UNIVERSITY OF CAMBRIDGE

Placentation

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ESHRE Tripartite SIG Campus
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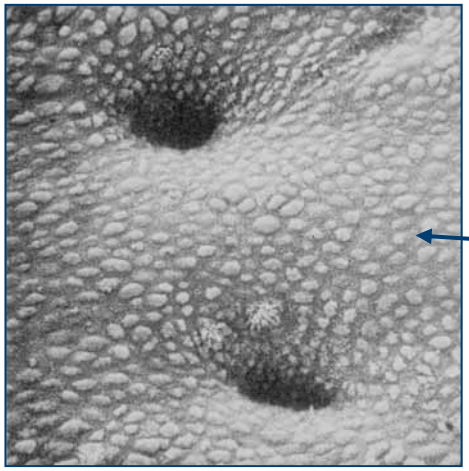
Centre for Trophoblast Research (www.trophoblast.cam.ac.uk)

Aims

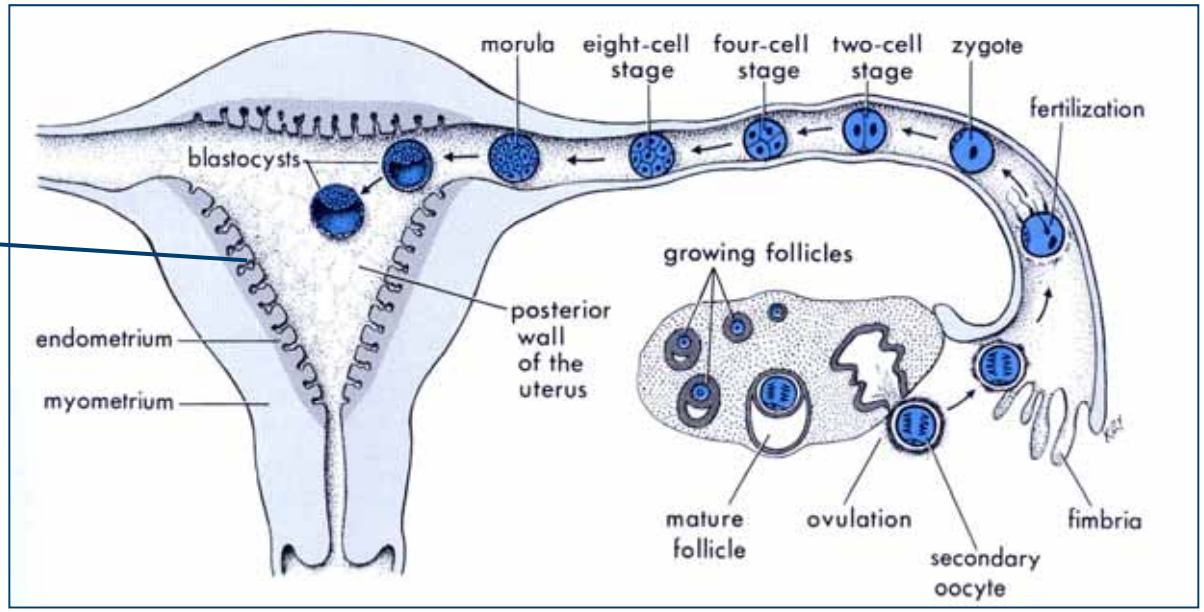
To consider human early placental development with particular reference to:

- **the role of the endometrial glands and their secretions in supporting the conceptus during the first trimester**
- **the possible regulation of those secretions by the conceptus**
- **the possible maintenance of potential trophoblast stem cells by the secretions**

The two sequential modes of nutrition for the conceptus

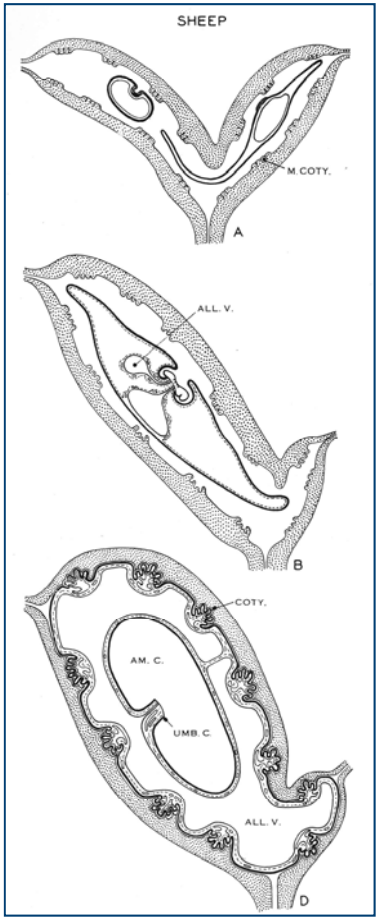


The human uterus has approximately 15 endometrial glands per mm^2



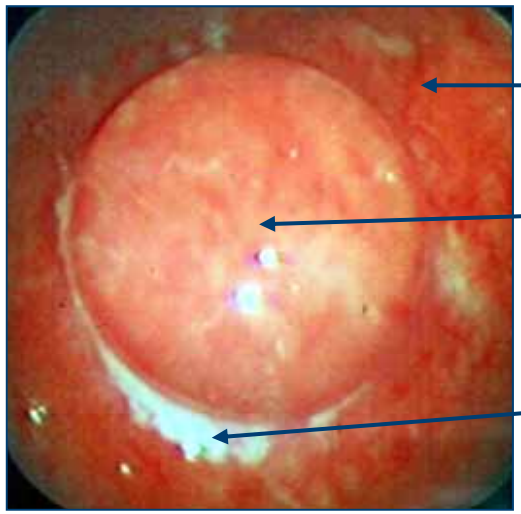
- Nutrition of the conceptus is initially histiotrophic in all species - the uptake of oviductal and uterine secretions by the trophoblast
- Later, in all mammals it switches to haemotrophic nutrition - exchange between the maternal and fetal circulations within the placenta

Histiotrophic nutrition in early pregnancy



Sheep, Cow
Horse, Pig

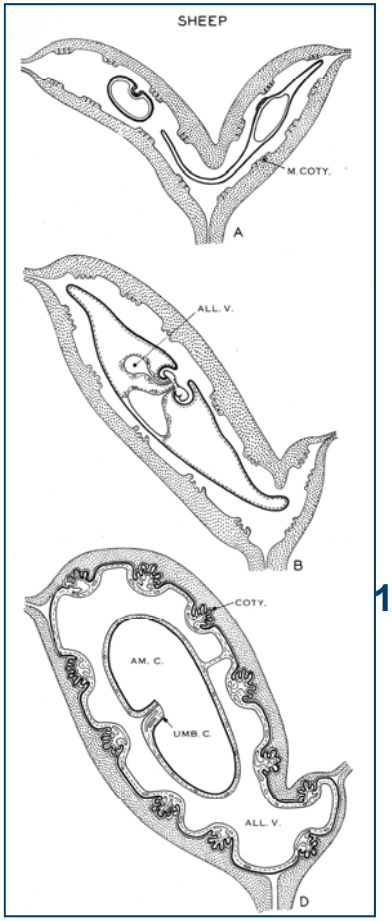
Courtesy of WA Allen



Endoscopic view of a horse conceptus at approximately day 35 of pregnancy

- In the majority of species the conceptus remains within the uterine cavity and is supported during the embryonic period by glandular secretions, 'uterine milk', from the endometrium

Histiotrophic nutrition in early pregnancy



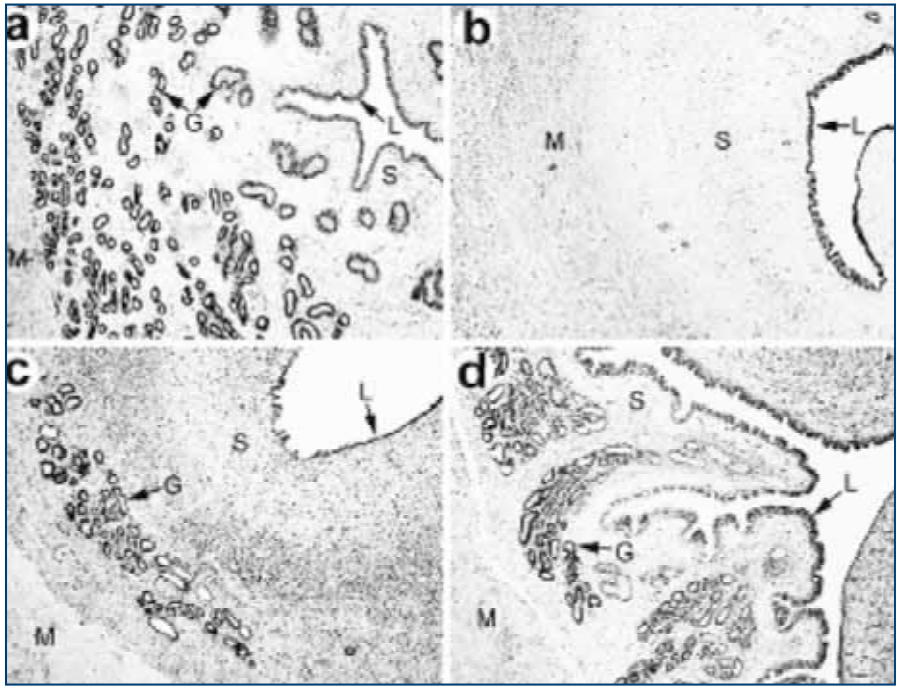
Sheep, Cow
Horse, Pig

Control
2 conceptuses

UGKO
1 non-expanded conceptus

Day 14 of pregnancy

Gray et al., 2001

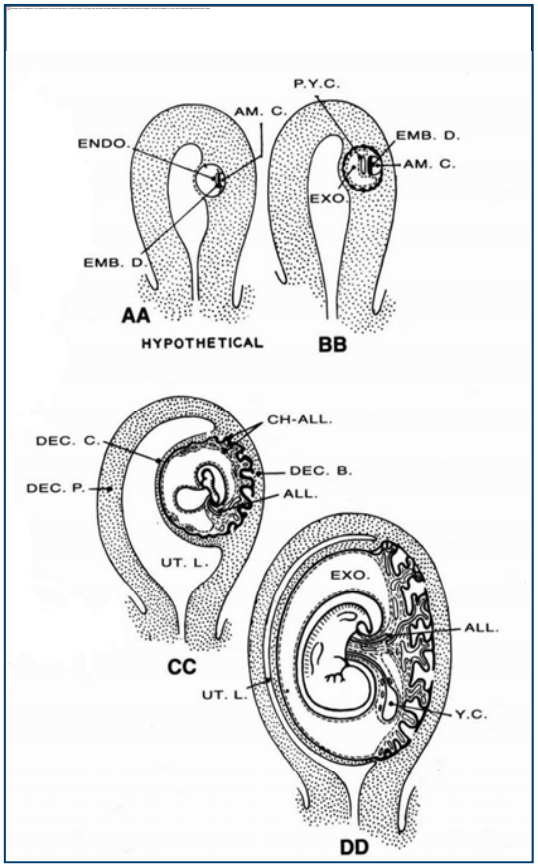


UGKO
no conceptus

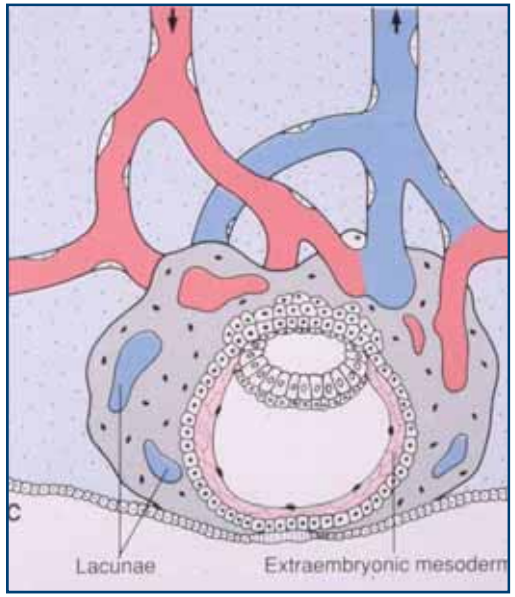
UGKO
1 expanded conceptus

- If development of the endometrial glands is suppressed experimentally in the sheep the conceptus fails to develop in a dose-dependent fashion

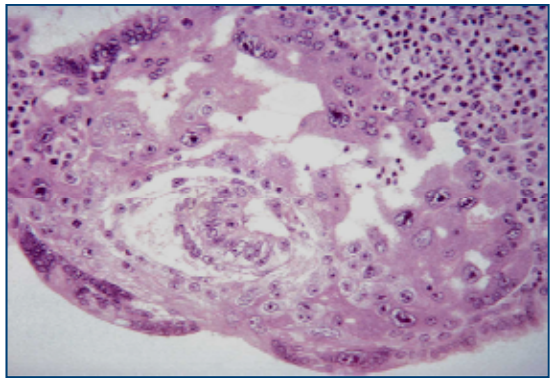
Histiotrophic nutrition in early pregnancy



Human
Great apes

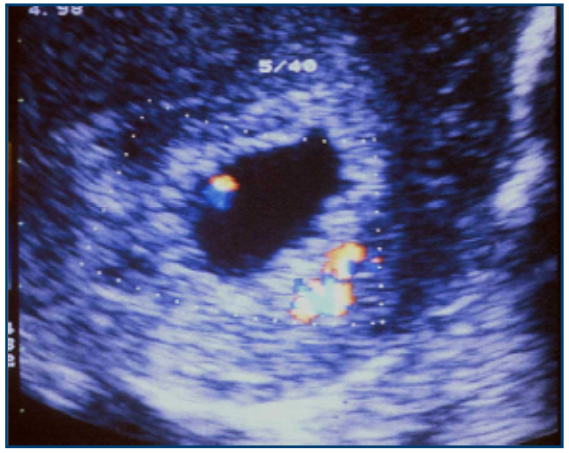


Day 9 post-fertilisation

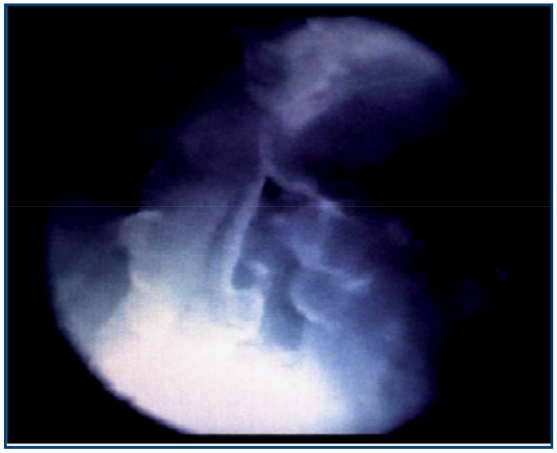


- In the human, the unique invasive form of implantation means that the conceptus is removed from the uterine lumen by day 9 post-fertilisation,
- Hence, the histiotrophic phase has always been considered to be brief

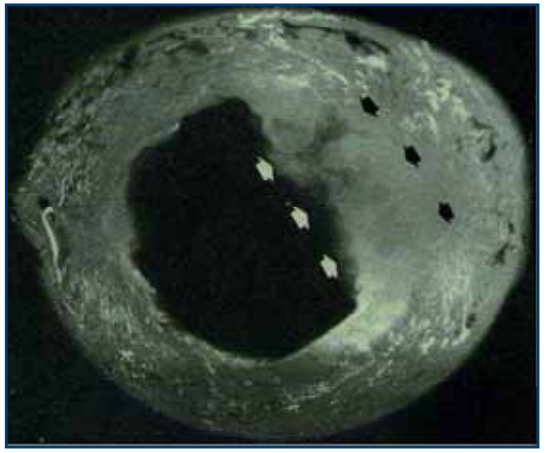
The maternal intraplacental circulation is not established until the end of the first trimester



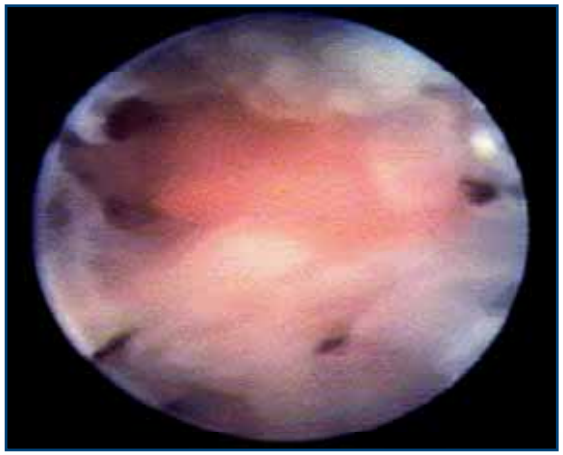
8 - 9 weeks

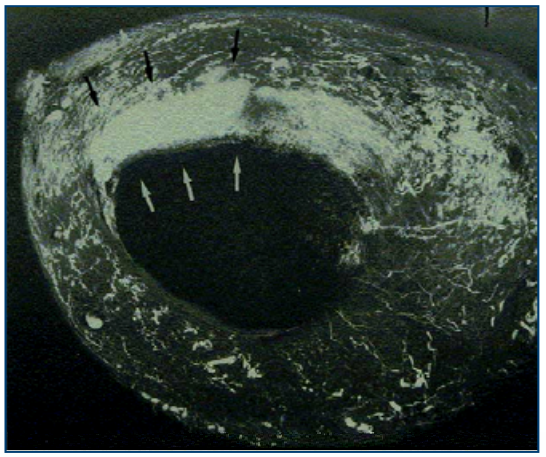


Hustin and Schaaps 1987



12 - 13 weeks

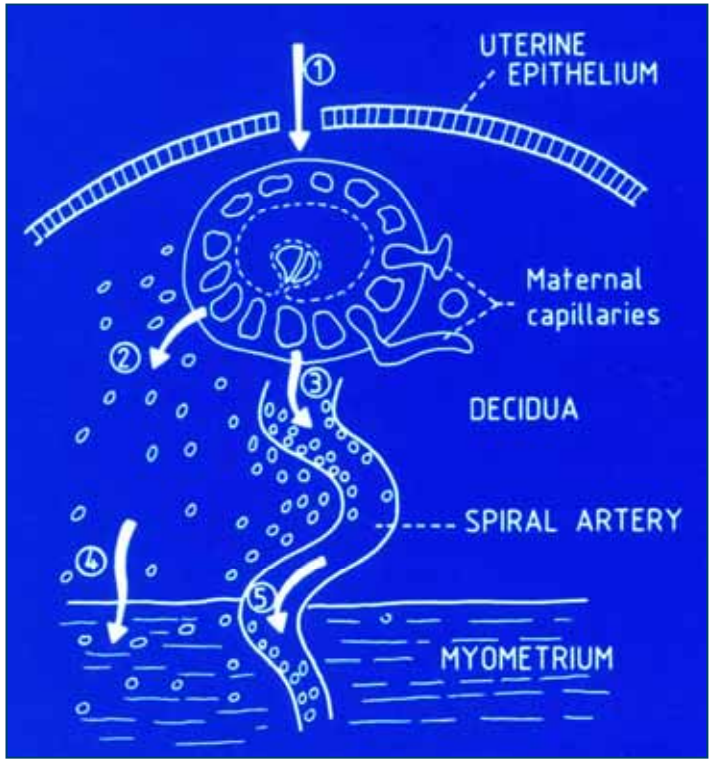


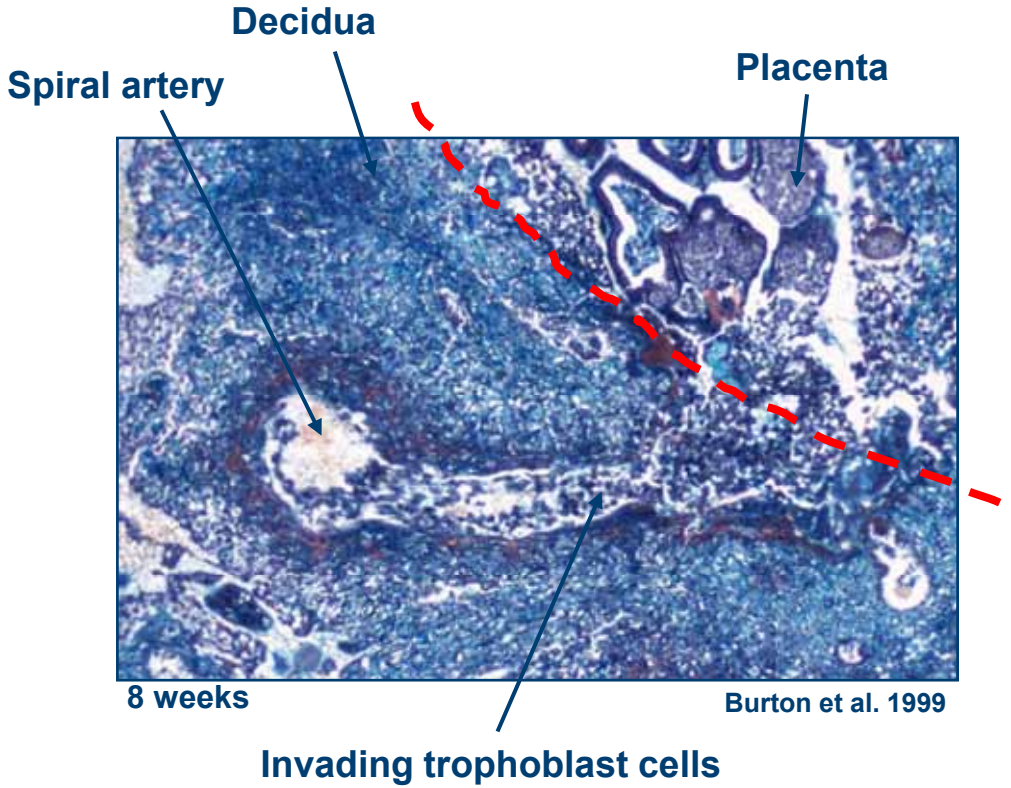


Doppler ultrasound, hysteroscopy, and perfusion of hysterectomy specimens all demonstrated an absence of significant maternal blood flow to the placenta prior to 10-12 weeks of pregnancy

The maternal spiral arteries are plugged by invading endovascular trophoblast in early pregnancy

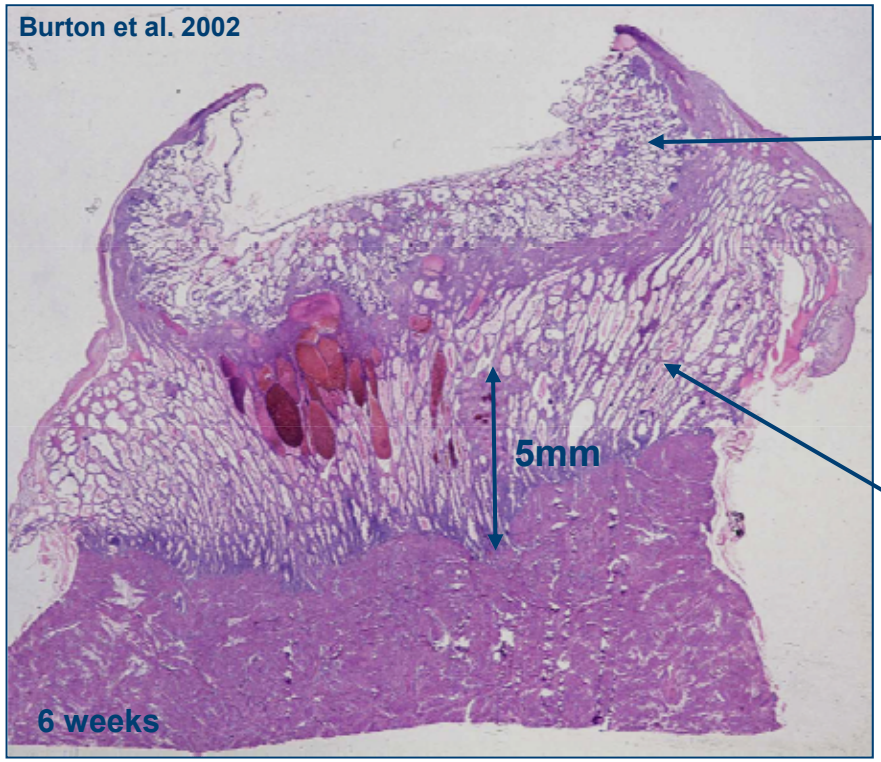
Pijnenborg

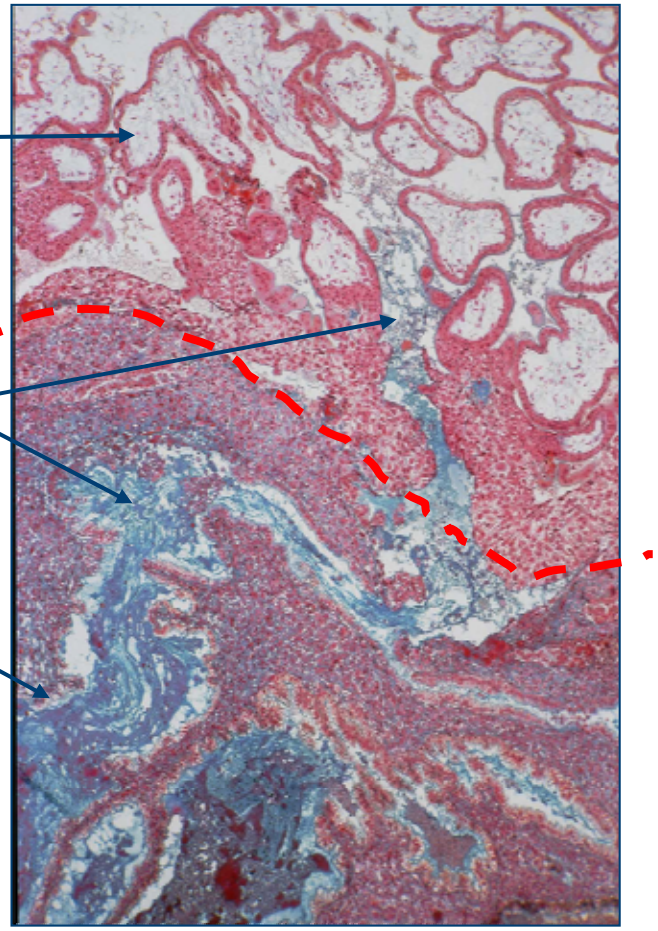




- During the first trimester the invading trophoblast cells plug the mouths of the maternal spiral arteries

Secretions from the endometrial glands support the conceptus during the first trimester

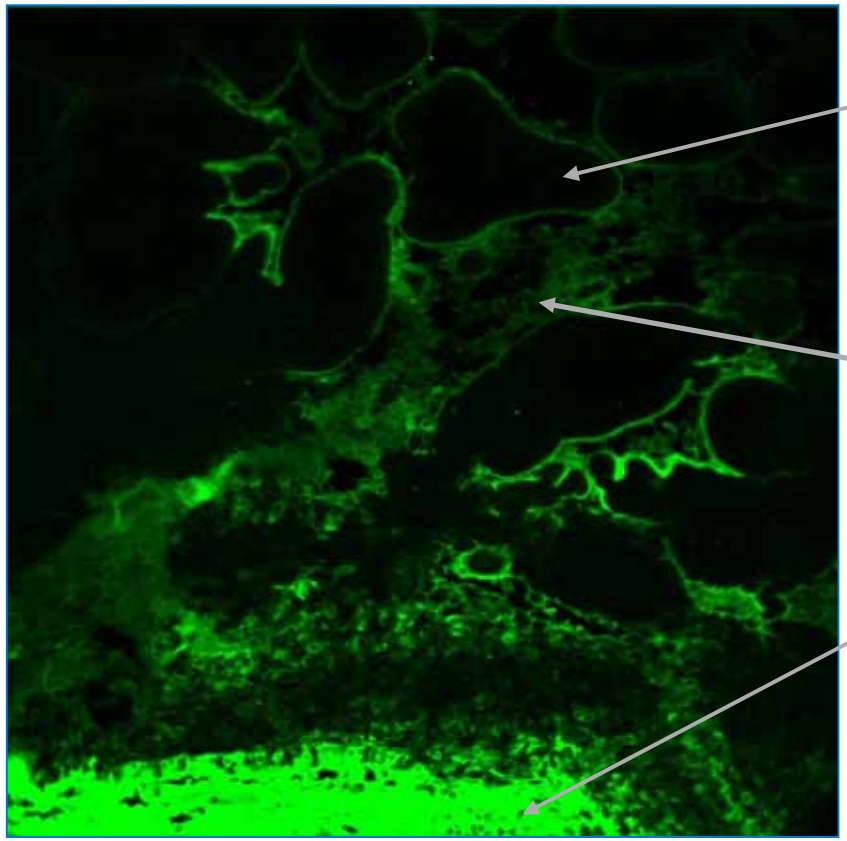




- Endometrium is still 5-6 mm thick at 6 weeks of gestation, with highly active glands that discharge into the placenta

Endometrial glands during the first trimester

Burton et al. 2002

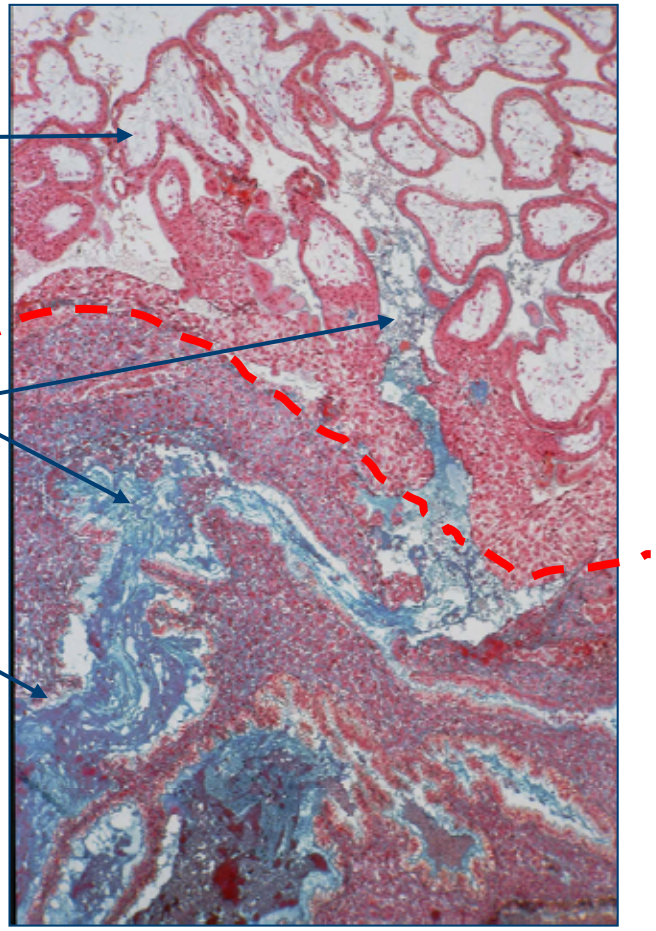


MUC-1

villus

'Uterine milk'

Endometrial gland



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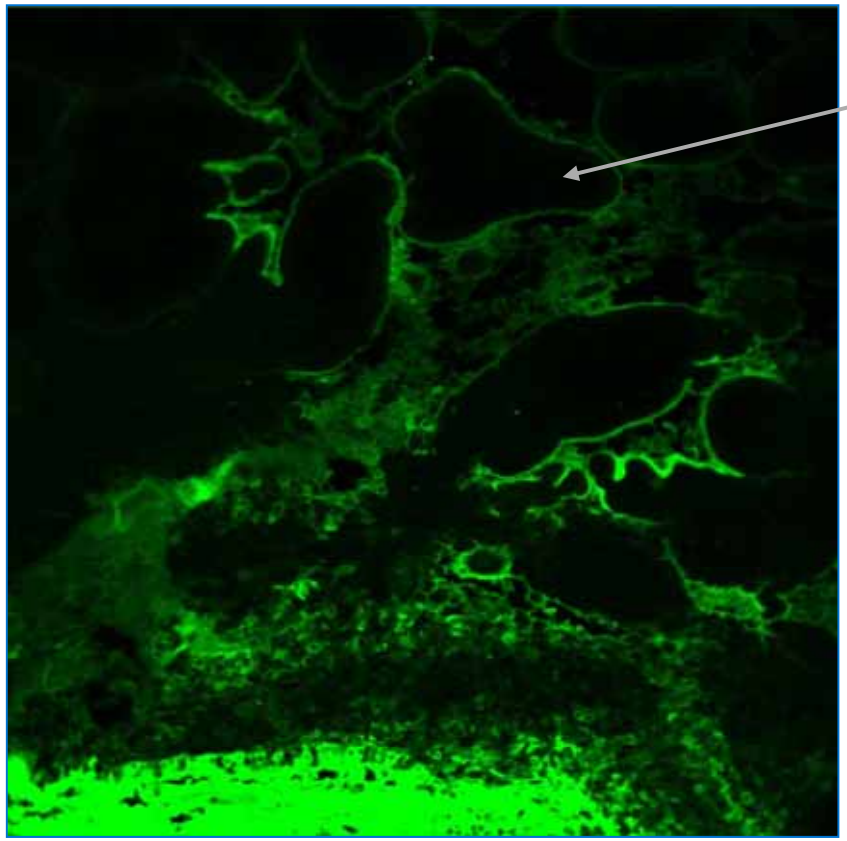
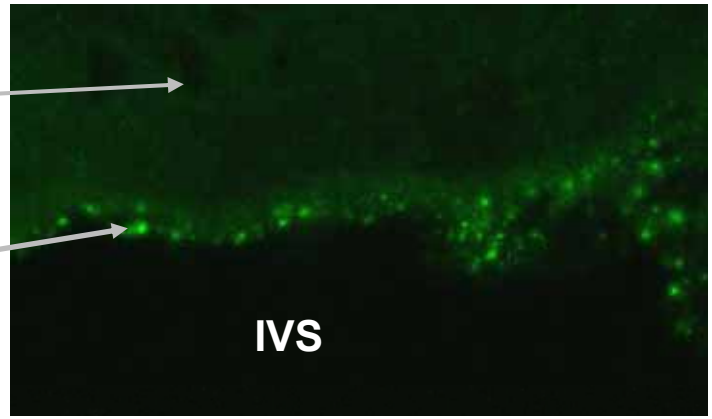
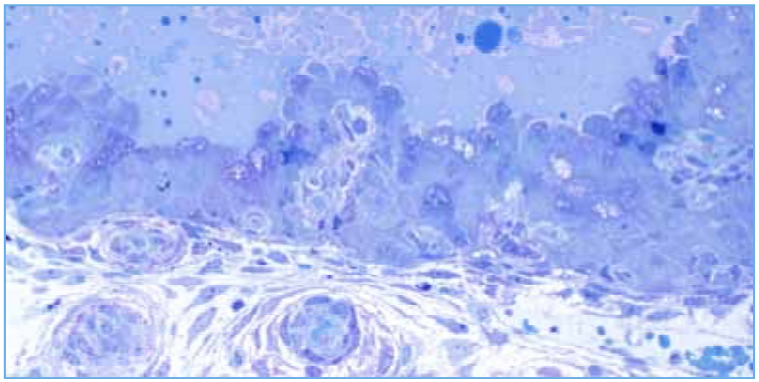


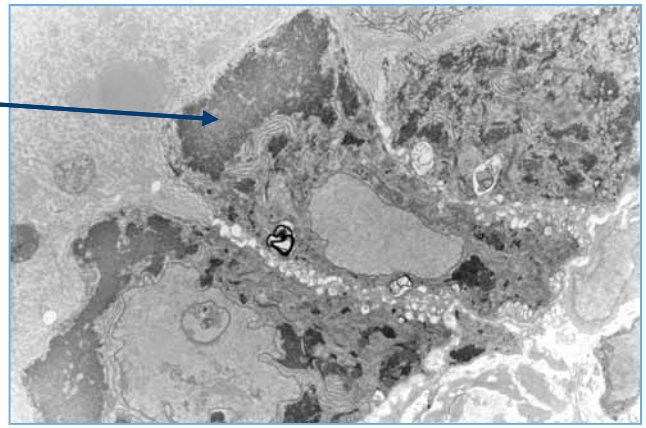
MUC-1



- Intense immunofluorescence for MUC-1 derived from the glands can be seen within the syncytiotrophoblast, suggesting phagocytic uptake
- Breakdown of these glycoproteins will release a rich supply of elements and amino acids

Endometrial glands during the first trimester

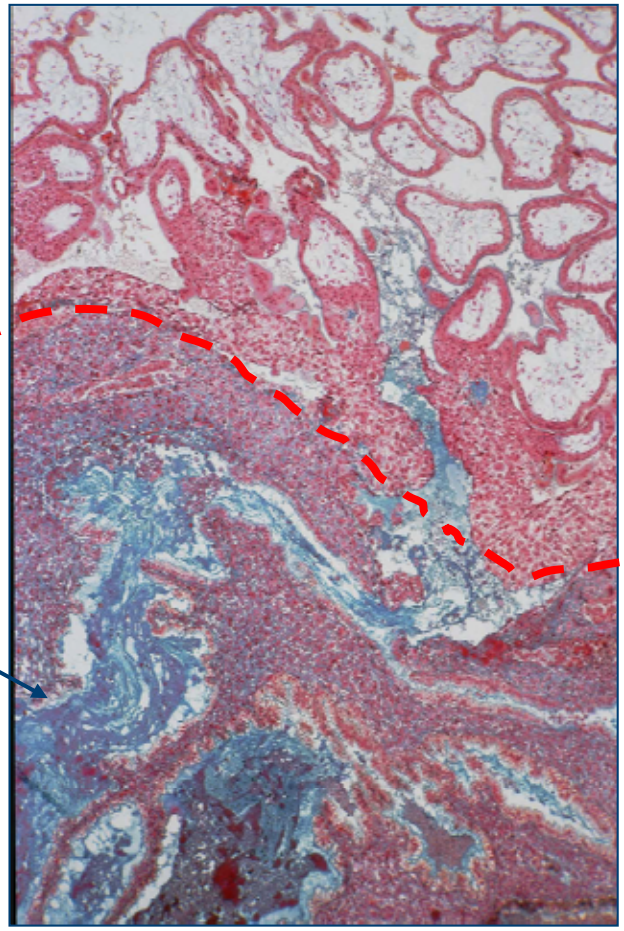




glycogen

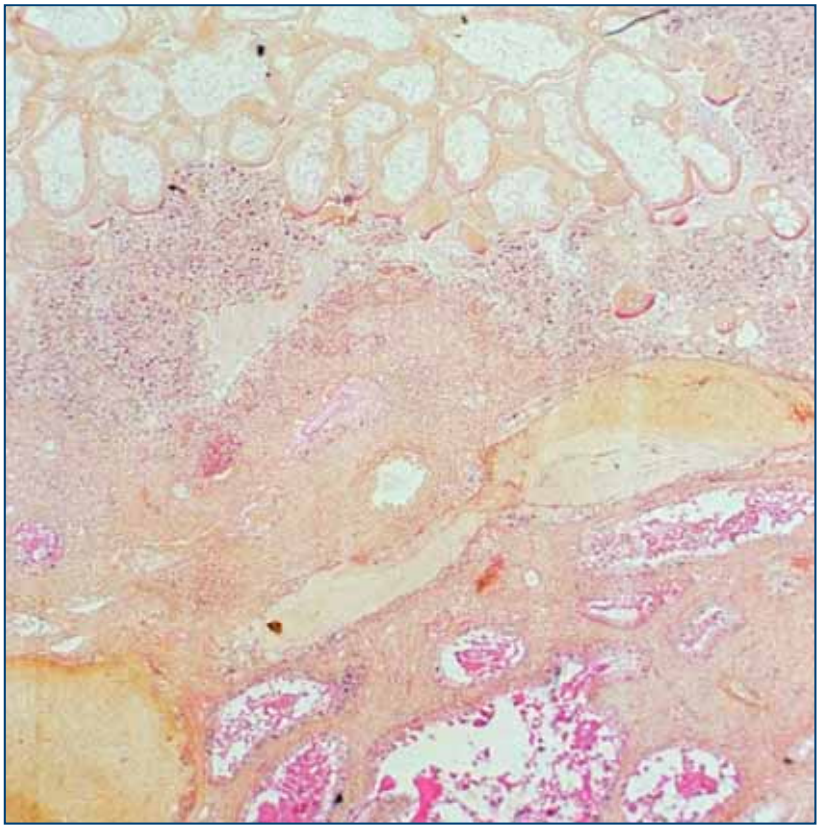
6 weeks

Endometrial gland



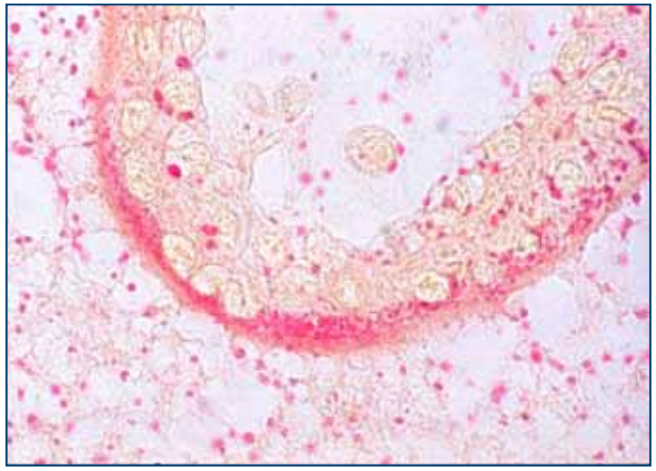
- At 6 weeks the gland epithelium is columnar, highly active, and similar morphologically to the secretory phase of the cycle

Secretions from the endometrial glands support the conceptus during the first trimester



Burton et al. 2002

6 weeks

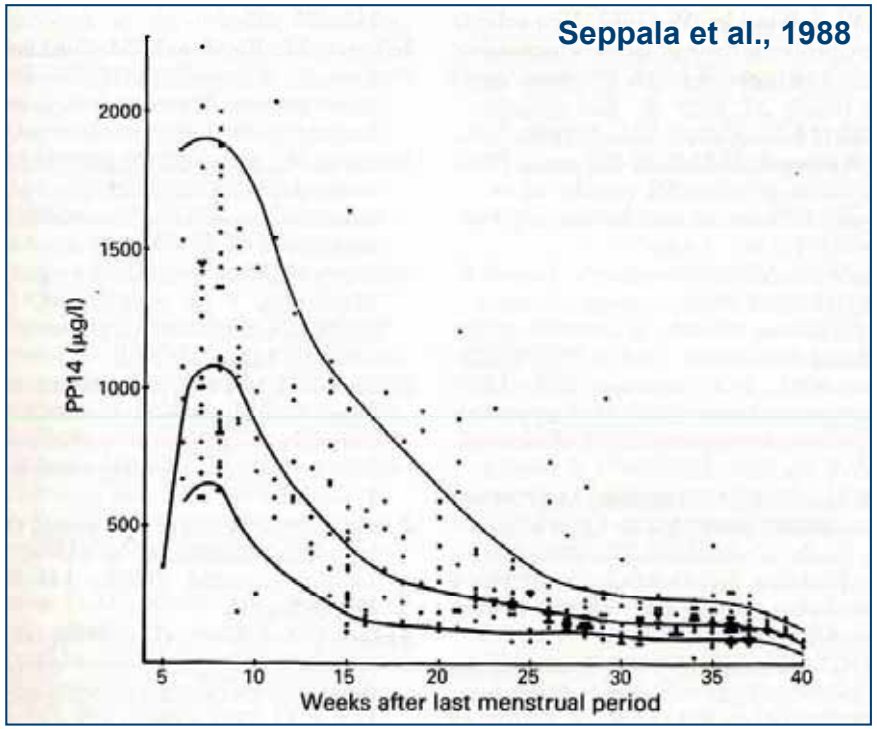


Glycogen in syncytiotrophoblast

- The glands are rich in glycogen, and this accumulates in the syncytiotrophoblast facing the openings of the glands

Glandular activity peaks during the first trimester

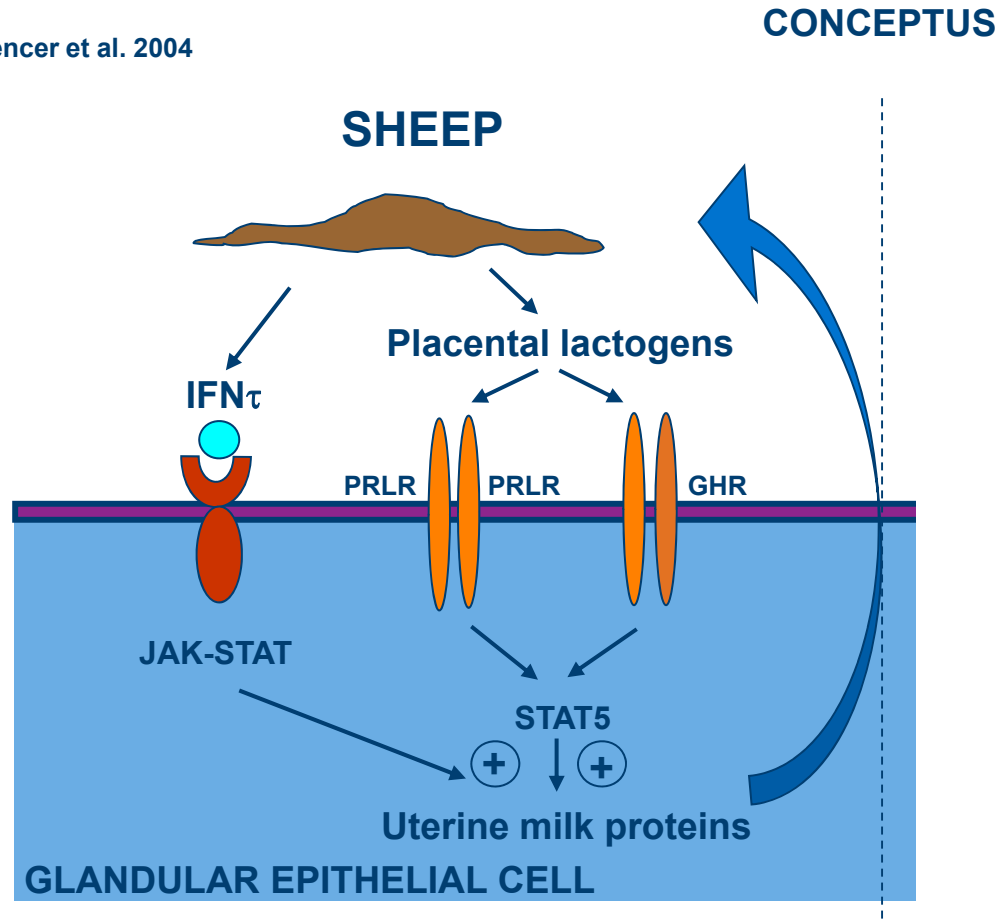
Concentration of maternal serum glycodelin (10, 90 centiles)



- Concentrations of glycodelin (PP14) peak in maternal serum at around 8 weeks, and rapidly decline after the first trimester
- Loss of glandular growth factors may reduce trophoblast stem cell support

Servomechanism regulating gland activity

Spencer et al. 2004



Servomechanism regulating gland activity

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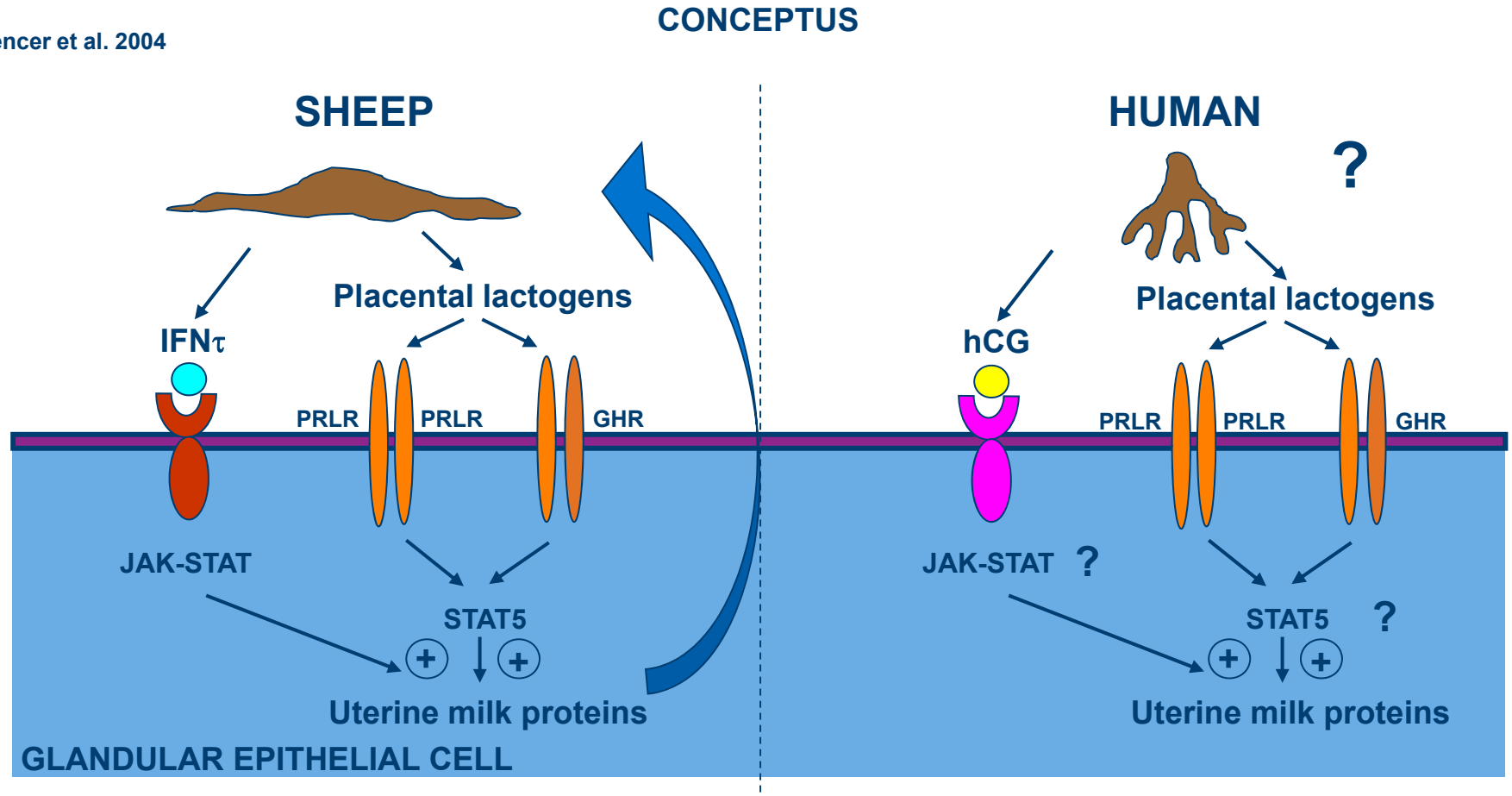
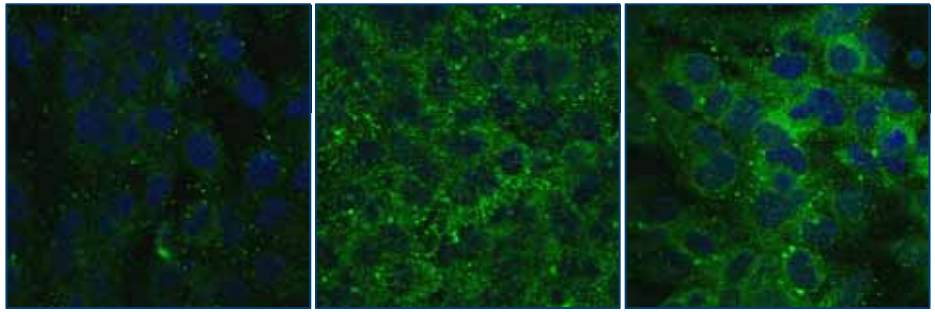


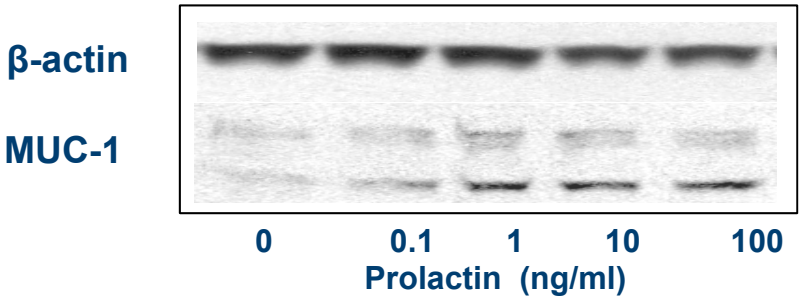
Servomechanism regulating gland activity

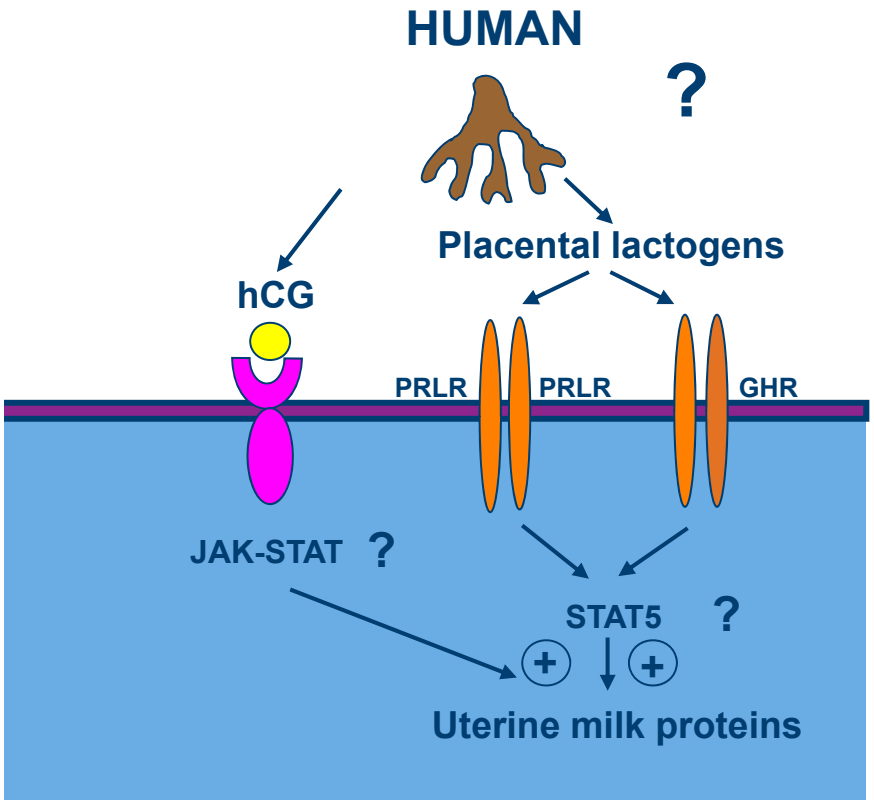
CONCEPTUS

MUC-1



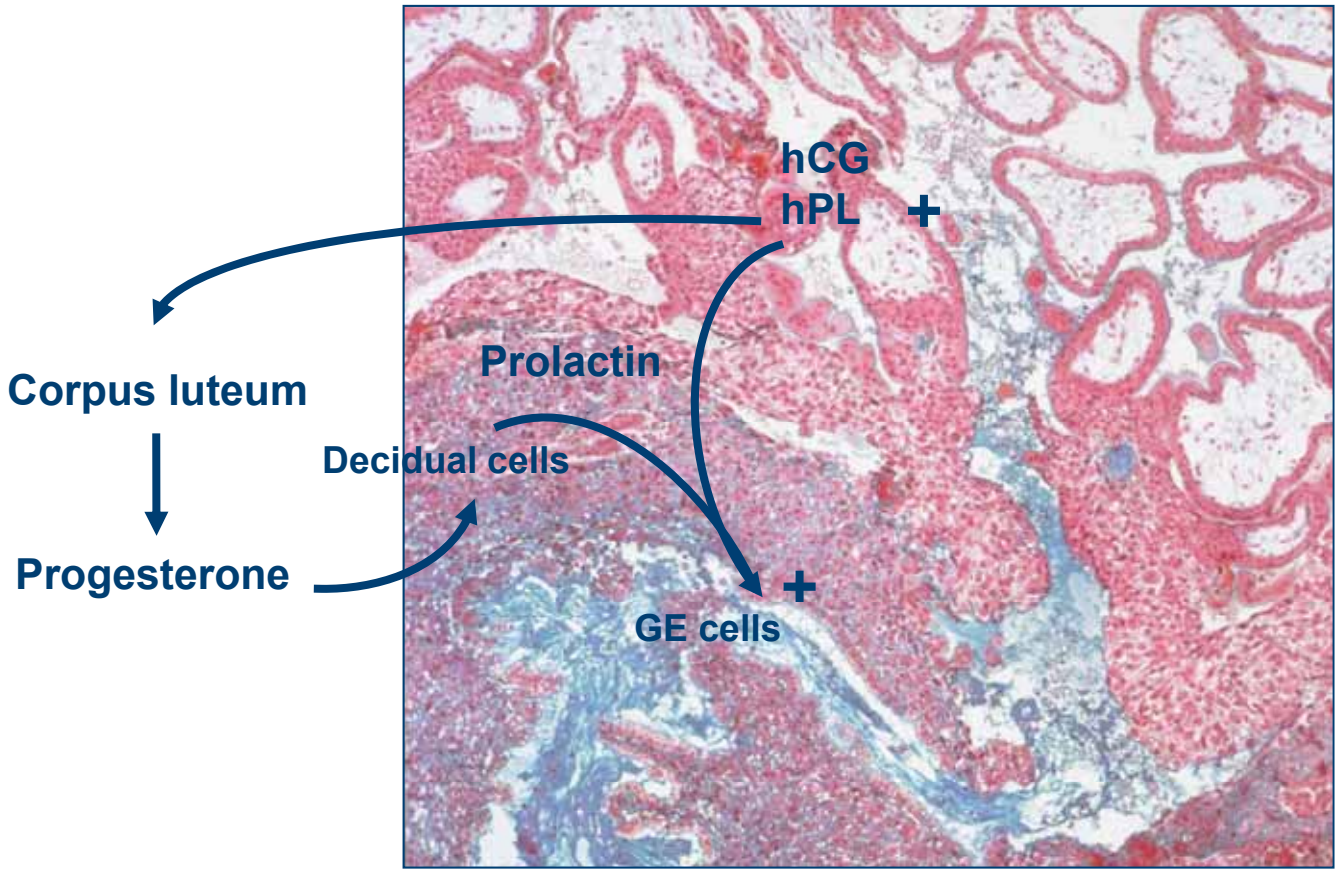
Vehicle control | 1 ng/ml | 100 ng/ml 3 hr





- **Addition of prolactin to a human endometrial gland cell line increases MUC-1, a 'milk protein' in a dose-dependent manner**

Potential servomechanism in the human

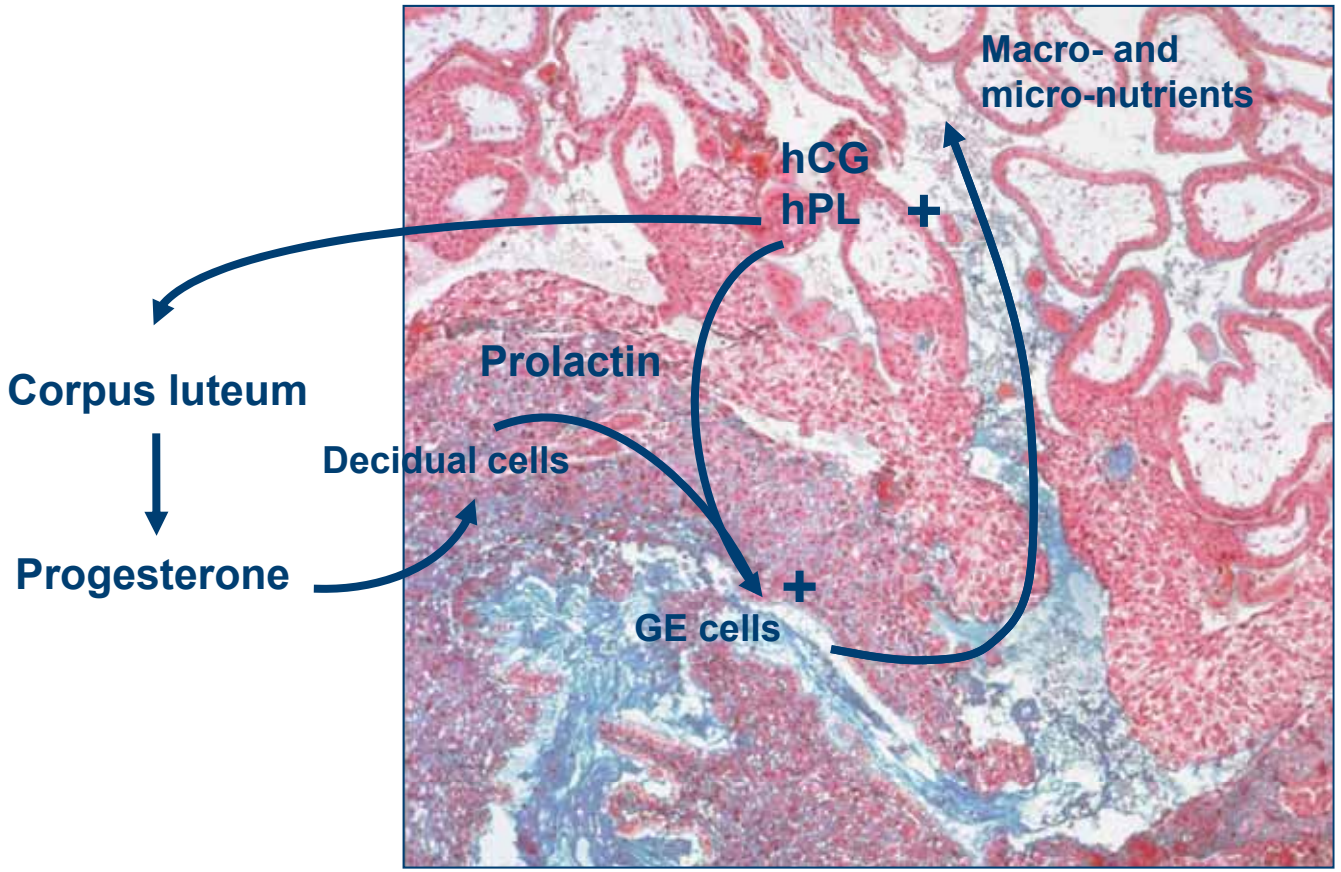


hCG, human chorionic gonadotropin

hPL, human placental lactogen

- hCG and hPL may stimulate the gland epithelial cells direct, or via prolactin secreted by the decidual cells in response to P4 from the CL

Potential servomechanism in the human



hCG, human chorionic gonadotropin

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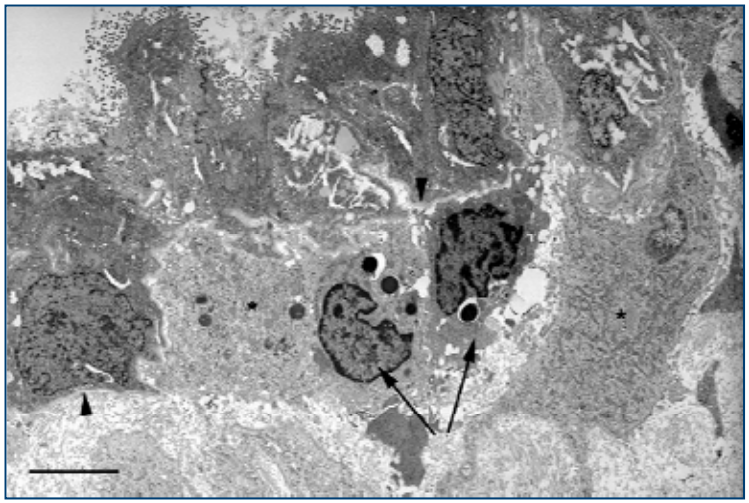
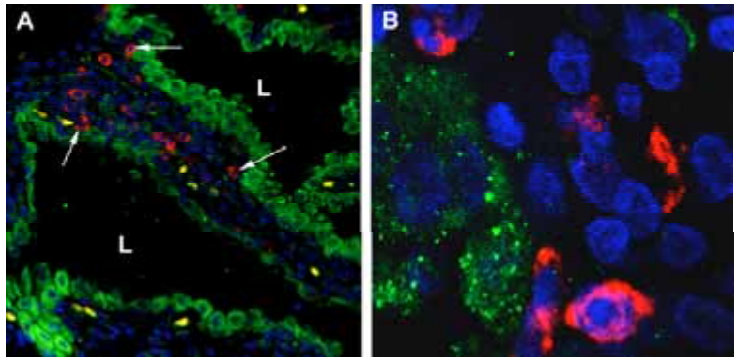
- hCG and hPL may stimulate the gland epithelial cells direct, or via prolactin secreted by the decidual cells in response to P4 from the CL

uNK cells are closely approximated to the glandular epithelium



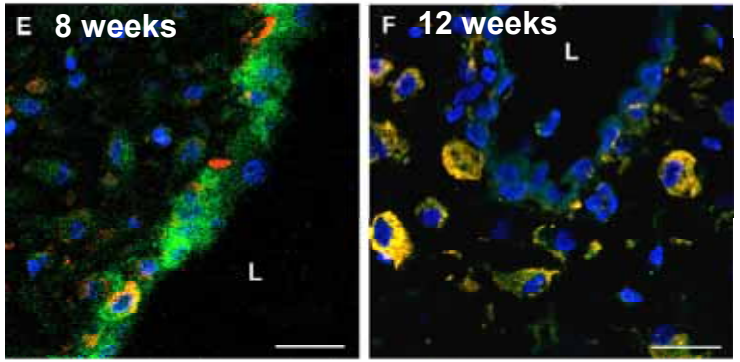
Hempstock et al. 2004

TTP



CD56

EGF



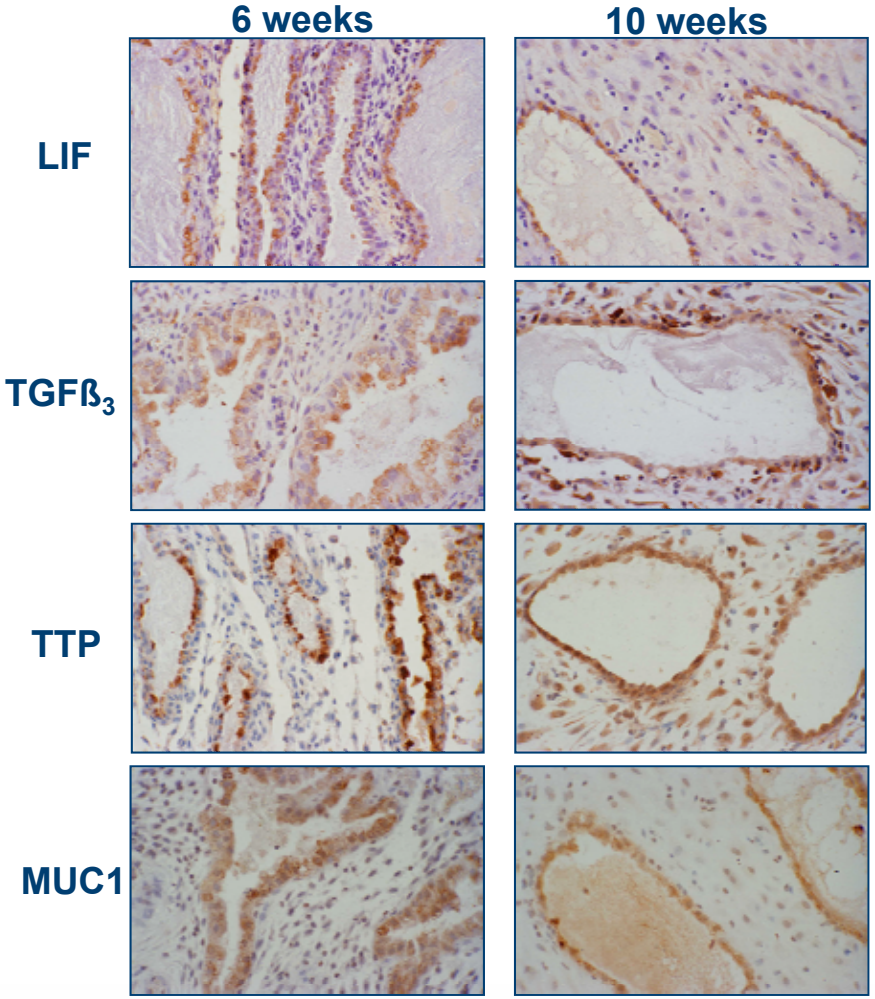
CD56

- uNK cells are closely approximated to the basal surface of the glandular epithelium
- uNK cells are immunopositive for EGF, suggesting they may play a role in maintaining the epithelium

Conclusions 1

- During the first trimester the placenta is not truly haemochorial but develops in a unique environment, characterized by histiotrophic support from the endometrial glands
- The endometrial secretions are taken up by the trophoblast where they are presumably broken down into amino acids and elements
- Prolactin from the decidual cells, and hPL and hCG from the placenta may stimulate secretory activity in the glands, similar to the servo-mechanism observed in other species
- Towards the end of the first trimester the activity of the glands subsides and haemotrophic nutrition takes over

Endometrial secretions are a source of growth factors and cytokines as well as nutrients



Growth factors (LIF, VEGF, EGF)

Regulation of cell proliferation and differentiation

Cytokines ($TGF\beta_3$)

Modulate trophoblast invasion

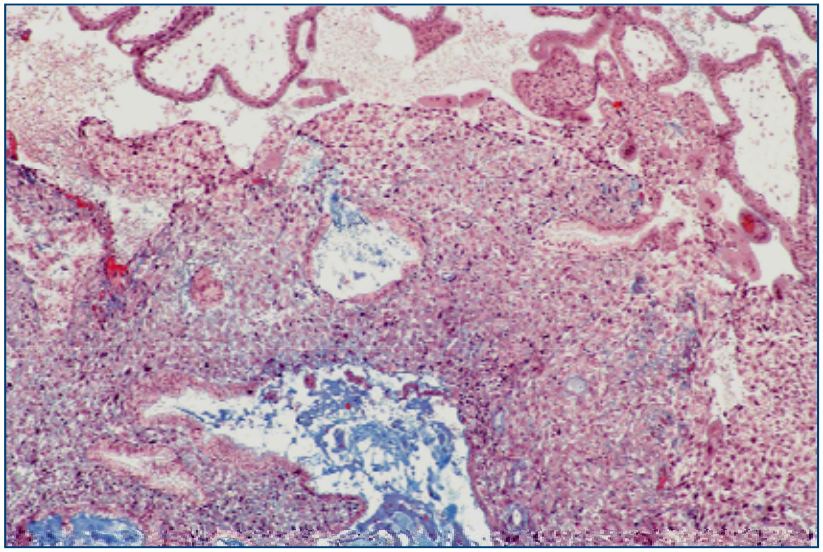
Transport proteins (TTP, lactoferrin)

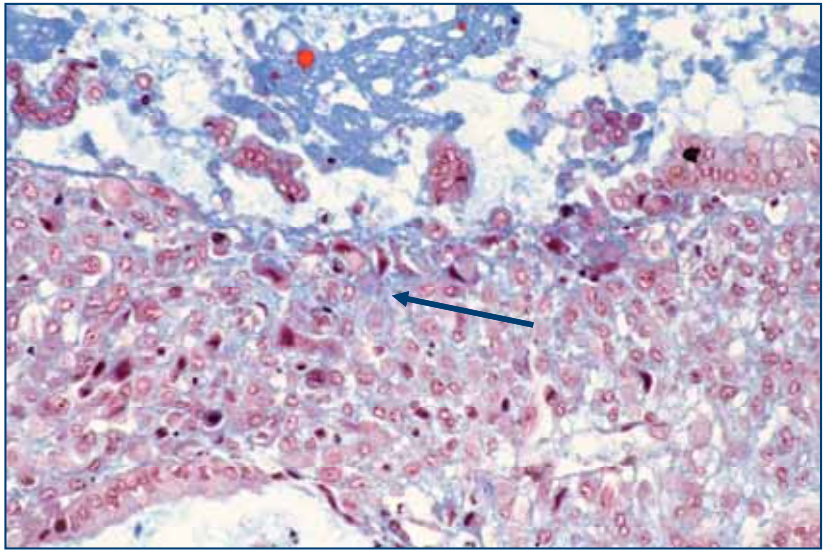
Transport of macro- and micro-nutrients

Glycoproteins (MUC1, Glycodelin, uteroglobin)

Regulation of immune interactions, cell migration

Immunomodulatory roles

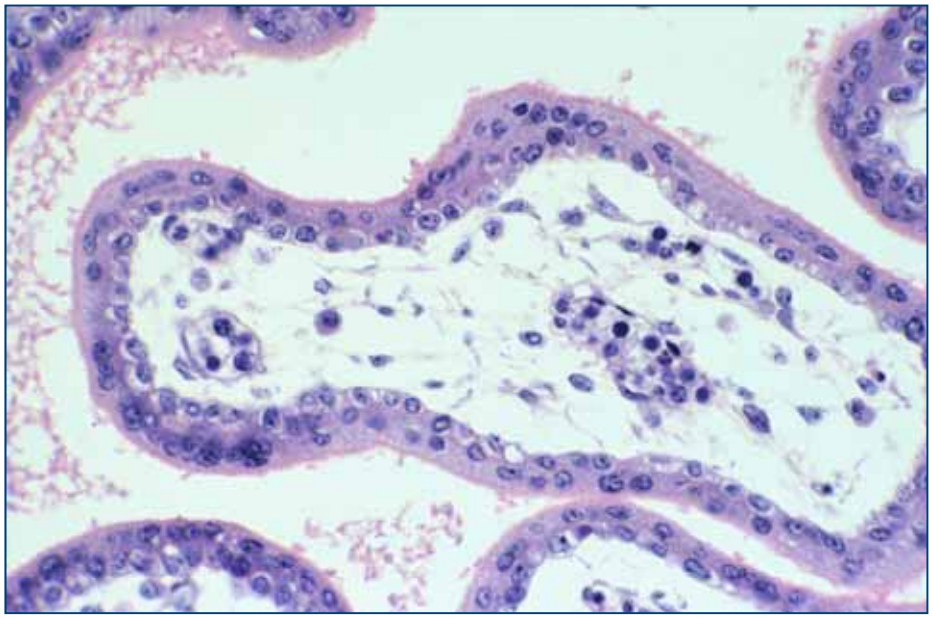


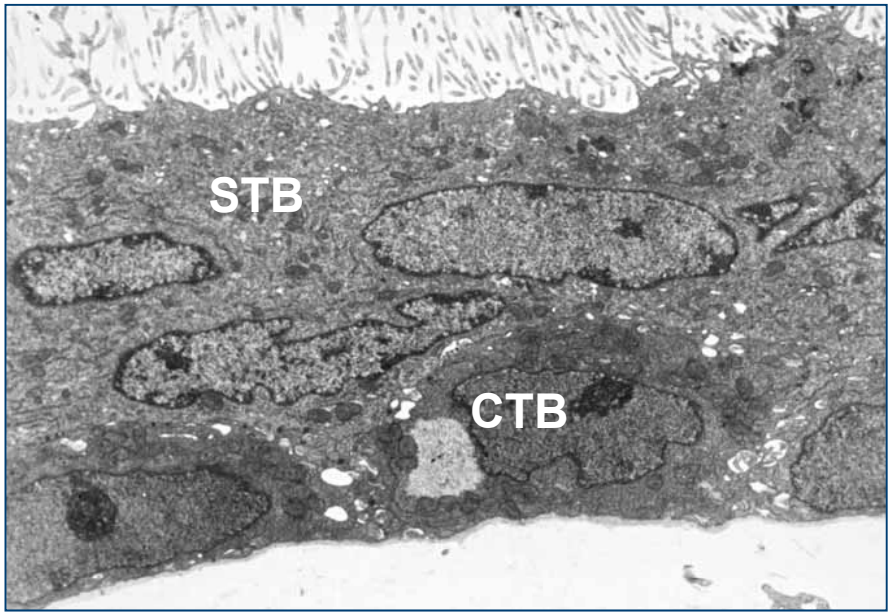


- Secretions are also released into the endometrial stroma at the maternal-fetal interface due to disruption of the gland walls
- Glycodelin suppresses cytotoxicity of uNK cells *in vitro*, reduces secretion of interleukins by lymphocytes and can trigger apoptosis in T cells
- Uteroglobin inhibits neutrophil and monocyte chemotaxis *in vitro*

Placental histology

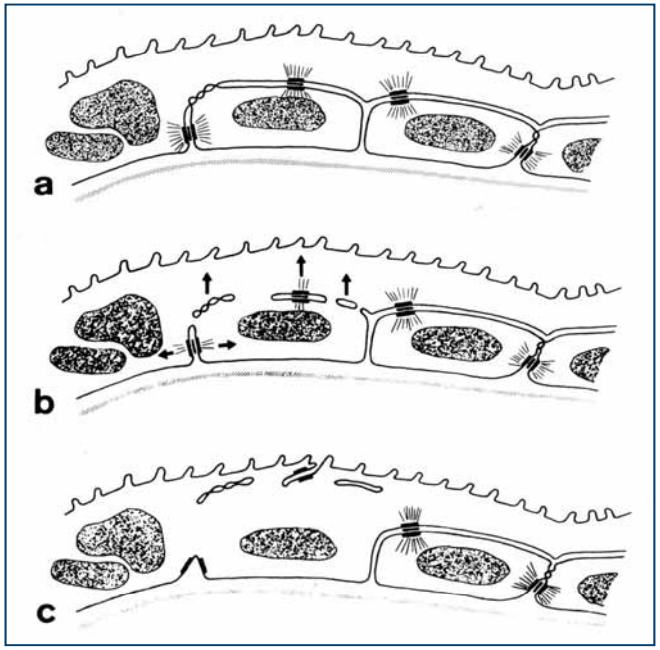
6 weeks gestational age

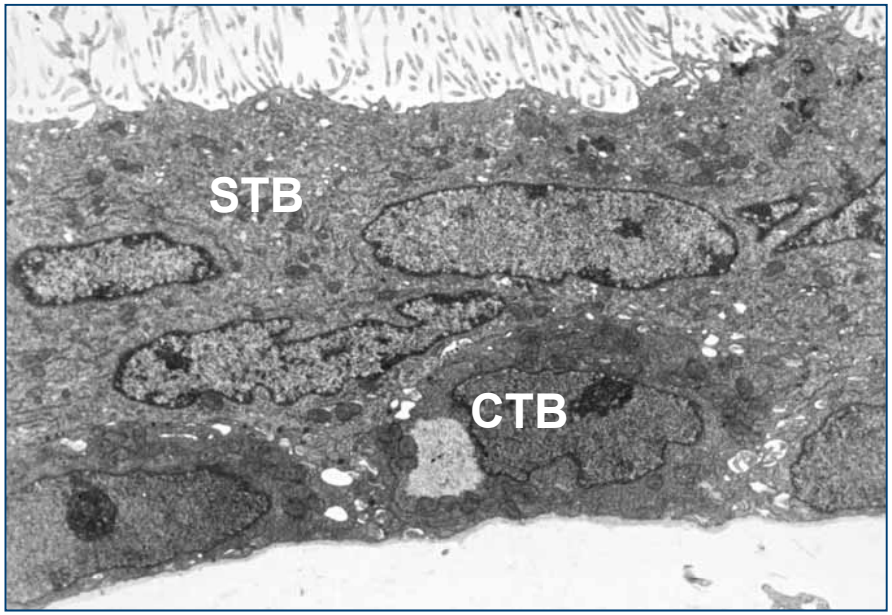




- The trophoblast consists of two layers in the first trimester, an outer multinucleated syncytiotrophoblast (STB) and an inner layer of uninucleate cytotrophoblast cells (CTB)
- Only the CTB cells are proliferative, and expansion of the STB layer is due to fusion and incorporation of CTB cells

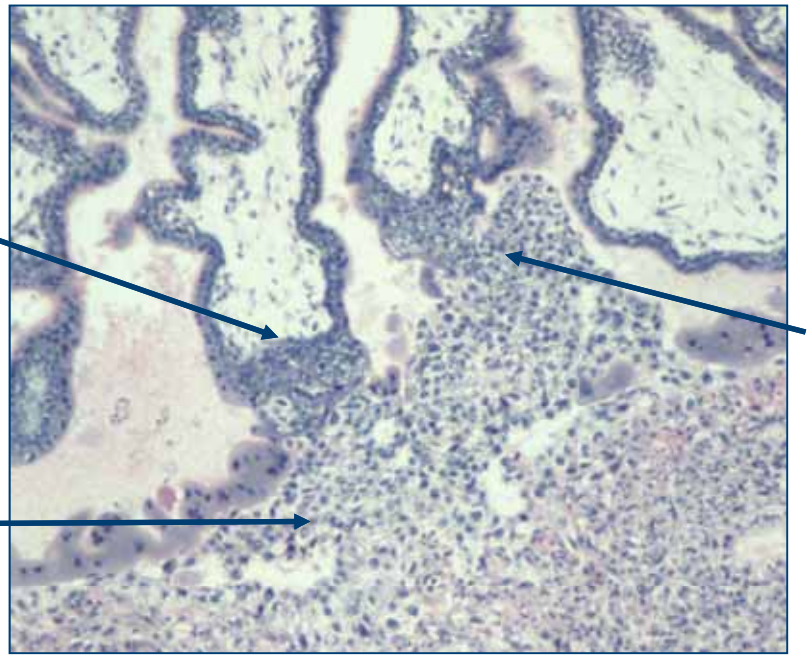
Placental histology





- Proliferation of CTB cells is therefore essential for trophoblast and villous growth
- It is also essential for formation of the cytotrophoblastic shell, which forms the materno-fetal interface during the first trimester

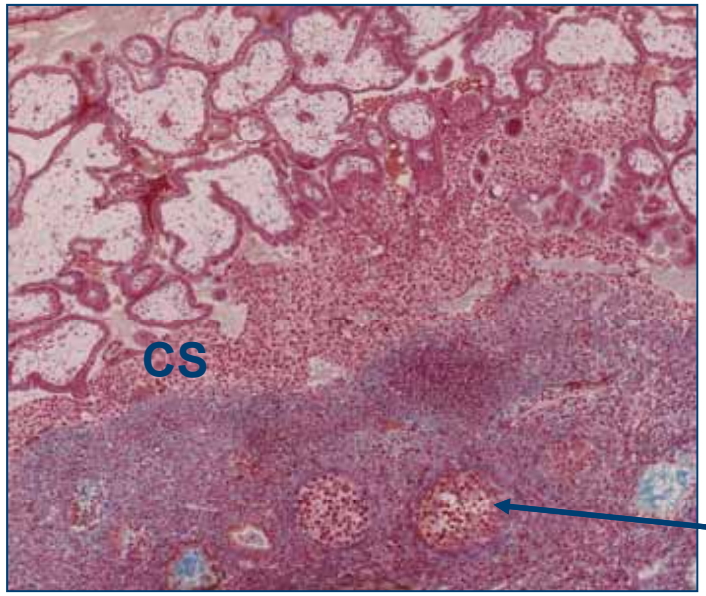
Placental histology

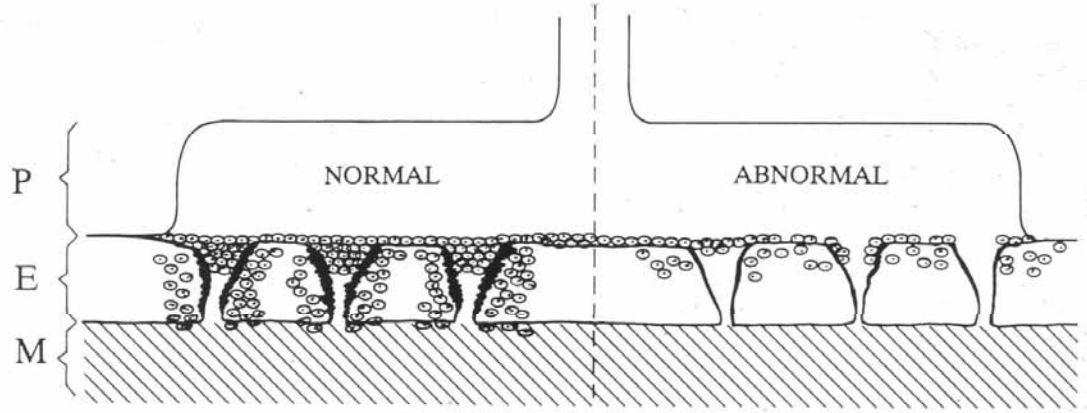


- Proliferation of CTB cells is therefore essential for trophoblast and villous growth
- It is also essential for formation of the cytotrophoblastic shell, which forms the materno-fetal interface during the first trimester, and the supply of extravillous trophoblast cells which invade into the decidua

Deficient formation of the cytotrophoblast shell is associated with miscarriage

Normal 6 week pregnancy



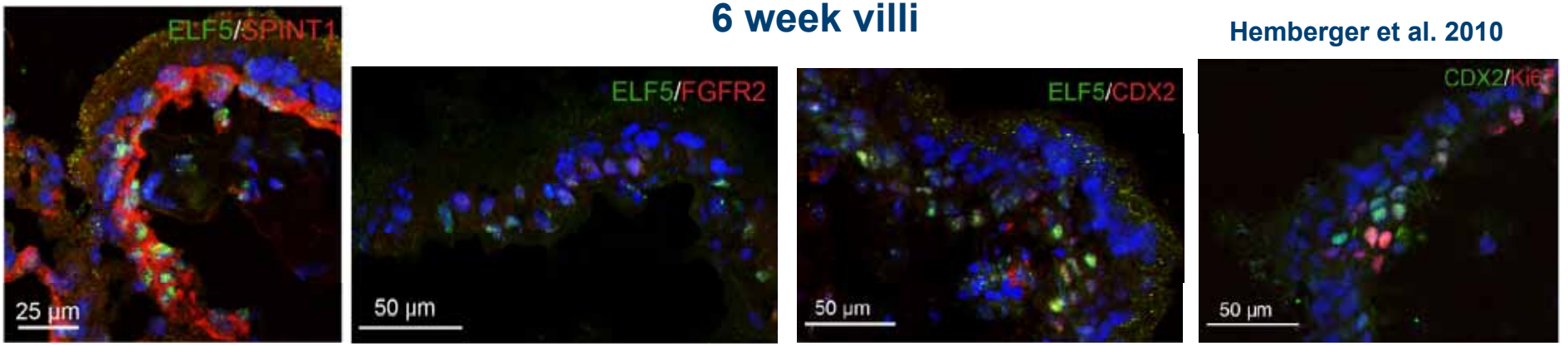


Hustin et al. 1990

Plugged artery

- The cytotrophoblastic shell (CS) is thin or incomplete in approximately 70% cases of spontaneous miscarriage, independent of the karyotype, leading to poor plugging of the arteries and consequently early and disorganised onset of the maternal blood flow to the placenta

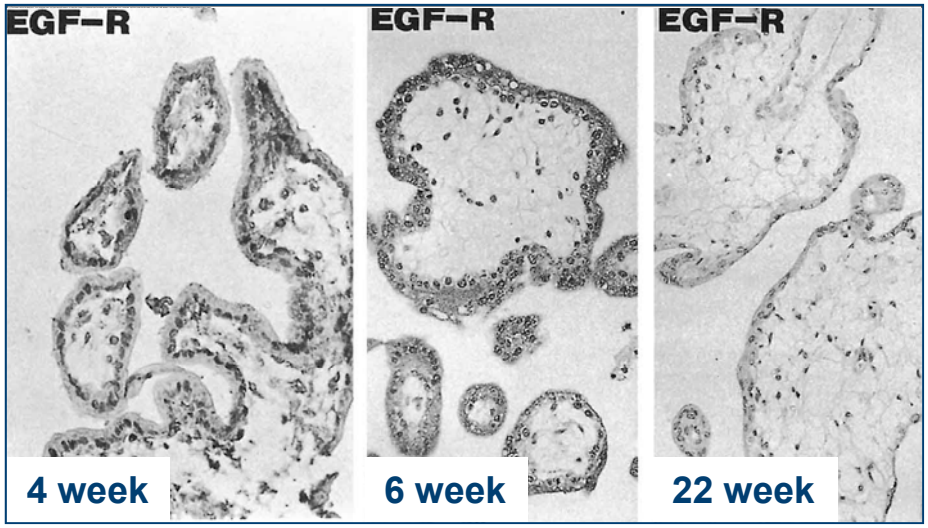
Growth factors from the endometrial glands may play a role in maintaining placental stem cells

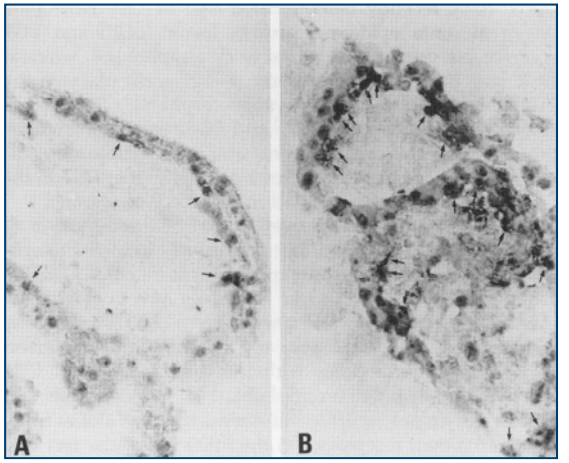


- ELF5, a transcription factor essential for trophoblast stem cell self-renewal, localizes to a sub-set of villous CTB (CTB are marked by SPINT1)
- ELF5 is activated by FGF, and co-localizes with the FGFR2
- ELF5 co-localizes with CDX2, a transcription factor essential for the trophoblast lineage
- CDX2 is preferentially localized to mitotically active cytotrophoblast cells (Ki67-positive)

Growth factors from the endometrial glands may play a role in regulating placental development

Maruo et al. 1997





Ki-67

4-5 weeks

control

EGF 100µg/L

- The receptor for Epidermal Growth Factor (EGF) is present on the cytotrophoblast cells at 4 weeks of pregnancy
- Addition of exogenous EGF to placental tissues causes increased proliferation

Glandular activity is increased in early pregnancy

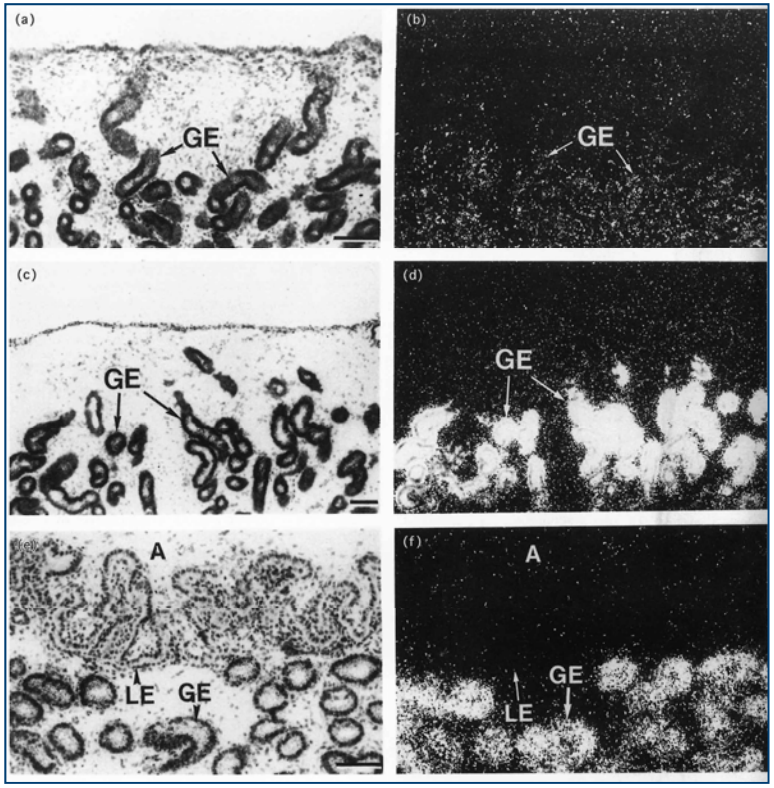
Bright-field

Dark-field

Day 33

Day 38

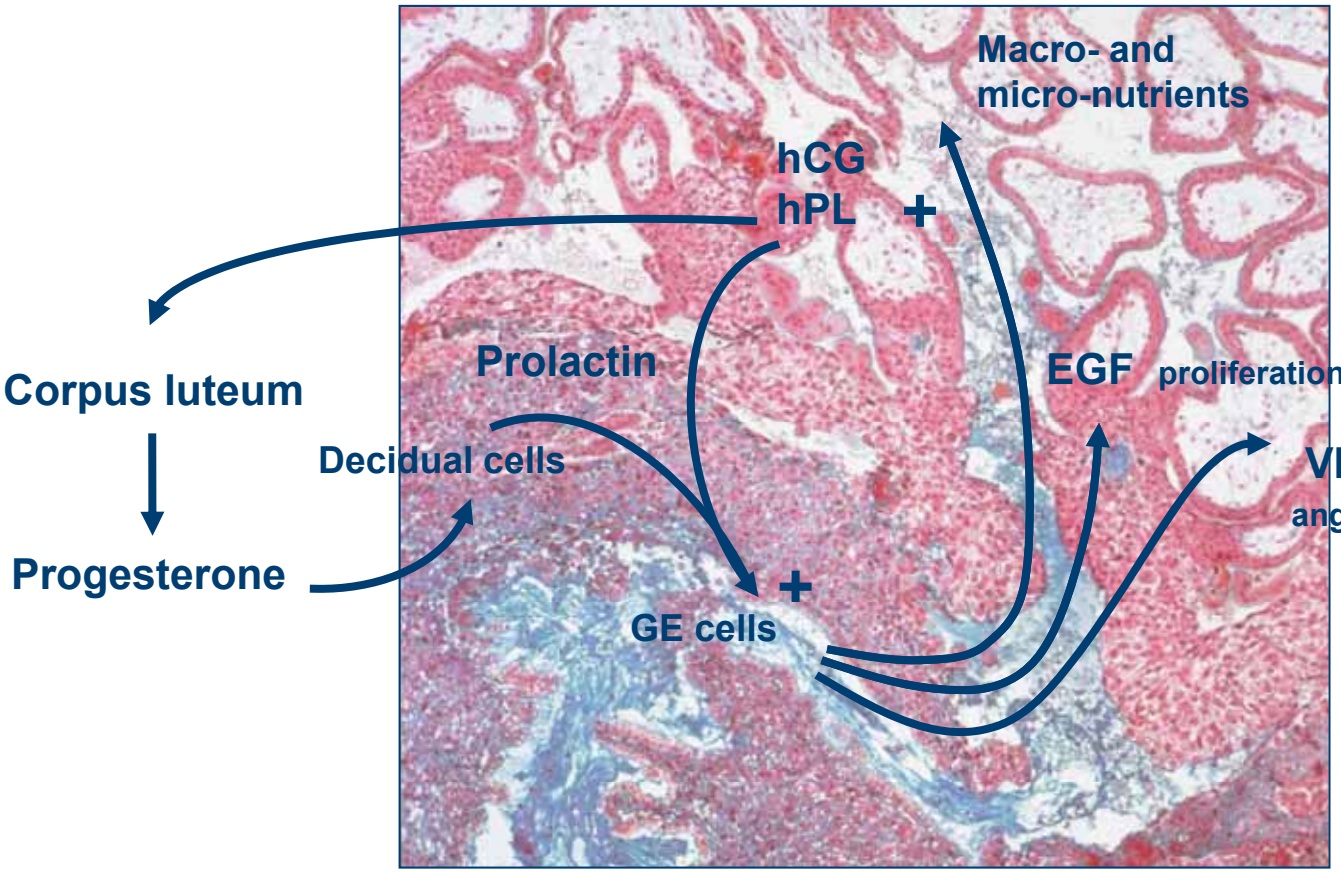
Day 60



ISH for EGF in the horse endometrium (Lennard et al. 1998)

- Expression of EGF increases in the glandular epithelial cells (GE) but not luminal epithelial cells (LE) during early pregnancy in the horse
- Expression of uterine milk proteins increases in pregnancy in the sheep

Potential servomechanism in the human



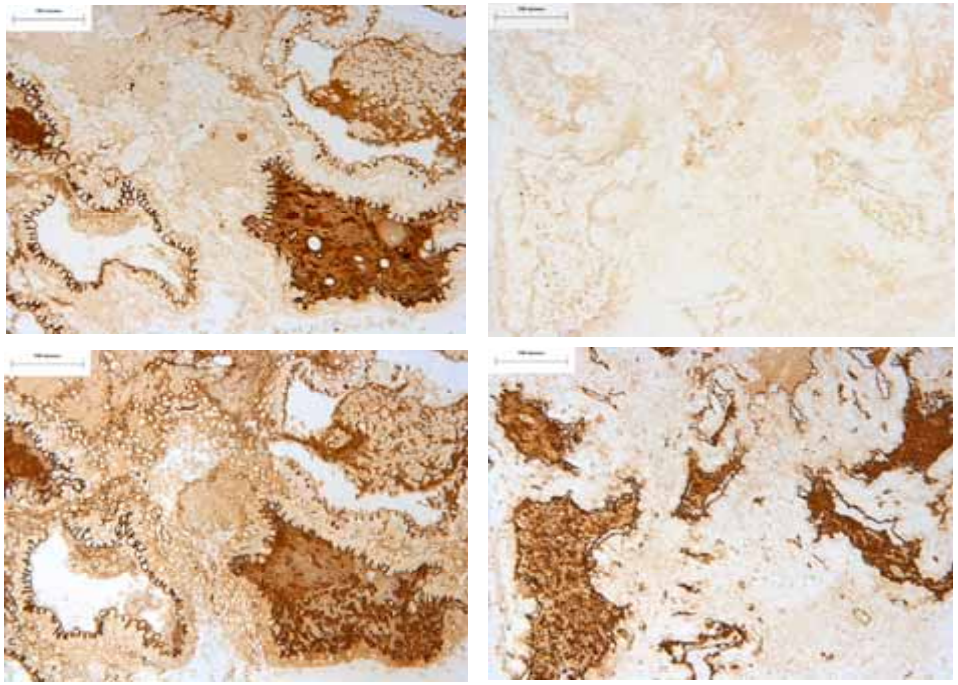
hCG, human chorionic gonadotropin

hPL, human placental lactogen

- In addition to nutrients, the glands also secrete an array of growth factors and cytokines that may regulate placental development

Changes in sialylation during early pregnancy

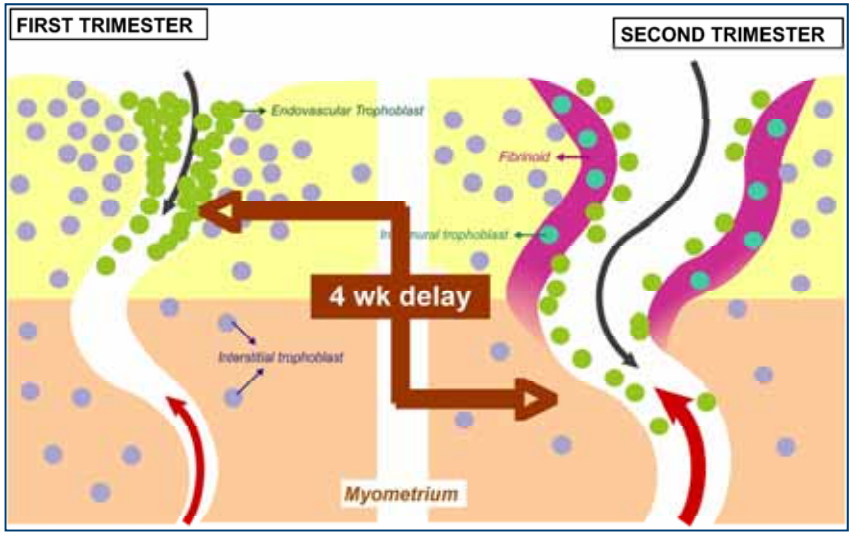
Jones et al. 2010



- During early pregnancy terminal sialylation is reduced, exposing galactose side-chains.
- Loss of sialylation ensures that the secretions/growth factors are rapidly cleared from the maternal circulation, protecting the mother from the dangers of overstimulation of her endothelial and other cells

Onset of the maternal intraplacental circulation

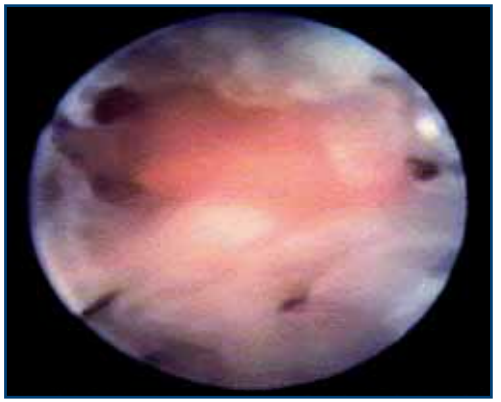
Pijnenborg et al. 2006



Hustin & Schaaps 1987

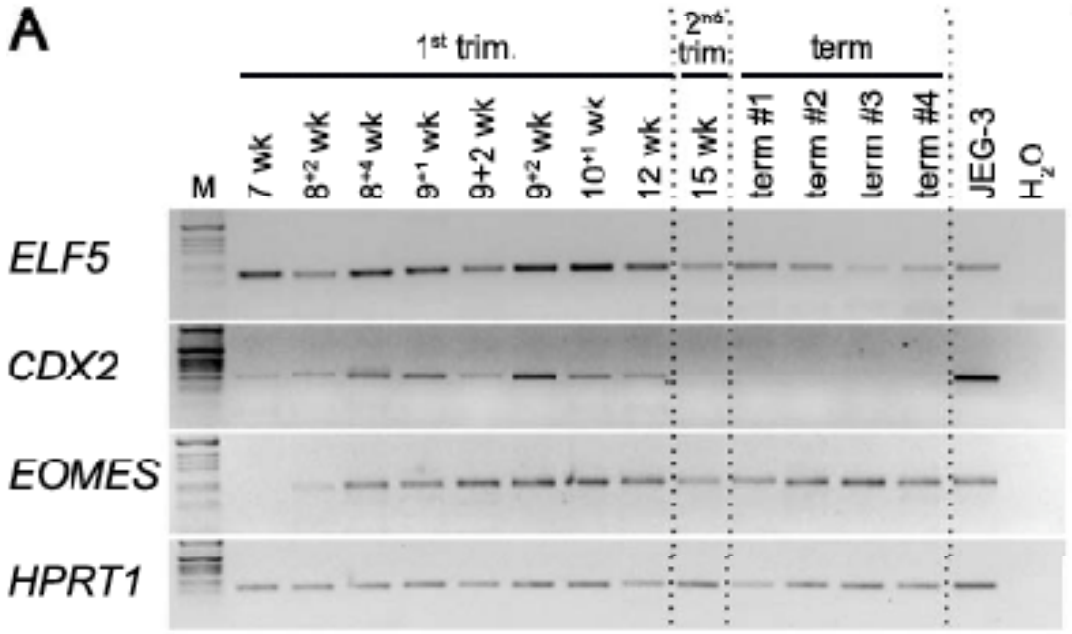
8 - 9 weeks

12 - 13 weeks



- Onset of the maternal arterial circulation occurs towards the end of the first trimester due to unplugging of the spiral arteries

Markers of trophoblast proliferation decline at the end of the first trimester

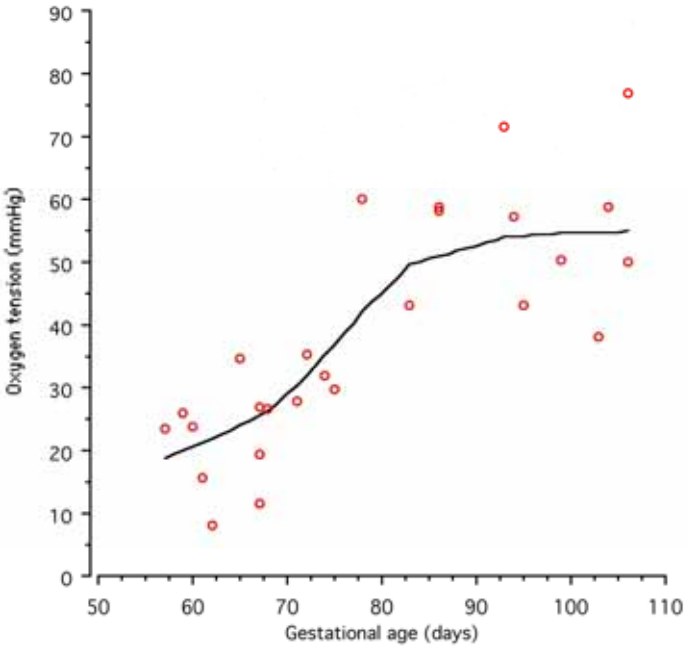


Hemberger et al. 2010

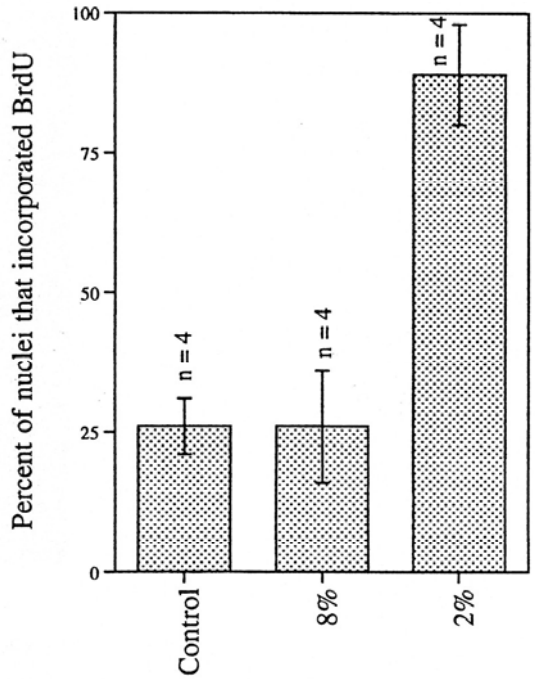
- RT-PCR demonstrates dramatic reduction in ELF5 and CDX2 expression after the end of the first trimester

The oxygen transition and trophoblast proliferation

Jauniaux et al. 2000



Genbachev et al. 1996



- Onset of the maternal arterial circulation towards the end of the first trimester is associated with a three-fold rise in the oxygen concentration
- Low oxygen *in vitro* stimulates trophoblast proliferation

Conclusions 2

- During the first trimester a subset of the villous cytotrophoblast cells immunopositive for ELF5/CDX2 show high proliferative activity and may represent a stem cell compartment
- Growth factors from the glands may maintain this population in a proliferative state
- The low oxygen concentration within the placenta during the first trimester also stimulates cytrotrophoblast proliferation
- At the end of the first trimester there is a dramatic reduction in ELF5/CDX2 expression in the placenta, suggesting a diminution in proliferative capacity
- This reduction may reflect loss of glandular growth factor support and/or a rise in oxygen concentration associated with the onset of the maternal arterial circulation to the placenta, and hence haemotrophic nutrition

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Established knowledge

1. During the first trimester the human placenta is not haemochorial but is supported by secretions from the endometrial glands
2. These secretions provide a source of nutrients but also contain a number of growth factors that have the potential to stimulate trophoblast proliferation and placental differentiation
3. In many species there is a servo-mechanism by which signals from the conceptus lead to up-regulation of uterine milk proteins and growth factors
4. Changes in sialylation of the secretions ensure that any growth factors that enter the maternal circulation (via the uterine veins) will be cleared in one pass through the liver, creating a safe stimulatory microenvironment in the placenta
5. Loss of the secretions or changes in oxygen concentration may cause the drop in proliferative potential in the trophoblast observed at the end of the first trimester

Research questions

1. What is the composition of the endometrial secretions in early pregnancy?
2. Does a servo-mechanism exist in the human, and if so what are the regulating signals?
3. What accounts for the reduction in proliferative potential of the trophoblast seen at the end of the first trimester?
4. Does poor glandular function contribute to miscarriage through reduced trophoblast stimulation?