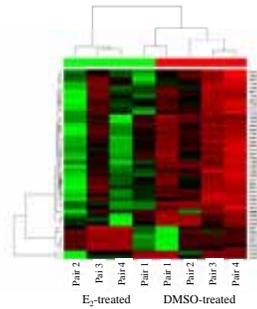


uNK array: data analysis

Principal component analysis

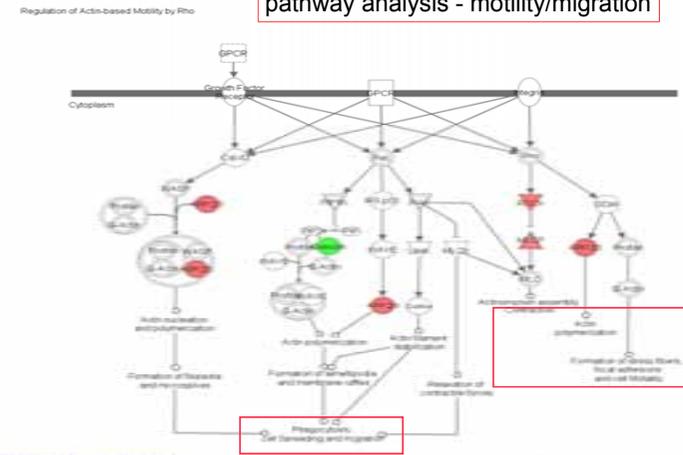


Pearsons Metric Clustering



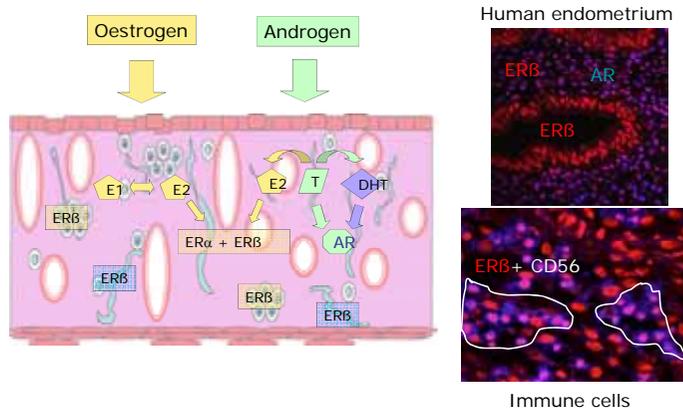
Threshold:  $p < 0.005$ ;  $FC \geq 1.5$  = 46 genes

Bioinformatics - Ingenuity pathway analysis - motility/migration



Elaine Marshall

Endometrial dynamics – Summary



Conclusions

- Expression of sex steroid receptors in human endometrium is spatially and temporally regulated
- Stromal cell decidualisation is associated with alterations in steroid biosynthetic capacity
- Androgens may play a role in modulating stromal cell differentiation and survival
- ERR's are expressed in human endometrium
- $ERR\alpha$  may play a role in decidualisation by altering expression of genes involved in energy metabolism
- The impact of oestrogens on endometrial endothelial and immune cells (uNK) are  $ER\beta$ -mediated

**Thanks to:**

*Saunders Team*  
 Frances Collins  
 Karen Kerr  
 Audrey Silvestri  
 Erin Greaves  
 Douglas Gibson  
 Elaine Marshall  
 Jacqui Lowrey  
 Carol Fitzgerald  
 Nozomi Itani  
 Vincent Bombail

Hilary Critchley  
 Dedicated Research Nurses  
 Histology Core Service

wellcome trust

**Sex steroid receptors**

- Dynamic spatial, temporal changes in expression of steroid receptors essential for co-ordinated cascade of gene expression
- Key processes steroid regulated
- Differences between functional and basal layers
- Evidence of local biosynthesis of ligands

Philippa Saunde

**Future challenges**

- Modelling human endometrial function
  - Functional vs basal
  - Progenitors and regeneration
  - Angiogenesis and differentiation
  - Rodent models – good/bad/indifferent?
  - Human tissue xenografts vs primates?
- Harnessing new technologies to inform our studies
  - Proteomics vs genomics
  - In silico networks