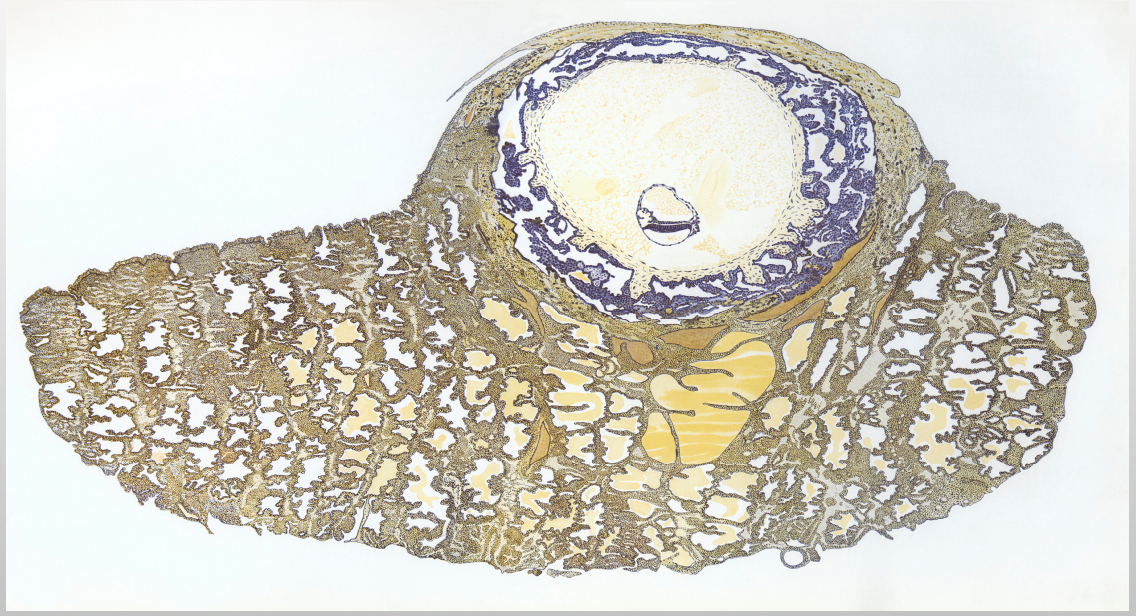
MANCHESTER 1824

The University of Manchester

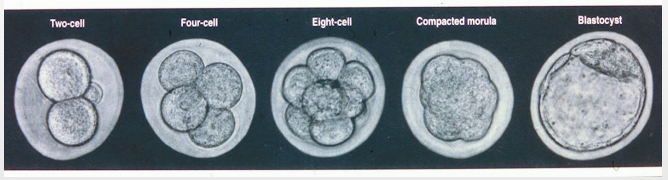
The uterus and implantation



John Aplin
University of Manchester

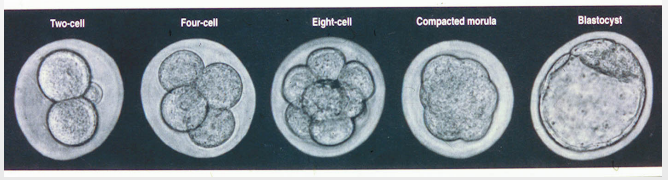
Stages of implantation

- Development to blastocyst
- Hatching



Stages of implantation

- Development to blastocyst
- Hatching
- Apposition of the blastocyst



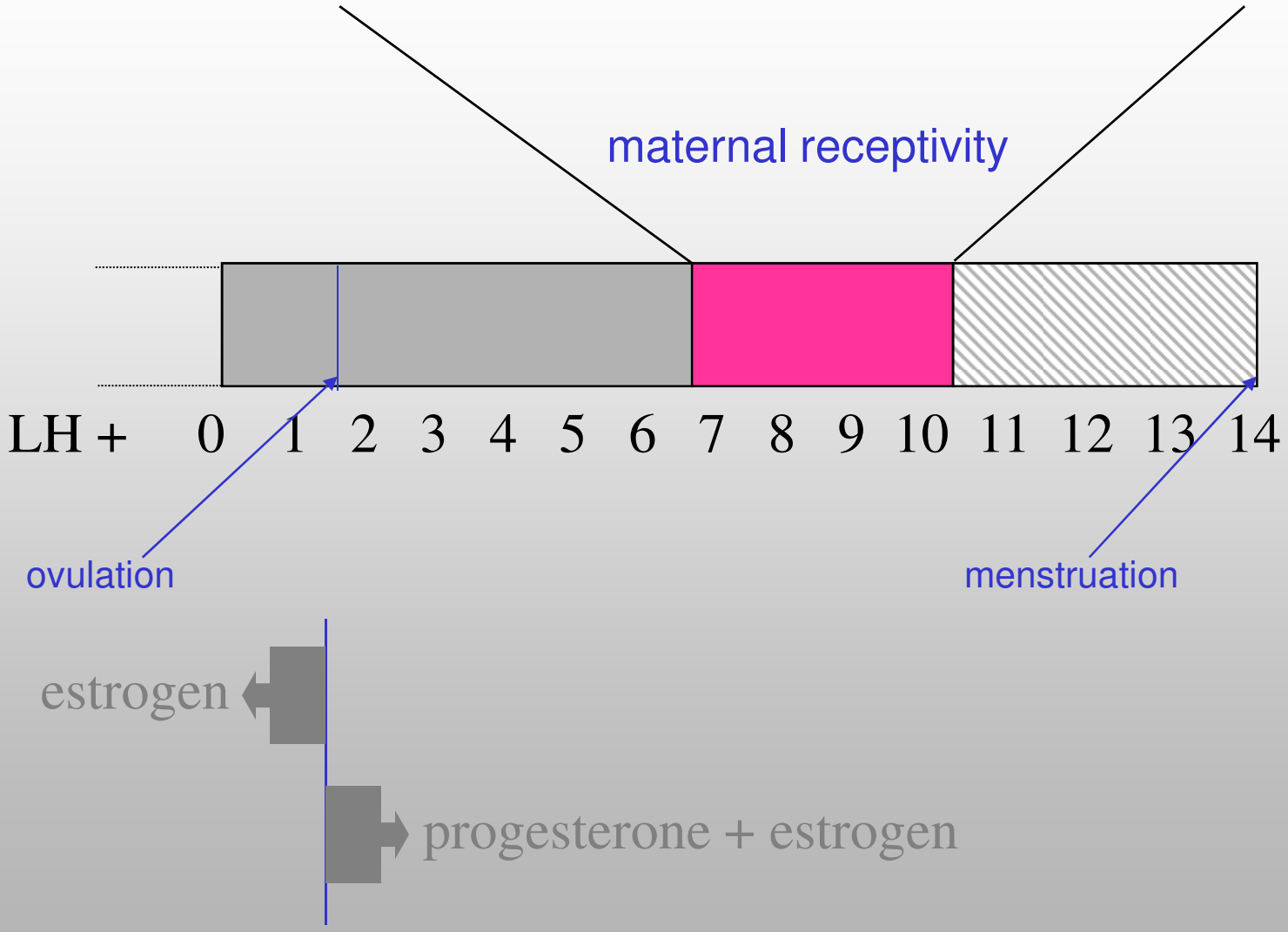
Stages of implantation

- Development to blastocyst
- Hatching
- Apposition of the blastocyst
- Attachment to the epithelial surface
- Penetration of the epithelial layer

Stages of implantation

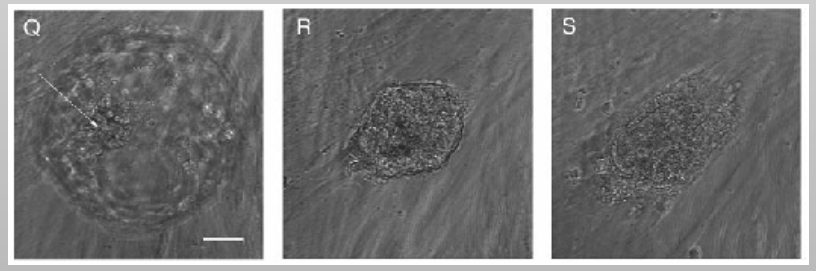
- Development to blastocyst
- Hatching
- Apposition of the blastocyst
- Attachment to the epithelial surface
- Penetration of the epithelial layer
- Invasion of stroma
- Invasion and transformation of spiral arteries

Implantation window



The window is maternally controlled

- Unimplanted mouse embryos flushed after transfer to one recipient can implant into a second recipient in her receptive phase
- Hatched human blastocysts 'implant' efficiently on endometrial stromal cells



Mardon et al
Human Reproduction
18, 283, 2003

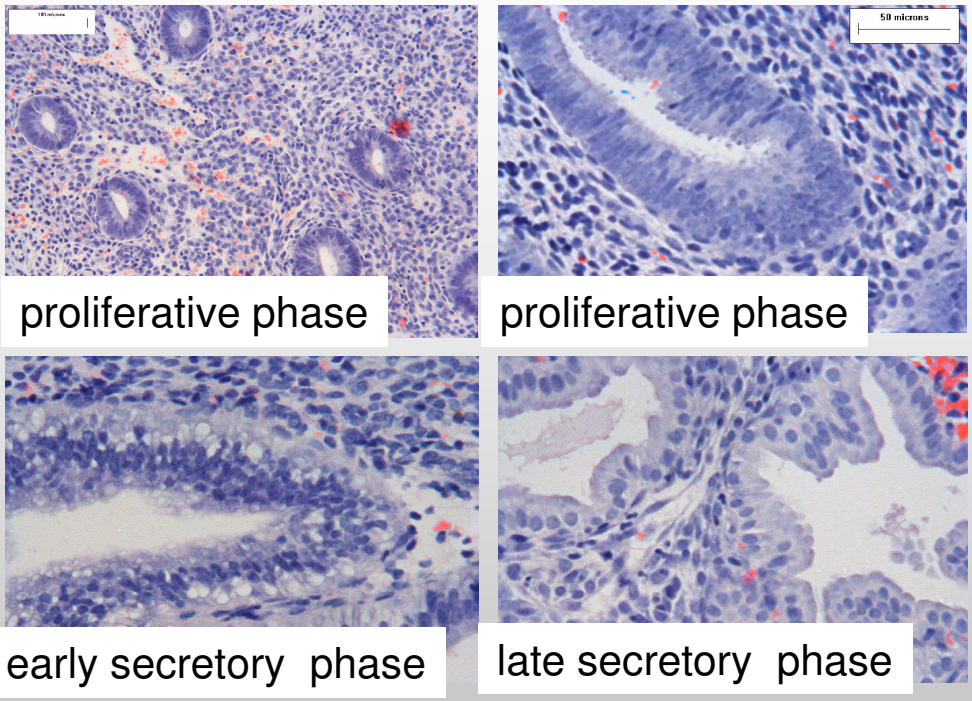
- *Control seems to be exerted by the endometrial epithelium*

Control of implantation

The barrier hypothesis

The maternal luminal epithelium is *specifically non-receptive* outside the window phase

Endometrial histology



•The Noyes criteria (Noyes, Hertig & Rock Fert Steril 1, 3-11,1950)

•Histological changes quantified using morphometric methods and applied to a fertile control group in the period LH+2 to LH+ 7 show highly reproducible daily changes

•Li TC, Rogers AW, Dockery P, Lenton EA, Cooke ID. A new method of histologic dating of human endometrium in the luteal phase. Fertil Steril. 1988 Jul;50(1):52-60.

But …

- Coutifaris C, Myers ER, Guzick DS, Diamond MP, Carson SA, Legro RS, McGovern PG, Schlaff WD, Carr BR, Steinkampf MP, Silva S, Vogel DL, Leppert PC; NICHD National Cooperative Reproductive Medicine Network.

  Histological dating of timed endometrial biopsy tissue is not related to fertility status.

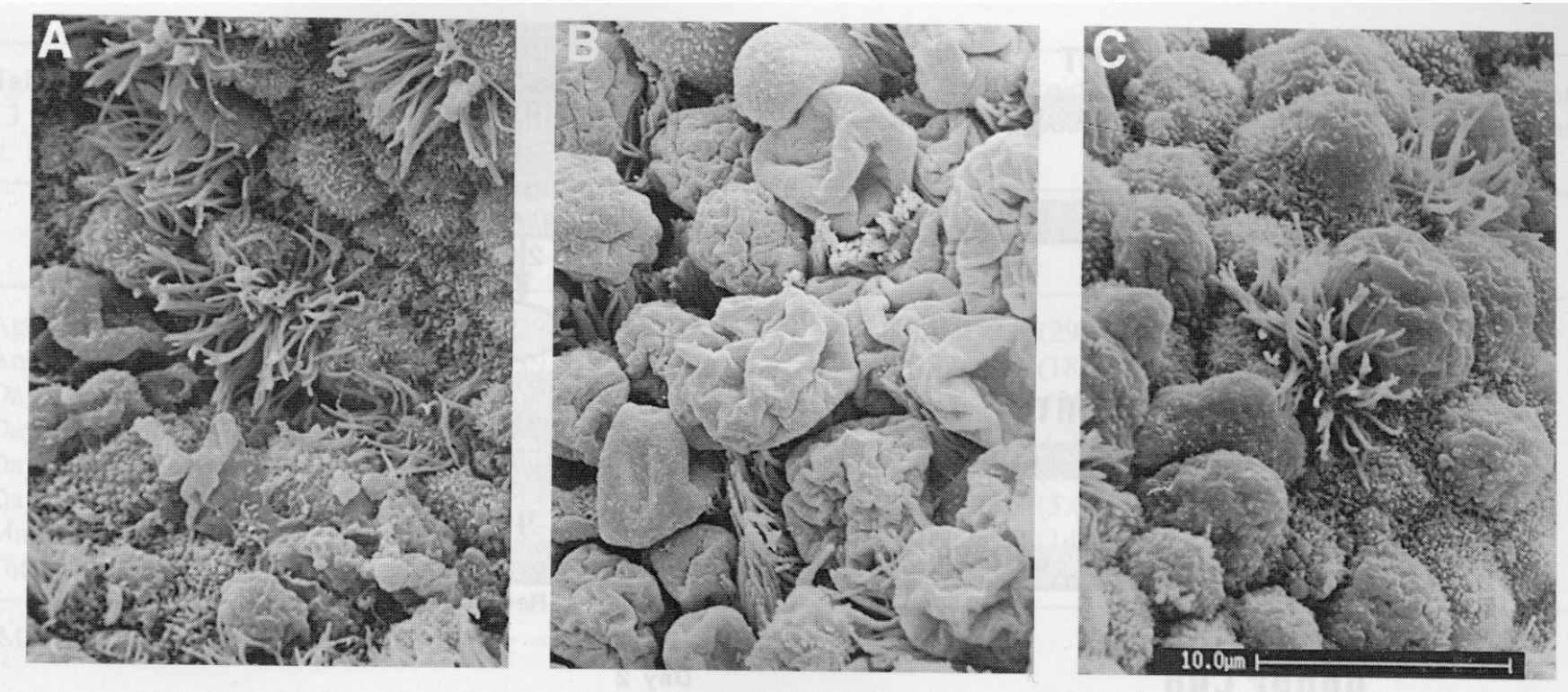
  Fertil Steril. 2004 Nov;82(5):1264-72.

- Myers ER, Silva S, Barnhart K, Groben PA, Richardson MS, Robboy SJ, Leppert P, Coutifaris C; NICHD National Cooperative Reproductive Medicine Network.

  Interobserver and intraobserver variability in the histological dating of the endometrium in fertile and infertile women.

  Fertil Steril. 2004 Nov;82(5):1278-82.

‘Uterodomes’ or ‘Pinopodes’ (bulbous protrusions) develop at the luminal surface of the endometrium at approximately the time of implantation



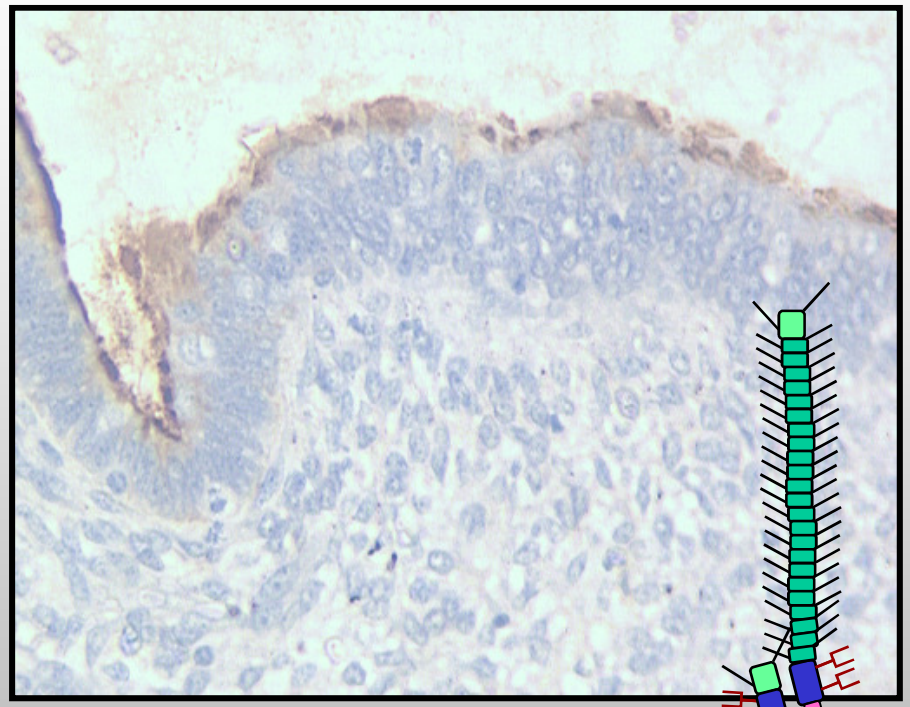
Pre-receptive microvilli and forming uterodomes

‘Receptive’ (uterodomes)

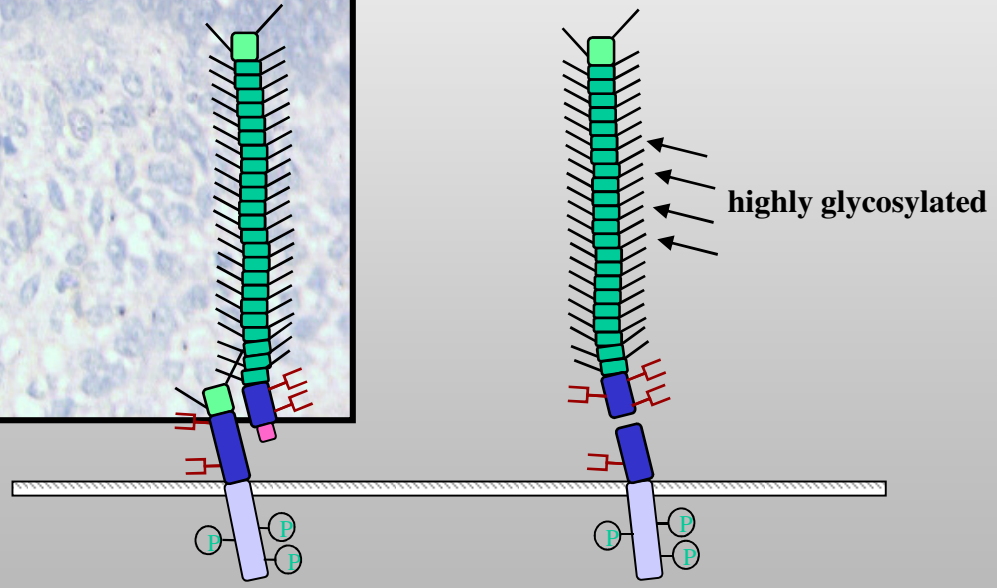
Post-receptive uterodomes regressing; microvilli reappearing

Nikas et al Endometrial pinopodes indicate a shift in the window of receptivity in IVF cycles. Human Reproduction, Vol. 14, No. 3, 787-792, 1999;

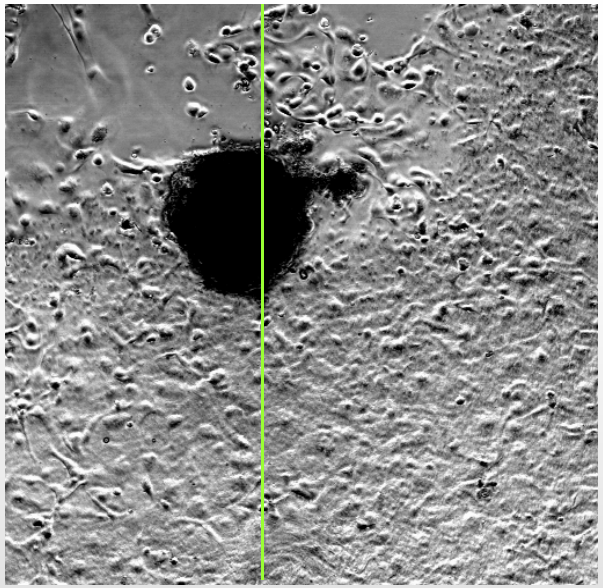
MUC1 is expressed at the endometrial cell surface in the implantation phase

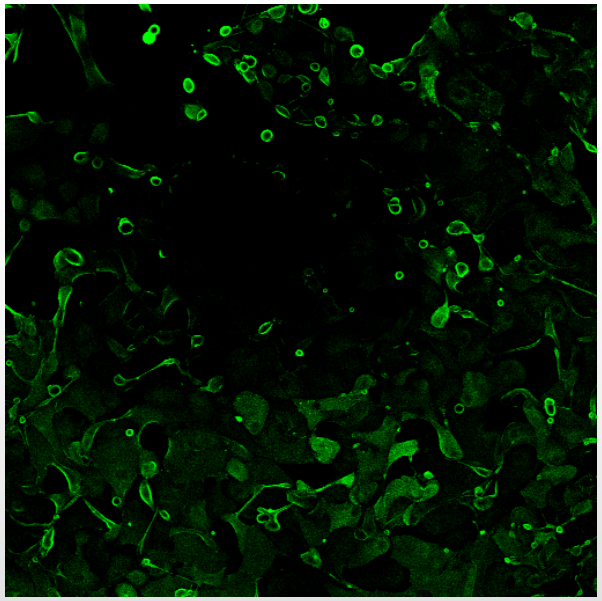


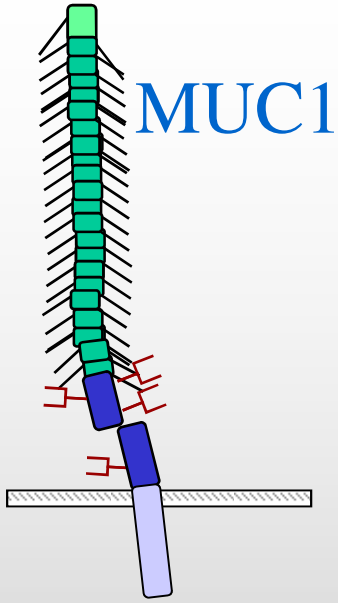
MUC1 is an anti-adhesion molecule



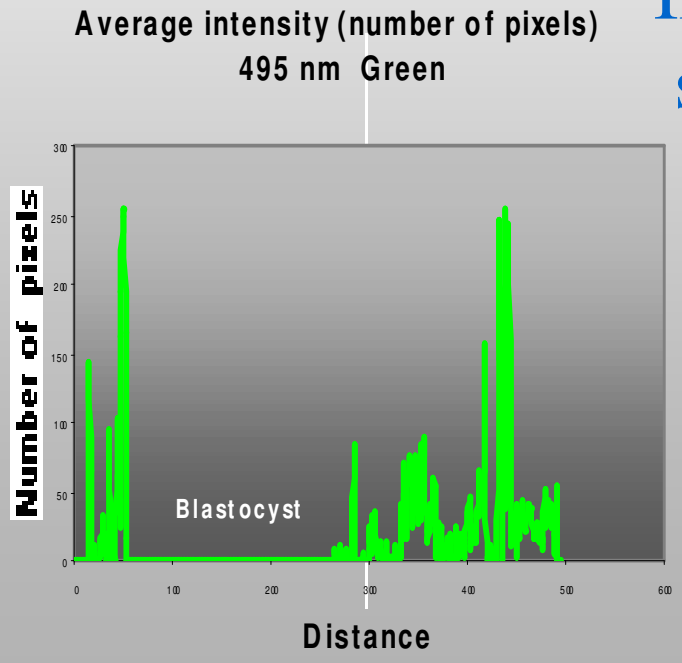
M. MESEGUER, J.D. APLIN, P. CABALLERO-CAMPO, J.C. MARTÍN, J. REMOHÍ, A.PELLICER, C. SIMÓN (2001) Hormonal and embryonic regulation of human endometrial MUC1. Biology of Reproduction 64, 181-192.



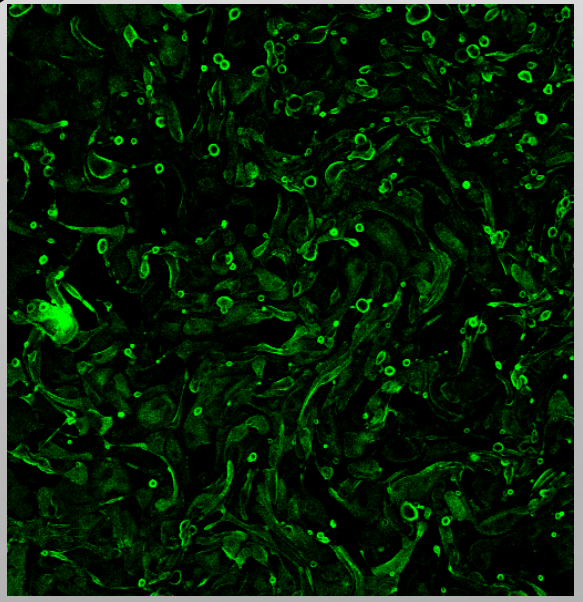




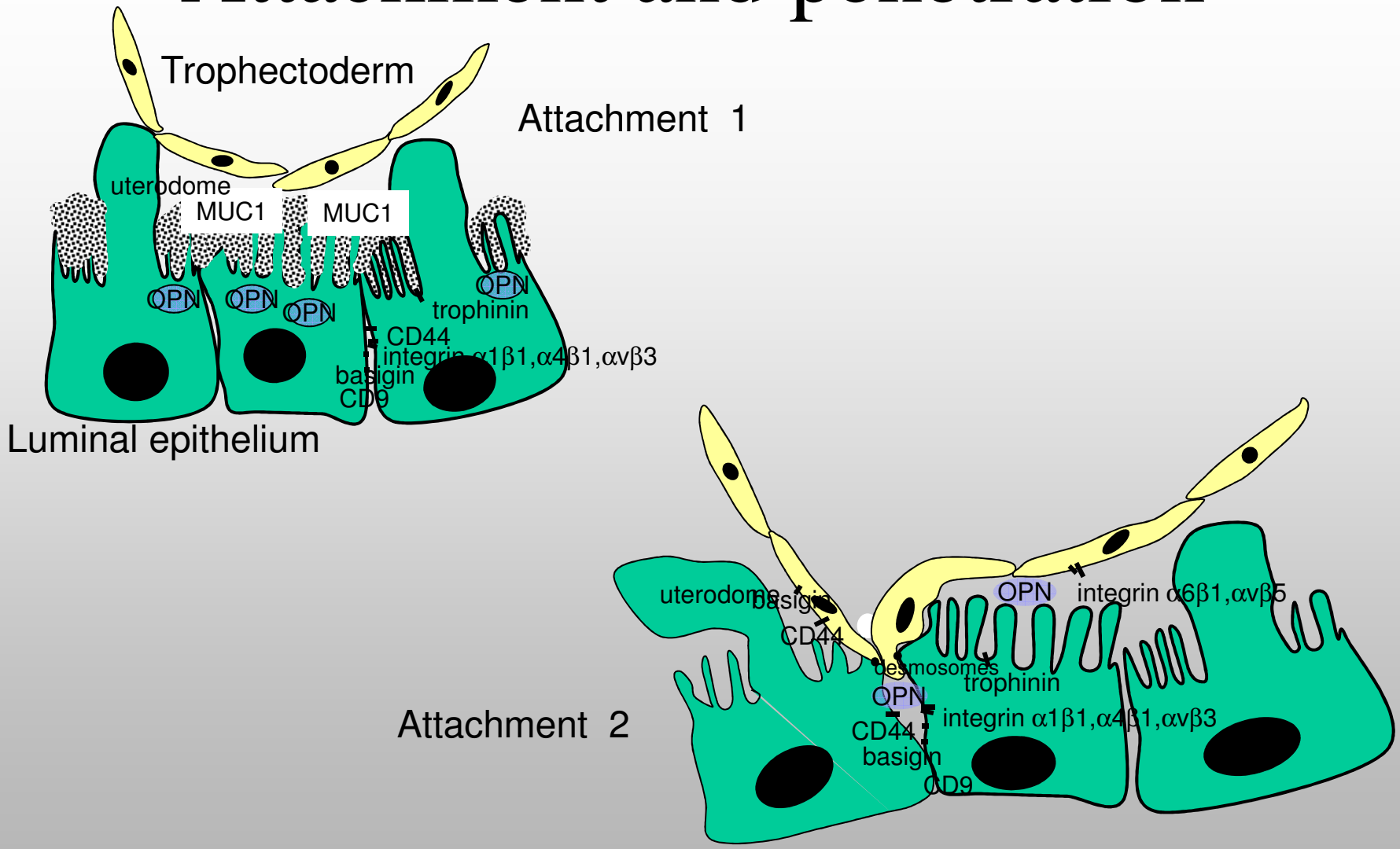
Implantation site in vitro



Non-site



Attachment and penetration



Aplin JD. Embryo implantation: the molecular mechanism remains elusive. Reprod Biomed Online. 2006 Dec;13(6):833-9.

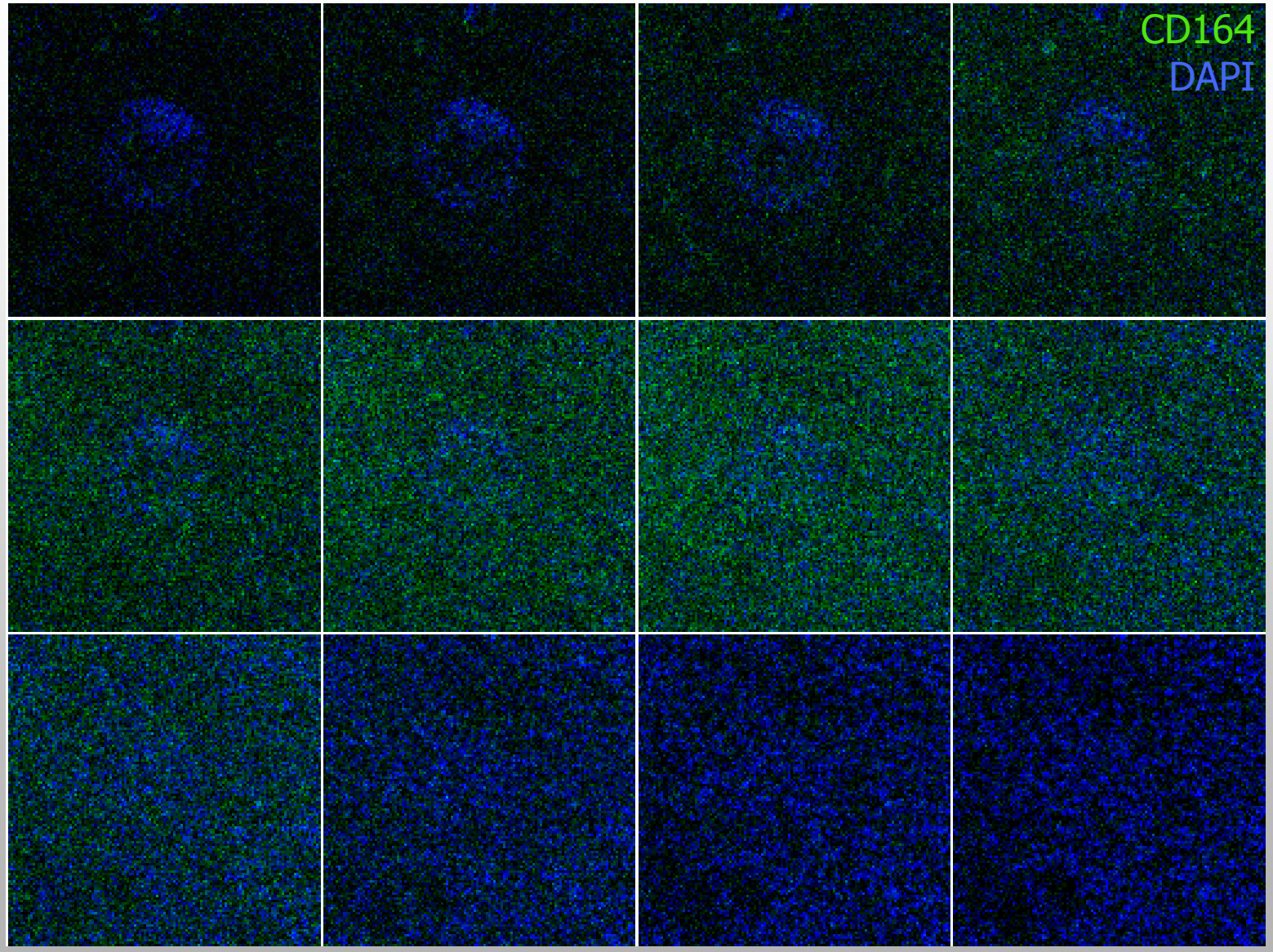
Molecular markers of cyclic change do not predict pregnancy outcome in subsequent cycles

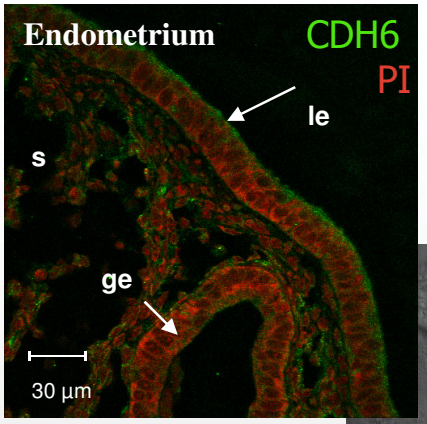
- Ordi J, Creus M, Casamitjana R, Cardesa A, Vanrell JA, Balasch J. Endometrial pinopode and alphavbeta3 integrin expression is not impaired in infertile patients with endometriosis. J Assist Reprod Genet. 2003 Nov;20(11):465-73.

- Ordi J, Creus M, Quinto L, Casamitjana R, Cardesa A, Balasch J. Within-subject between-cycle variability of histological dating, alpha v beta 3 integrin expression, and pinopod formation in the human endometrium. J Clin Endocrinol Metab. 2003 May;88(5):2119-25.

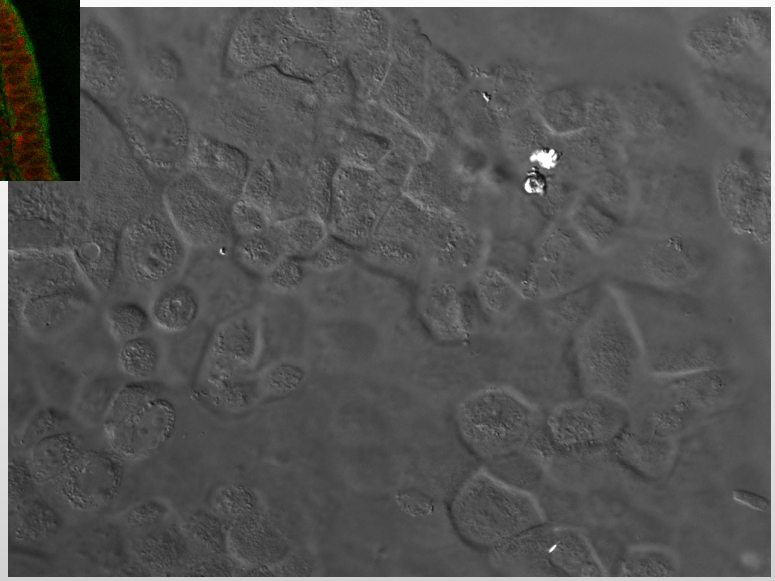
- Ordi J, Creus M, Ferrer B, Fabregues F, Carmona F, Casamitjana R, Vanrell JA, Balasch J. Midluteal endometrial biopsy and alphavbeta3 integrin expression in the evaluation of the endometrium in infertility: implications for fecundity. Int J Gynecol Pathol. 2002 Jul;21(3):231-8.

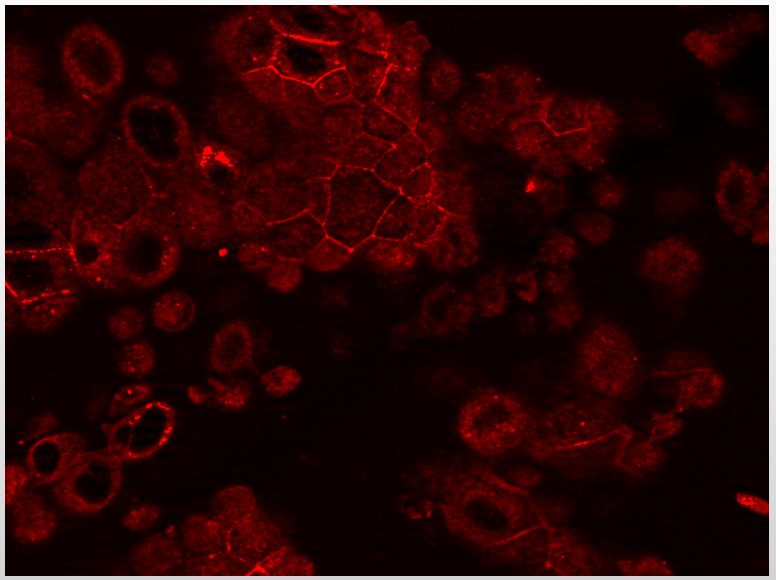
CD164

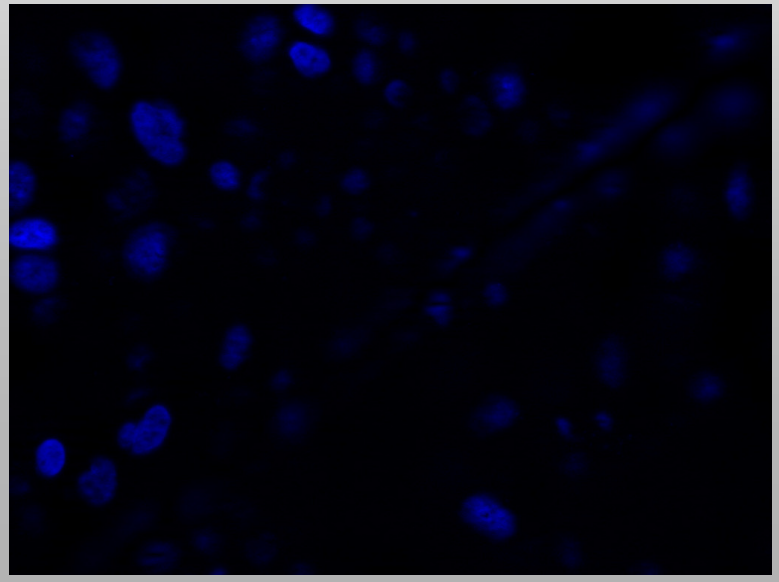


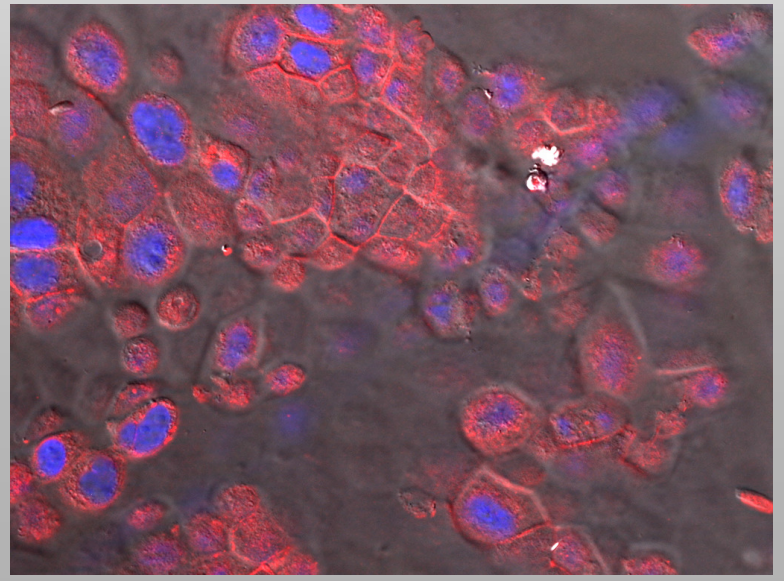


Cadherin 6 – a new candidate adhesion molecule derived using a proteomics approach



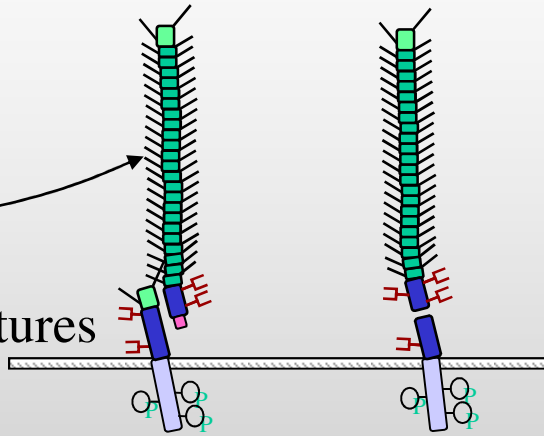






Endometrial glycoprotein glycans

- A small subset of specific structures is highly sensitive to progesterone-induced differentiation (cycle stage)
- AND to tissue pathology: endometriosis, infertility…
- MUC1-associated glycans are found amongst these structures



- They include:
  - B72.3 epitope (sialyl Tn); Dolichos biflorus-binding glycans; 214D4 epitope; keratan sulphate (D9B1 and 5D4 epitopes)
- These glycans are regulated *independently* of MUC1 core protein

C.J.P.JONES, A.T. FAZLEABAS, P.B. MCGINLAY, J.D. APLIN (1998) Cyclic modulation of epithelial glycosylation in human and baboon (Papio anubis) endometrium demonstrated by the binding of the agglutinin from dolichos biflorus (DBA). Biology of Reproduction 58, 20-27

Unusual implantation sites: can we learn anything?

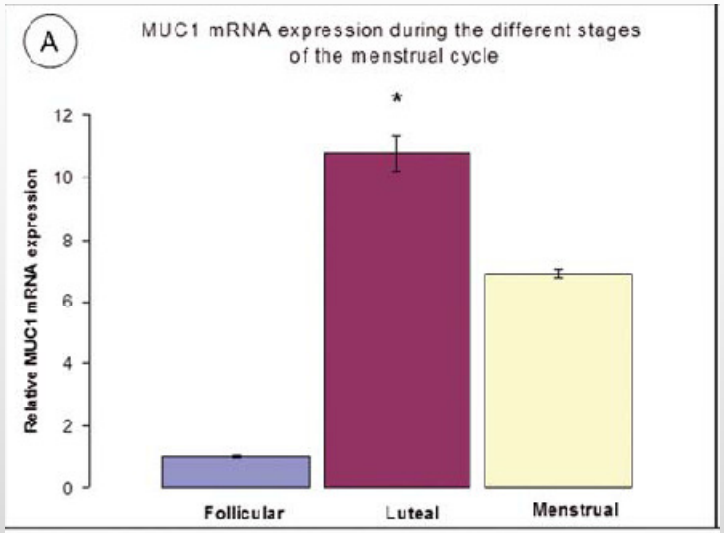
- Tubal implant
- Septum
- Post-Caesarian scar

Tubal implants

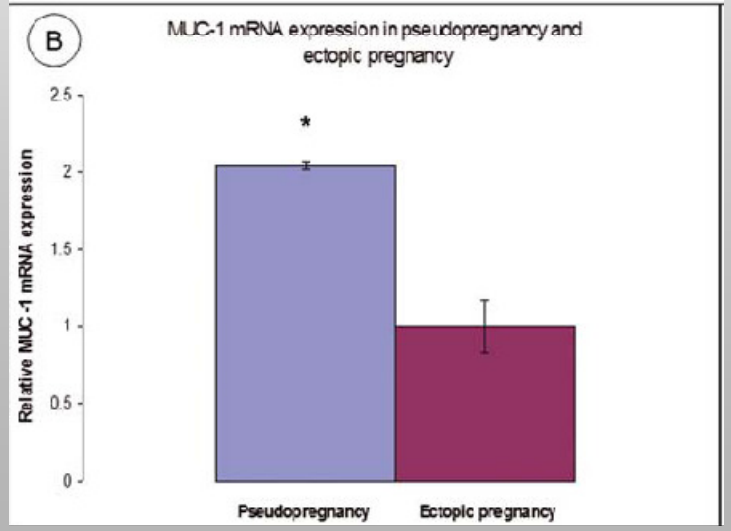
- Hypothesis: higher tubal receptivity in women bearing ectopic implants
- Fallopian tubes from menstrual cycle stages (n=24), tubes bearing an ectopic pregnancy (n=15) and pseudopregnant tubes (n=6)
- Expression of the surface barrier molecule MUC1 is less (mRNA and protein) in tubal epithelium from ectopic pregnancy than in pseudopregnant tubes

qRT-PCR of MUC1 in tubal tissue

cycle



pseudopreg vs ectopic



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- The glycan epitope 214D4 was *absent* from epithelial cytoplasm in tubal tissue adjacent to ectopic implants

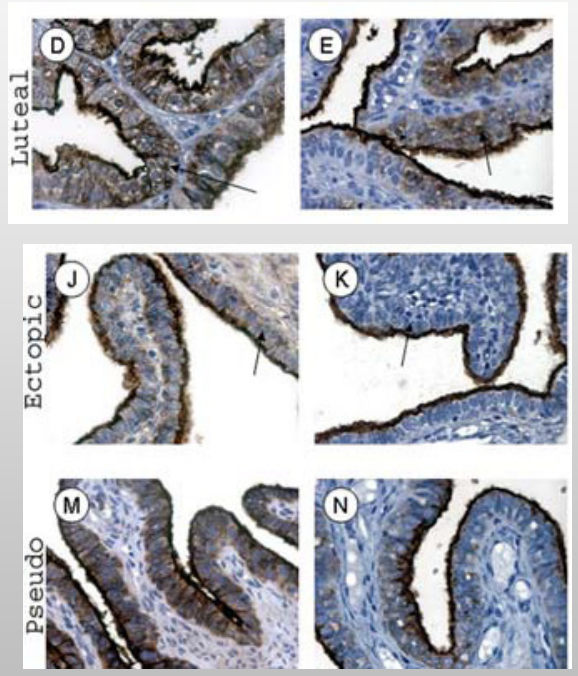
Tubal tissue

MUC1 core protein glycan 214D4

Luteal

Ectopic

Pseudo



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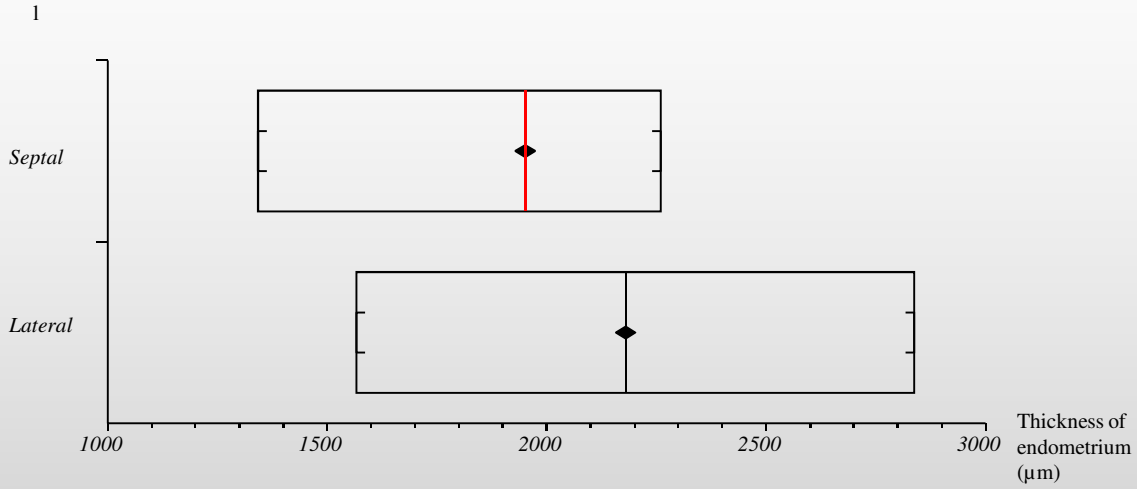
- The glycan epitope 214D4 was absent from epithelial cytoplasm adjacent to ectopic implants.
- Data suggest a possible decrease in tubal epithelial barrier function associated with ectopic sites

The expression of MUC1 in human Fallopian tube during the menstrual cycle and in ectopic pregnancy
M Al-Azemi, B Refaat, JD Aplin, W Ledger, Human Reproduction 24, 2582, 2009

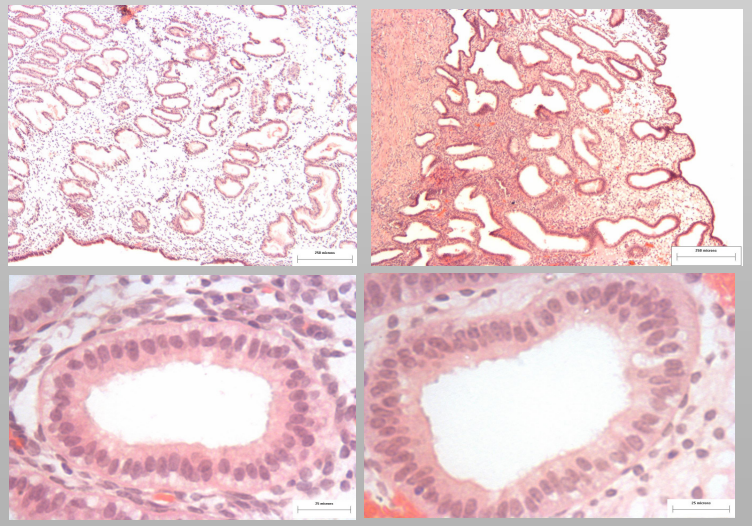
Uterine septum

- Septate uterus is linked with an increased incidence of recurrent pregnancy loss
- Hypothesis: higher receptivity in the septum
- Endometrial biopsies were performed in 8 women with septate uterus and a history of recurrent miscarriage, during hysteroscopy in the postovulatory phase of the cycle
- Samples were taken from the endometrium of the septal and lateral wall
- Paraffin-embedded sections were examined for endometrial morphology and a panel of tissue markers

Septum vs lateral wall



Lateral wall **Septal wall**



Normal morphology

Uterine septum

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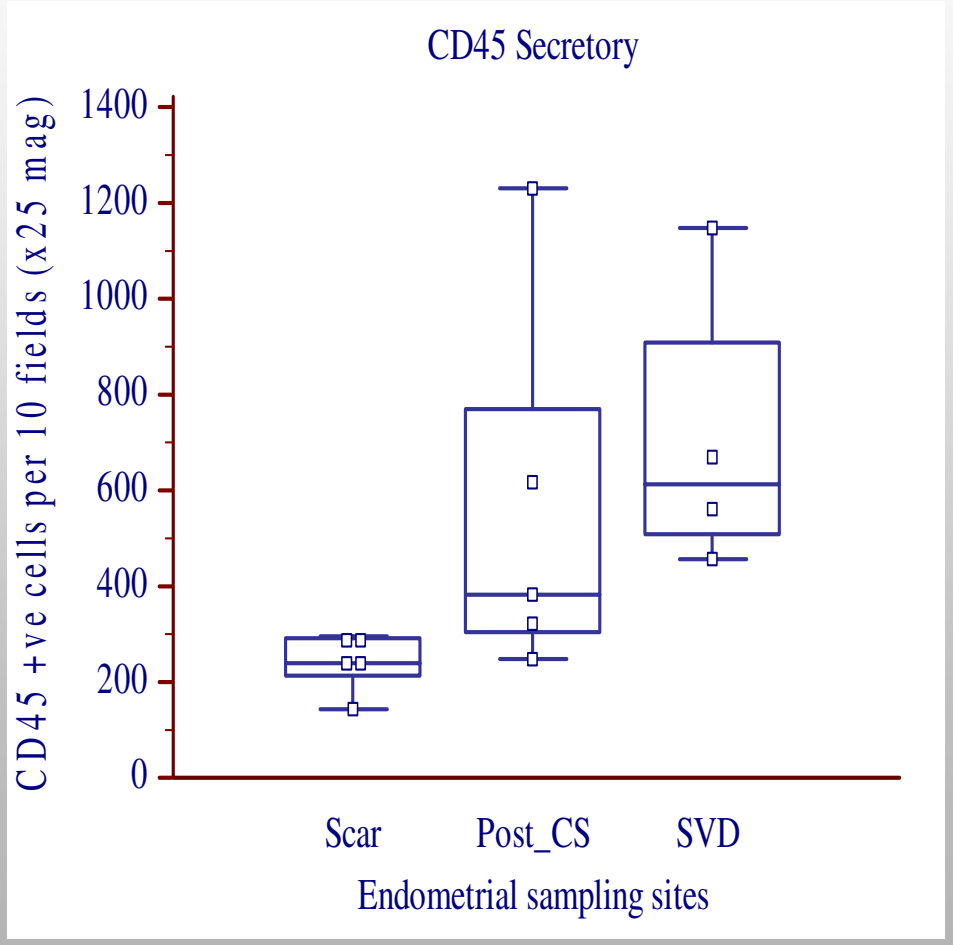
- Samples were taken from the endometrium of the septal and lateral wall
- Paraffin-embedded sections were examined for endometrial morphology and a panel of tissue markers
- Septal endometrium is slightly thinner (ns) but otherwise normal
- Pregnancy loss may be associated with abnormalities in the later vascular stages of implantation

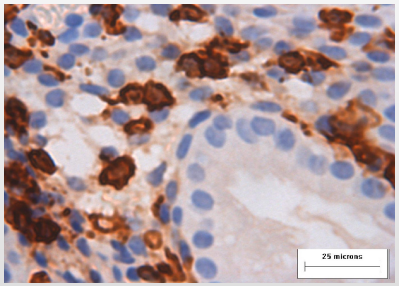
Exalto, Paranthaman, Aplin, unpublished

Post-Caesarian scar

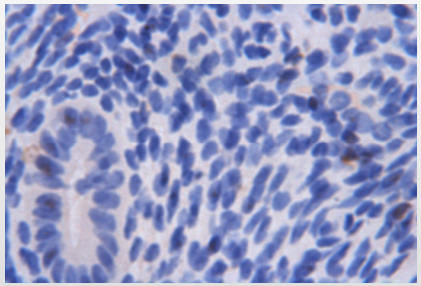
- Preferential implantation or more rapid trophoblast invasion through scar tissue can cause dehiscence as the conceptus grows
- Premenopausal women with history of CS (n=11) or vaginal delivery (n=16).
- Endometrial curettings were taken at the CS site and posterior wall of women with previous CS, and posterior wall endometrium of women with previous vaginal delivery.
- A panel of endometrial markers was applied.

Fewer leukocytes in scarred uterus





Secretory phase



Post-Caesarian scar

CD45+ cells = brown

Post-Caesarian scar

- Preferential implantation or more rapid trophoblast invasion through scar tissue can cause dehiscence as the conceptus grows
- Premenopausal women with history of CS (n=11) or vaginal delivery (n=16).
- Endometrial curettings were taken at the CS site and posterior wall of women with previous CS, and posterior wall endometrium of women with previous vaginal delivery.
- There were significantly fewer leukocytes present in the scarred endometrium (both sites) than in control endometrium in the secretory phase ($p<0.05$).
- Could scarring affect leukocyte recruitment with effects downstream on invasion?

The effect of Caesarean section on the endometrium.
J Ben-Nagi , A Walker, D Jurkovic, J Yazbek, JD Aplin, Int J Obstet Gynecol, 106:30, 2009

Redefining receptivity

Once the epithelial barrier has been overcome….

…..it may be that the uterine vasculature and stroma carry out subsequent barrier (or ‘interrogative’) functions towards the implanting conceptus

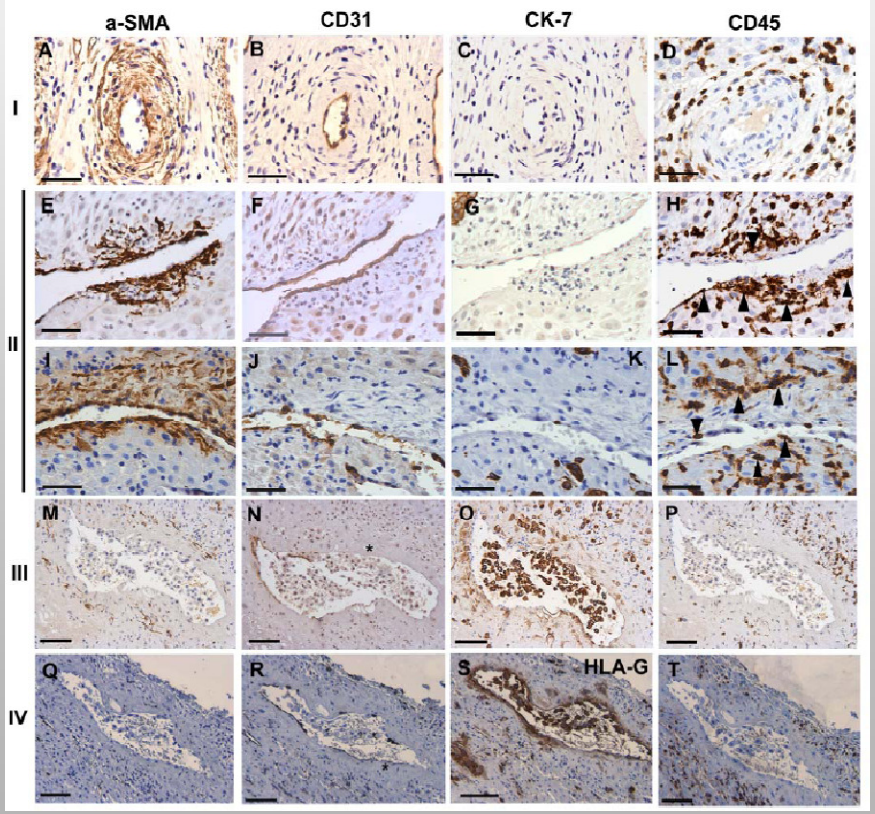
Redefining receptivity

- Different vascular beds differ in receptivity to invading trophoblast

I.P.Crocker, M.Wareing, G.R.Ferris, C.J.P.Jones, J.E.Cartwright, J.D.Aplin (2005). The effect of vessel structure, oxygen and tumor necrosis factor alpha on trophoblast invasion of maternal arteries in vitro. J Pathol, **206**, 476-485.

Redefining receptivity

- Endometrial leukocytes play a major role in remodelling spiral arteries during pregancy



Smith SD, Dunk CE, Aplin JD, Harris LK, Jones RL. (2009) Evidence for immune cell involvement in decidual spiral arteriole remodeling in early human pregnancy. Am J Pathol.174:1959-71