Does endometriosis alter the endometrial response to hCG?

Rob Sherwin, Andrew Sharkey
University of Cambridge

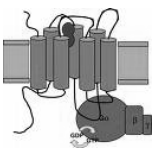
Asgi Fazleabas, Julie Hastings
Unversity of Illinois at Chicago

Does the embryo itself induce/contribute to endometrial receptivity?

- **'in a suitably primed endometrium' 'the changes associated with surface receptivity are only induced by a suitable embryonic signal'** (Lopata HR 11 Sup 1996)
- **HB-EGF up-regulated in murine lumen, 6 hr prior to embryo attachment** (SK Dey, Development 1994)
- **Embryo induces functional changes in endometrium**
  -before attachment

Does Chorionic Gonadotrophin alter endometrial gene expression

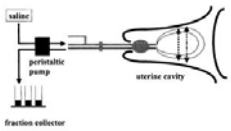
- **CG early embryo derived signal that is known to support the Corpus Luteum.**
- **CG secreted by 8 cell human embryo**
- **CG signals through LH/CG Receptor**
- **LH/CGR expressed in endometrium**
  - controversial



Paracrine actions of hCG

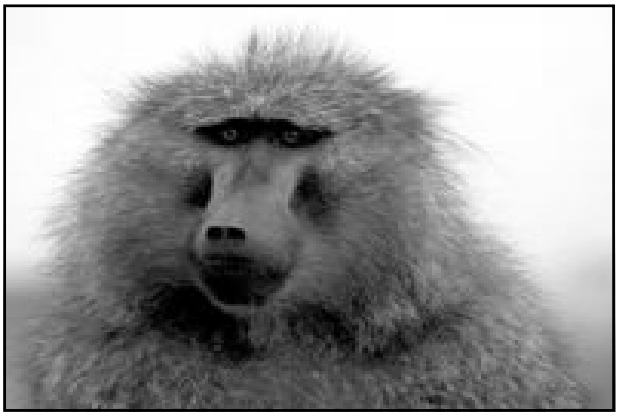
P Licht (HR Update 1998)

- **hCG infused via intrauterine microdialysis device (IUMD)**
- **LIF, VEGF up-regulated**
- **Prolactin, IGFBP-1 down-regulated**

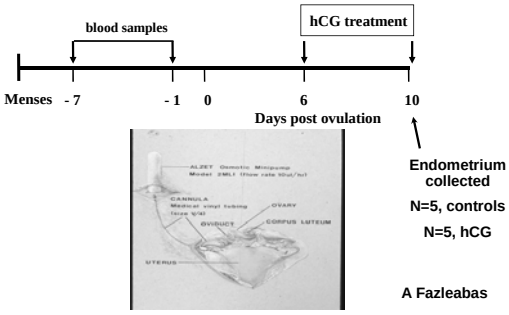


Hypothesis

- **The direct action of hCG on primate endometrium, would induce gene expression changes that would support embryo implantation.**



Treatment protocols for cycling Baboons



Endometrium collected

N=5, controls

N=5, hCG

A Fazleabas

Results of microarray analysis

- 61 genes differed by more than 2.5 fold ($p<0.01$)
- 48 transcripts increased after hGG and 13 decreased
- Real time PCR validation was performed for some genes

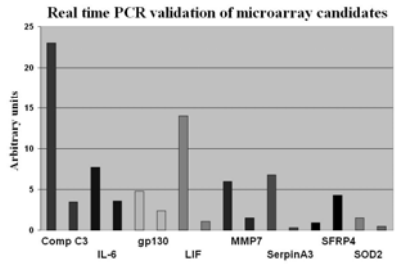
Genes down-regulated in response to CG treatment

Gene Name	Mean fold change
Secreted frizzled-related protein 4	0.2
NDRG family member 2	0.4
DnaJ (Hsp40) homolog, subfamily A, member 2	0.3
COX1 / prostaglandin-endoperoxide synthase 1	0.4
collagen, type IV, alpha 6	0.4
connexin 26	0.4
Apolipoprotein A1	0.4
claudin 11	0.4

Some of the genes up-regulated in response to CG treatment

Serpine A3	46.1
PP14	8.8
Heparanase	6.2
MMP-7	6.0
MMP-23A	6.0
interleukin 1 receptor-like 1	5.8
complement component 4A	5.8
chemokine (C-X-C motif) receptor 4	5.5
superoxide dismutase 2, mitochondrial	5.4
IL1b	4.5
placenta-specific 8	3.8
complement component 3	3.7
transforming growth factor beta regulator 1	3.7
PPAR-a	3.6
caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	3.4
glycoprotein hormones, alpha polypeptide	3.4
interleukin 6 signal transducer (gp130)	3.4
IL-8	3.3
VEGF-C	3.0
LIF	3.0

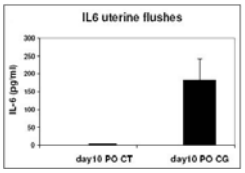
Taqman validation of candidates

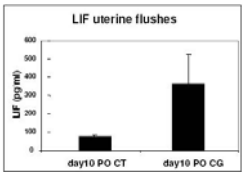


LIF+gp130 Upregulated

-known receptivity genes

ELISA assay for LIF and IL-6 protein abundance in uterine flushings





Conclusion-1

- **CG up-regulates LIF and IL6 mRNA & protein abundance**
  - **LIF and gp130 are known receptivity genes.**
- **Glycodelin, Complement C3 and C4A/B up-regulated-**
  - **may modulate peri-implantation and decidual immune environment.**
- **Up-regulation of MMP-7, MMP-23 and SERPINA3**
  - **implies a regulatory role for CG in implantation tissue remodelling.**

(Sherwin et al Endocrinology 2006)

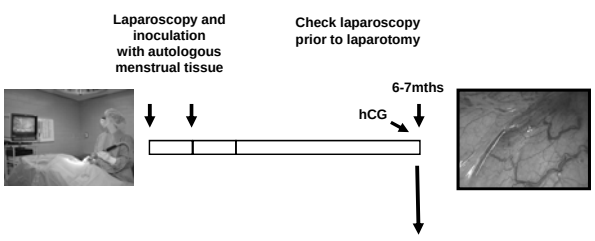
Is the endometrial response to hCG altered in baboons with endometriosis?

- **Hypothesis:**

  Endometrial gene expression in response to direct action of hCG, is altered in a primate model of endometriosis.
- **Supporting Observations:**

  Implantation rates reduced for patients undergoing IVF who have severe endometriosis **(Barnhardt Fert Ster 2003)**

In-Vivo Model of Endometriosis



Experimental Design

- **Baboons with induced endometriosis and control animals treated with hCG from day 6 to day 10 PO**
- **Morphological analysis**
- **Micro-arrays**
- **Real-time PCR and protein validation**

Results- plaque response

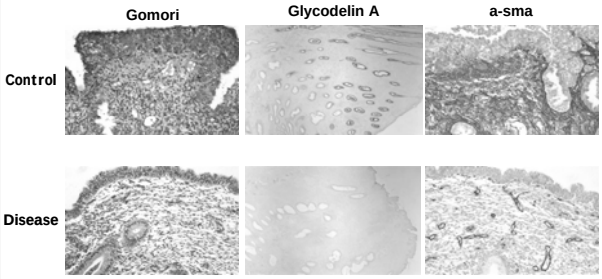
- **Controls**

  6/8 showed plaque response to hCG

- **Endometriosis model**

  1/5 showed plaque response to hCG

Endometriosis Suppresses the Endometrial Response to the Primate Embryonic Signal



NB: these responses are also seen in ovexed controls treated with hCG

Results- gene dysregulation

Six months after induction of endometriosis;

35 genes higher in endo
(DO NOT go down with hCG)

81 genes lower in endo
(DO NOT go up with hCG)

Are these genes that fail to respond to hCG?

Genes Dysregulated following hCG in animals with endometriosis

Gene Name	Fold Change
PTGS1	5.9
APOA1	3.3
PAPPA	3.3
SFRP4	3.0
CNTFR	2.8
HOXA9	2.3
WNT4	2.2

hCG downregulates
Higher in endosis

Gene Name	Fold Change
SERPINA3	0.12
MMP7	0.24
C3	0.26
ADAMTS8	0.30
IL1R2	0.35
SFRP1	0.36
SOD2	0.37
C1R	0.43

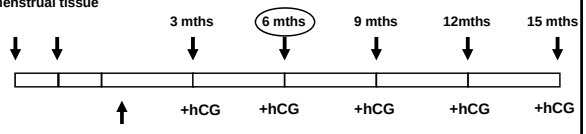
hCG upregulates
Lower in endosis

Blunted response to hCG after 6 months

Collection of additional eutopic endometrium

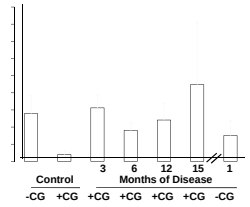
Laparoscopy and inoculation with autologous menstrual tissue

Harvest of eutopic endometrium by laparotomy, after hCG infusion, during the window of receptivity



Real Time PCR Validates Microarray

ApoA1

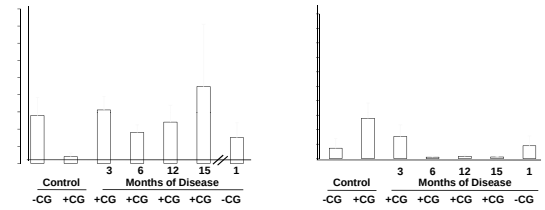


Kruskal –Wallis ANOVA, p<0.05

Real Time PCR Validates Microarray

ApoA1

SerpinA3



Kruskal –Wallis ANOVA, p<0.05

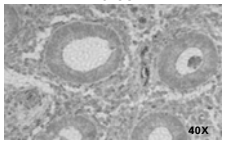
Transcripts fail to respond to CG in animals with endometriosis

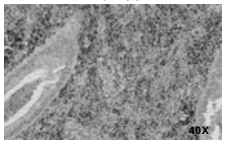
Immunohistochemistry Validates Microarray

Disease-free Control
No CG

Complement
Factor 3 (C3)

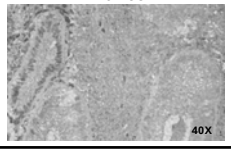
Disease-free Control
With CG





3 Months Endometriosis
With CG

6 Months Endometriosis
With CG



Conclusions

- In a primate model of endometriosis, the eutopic endometrial response to Chorionic Gonadotrophin is blunted (plaque, transcript and protein).
- Does this happen in humans?
- Some of the implantation failure seen in patients with endometriosis related infertility, may be caused by altered endometrial gene response to hCG and other embryo derived peptides.

How does this fit in with other receptivity mechanisms?

- Receptivity requires Oe then Progesterone
- LIF upregulates some genes increased by PR
  LIF+ P synergise in mice
- Embryonic signals such as hCG increase LIF etc
- Multiple mechs work together to achieve expression of critical genes

Thank you

Cambridge- Rob Sherwin, Andrew Sharkey
microarray facility

Chicago- Asgi Fazleabas
Julie Hastings
Patty Mavrogianis