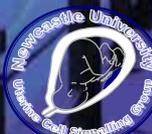


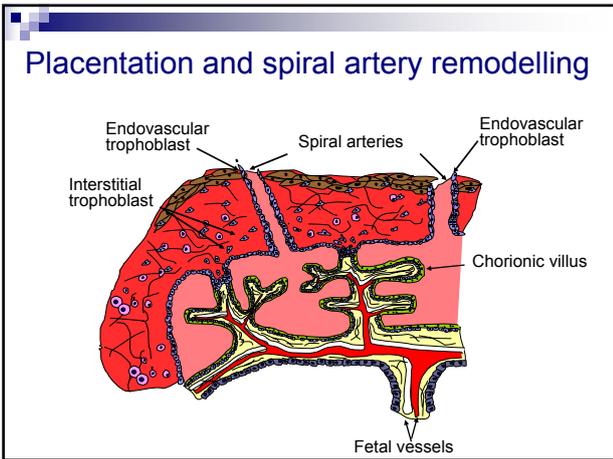
Newcastle University

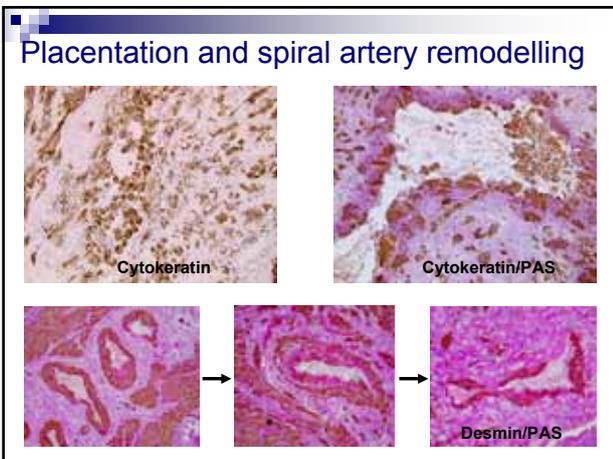
Uteroplacental interactions in early pregnancy

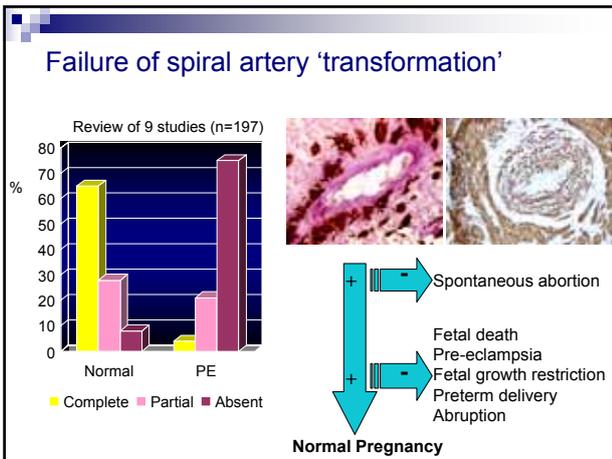
Judith N Bulmer
Gendie E Lash
Newcastle University

bbsrc
Bioscience Research Centre









Control of trophoblast invasion

VS

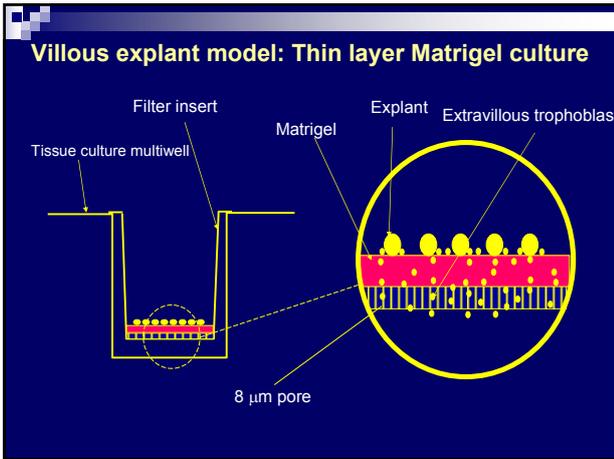
Extravillous trophoblast (EVT)

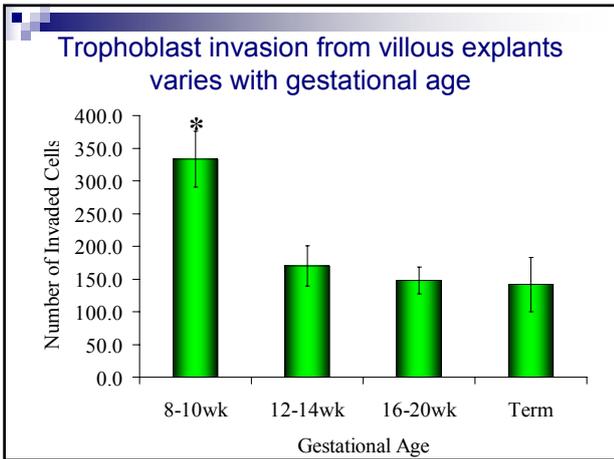
- Innately invasive
- Activity greatest in 1st trimester
- ECM degradation
 - MMPs -2 and -9
 - Urokinase plasminogen activator system

Decidua

- Regulatory
- uNKs a prominent population
- Role in EVT regulation
- Cytokine production
 - TNF- α , TGF- β 1, IFN- γ

- ### Investigation of trophoblast invasion in vitro
- Choriocarcinoma cell lines e.g. Jeg3, Jar, BeWo
 - EVT (like) cell lines eg HTR, PL4 and others
 - Cytotrophoblast cells
 - Placental villous explants
 - Purified EVT





Control of trophoblast invasion

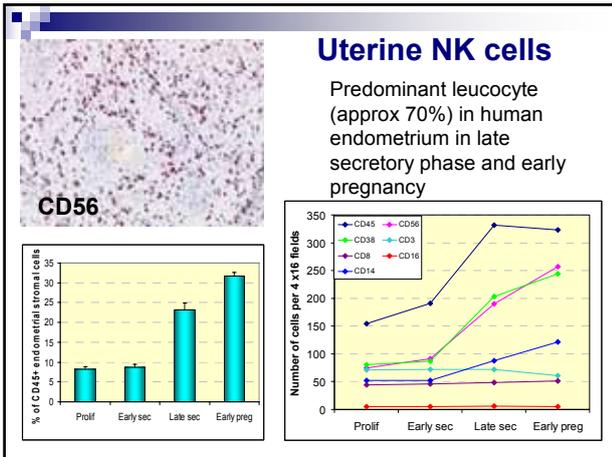
VS

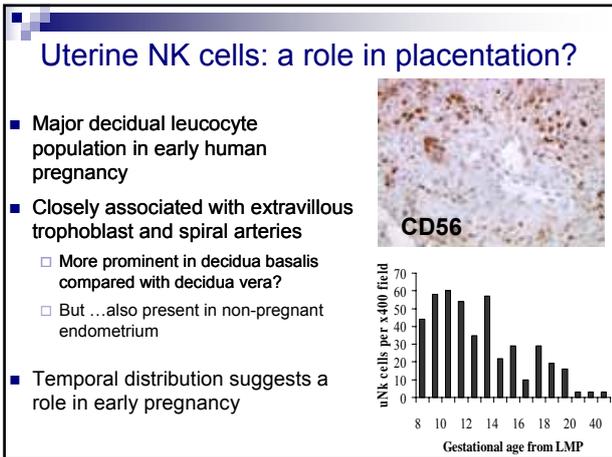
Extravillous trophoblast (EVT)

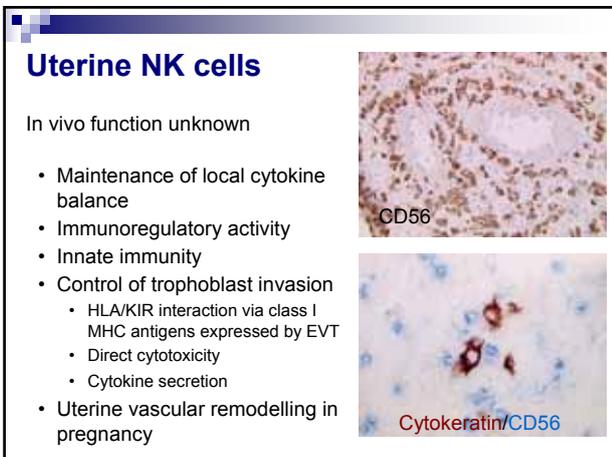
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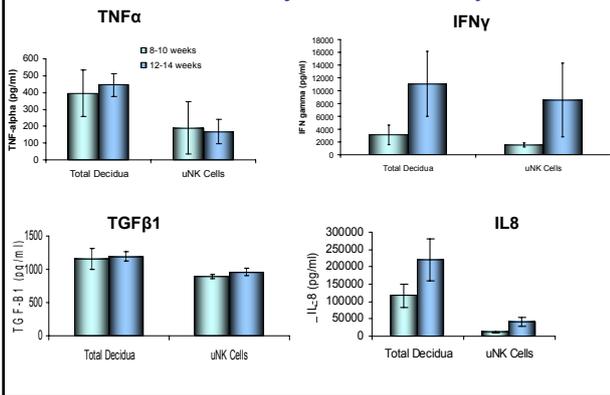


Cytokines produced by uNK cells

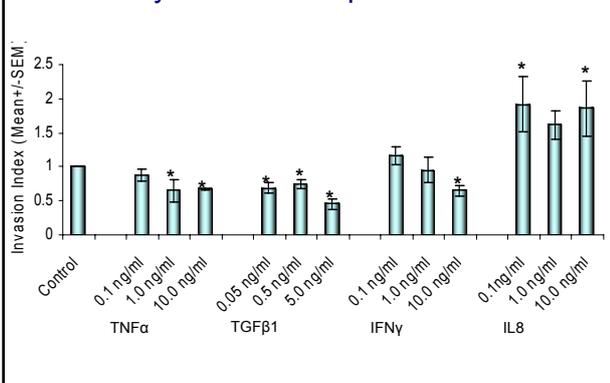
Cytokine	Regulator of Invasion
IL-1 β	No
G-CSF	No
GM-CSF	No
M-CSF	No
TNF- α	Variable effects depending on cell type
TGF- β 1	Variable effects depending on cell type
IFN- γ	Inhibitory
LIF	No

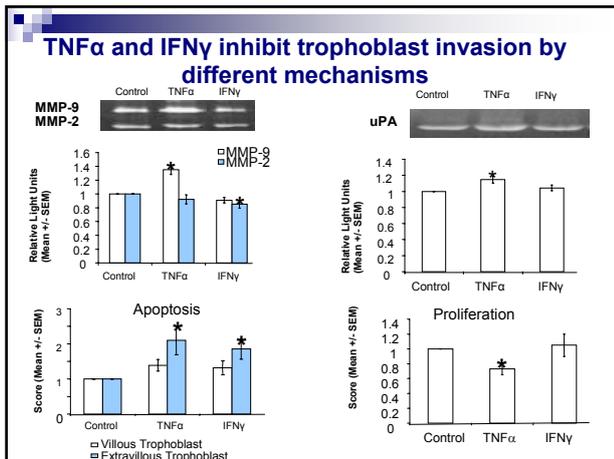
Saito *et al.*, Int Immunol 1993; Jokhi *et al.*, Cytokine 1997

uNK cells are a major source of cytokines

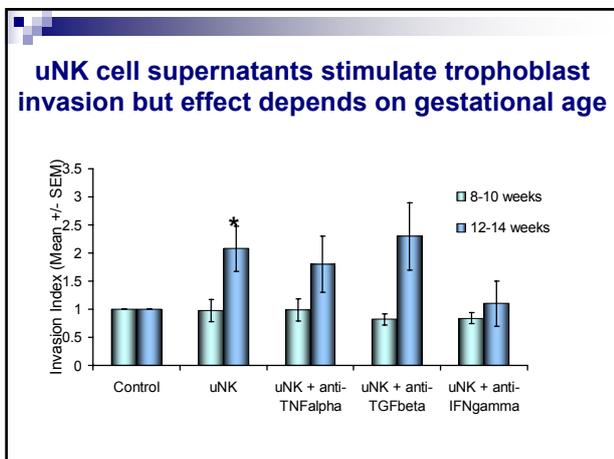


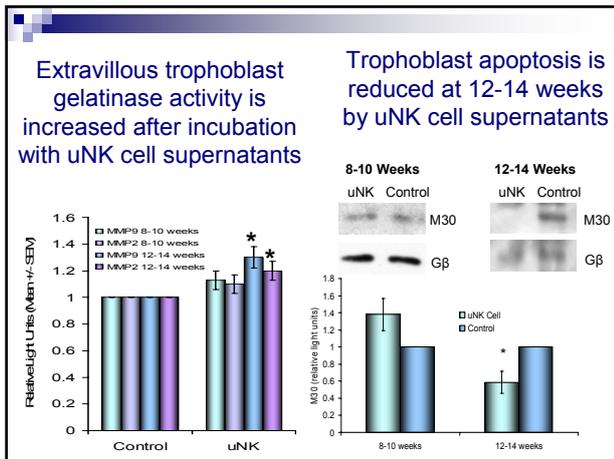
Effect of cytokines on trophoblast invasion





- ### Evidence for a role of uNK cells in regulating EVT invasion
- Hanna et al. Nature Medicine 2006
 - IL-15 stimulated uNK cells stimulated cytotrophoblast invasion
 - Stimulation of invasion attenuated by anti-IL-8 and anti-IP-10 antibodies
 - Hu et al. Journal of Immunology 2006
 - IL-15 stimulated uNK cells inhibited EVT migration
 - Inhibition blocked by anti-IFN- γ antibody





Uterine NK cell cytokine production

FASTQuant multiprotein analysis system (Whatman) Proteome profiler array (R&D Systems)

Quantitative
8 point standard curve
56 samples in triplicate
Up to 9 analytes

Semi-quantitative
4 samples in duplicate
Up to 59 analytes

Cytokine and chemokine expression profile of uNK cells (Proteome profiler array)

Little to No Expression	Moderate Expression	High Expression
C5A	sICAM-1	G-CSF
CD40 Ligand	IFN- γ	GM-CSF
IL-2	IL-1 α	GRO α
IL-4	IL-13	I-309
IL-5	IL-17E	IL-1 β
IL-10	IL-23	IL-1ra
IL-12p70	IL-27	IL-6
IL-17	MIP-1 β	IL-8
IL-32 α	RANTES	IL-16
IP-10		MIF
I-TAC		MIP-1 α
MCP-1		PAI-1
SDF-1		
TNF- α		
sTREM-1		

uNK cells and trophoblast invasion - summary

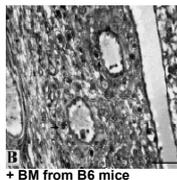
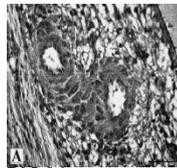
- uNK cells are a major regulator of EVT invasiveness at 12-14 weeks gestation – but not via TNF- α , TGF- β 1 or IFN- γ
- uNK cell regulation of EVT invasion is likely to involve a complex mixture of many cytokines
- EVT invasion is regulated both by altering levels of active proteases and by apoptosis

Human uterine NK cell function

- Control of trophoblast invasion – direct cytotoxicity
- Immunoregulatory functions
- Cytokine secretion
 - Control of trophoblast invasion
 - Immunoregulation
- Angiogenesis/priming of spiral arteries for trophoblast invasion

Uterine NK cells and Vascular Remodelling

- Mice deficient in uNK cells (RAG2-/-) or IFN- γ signalling (IFN- γ -/-) have implantation site abnormalities + failure of decidual artery remodelling (Ashkar et al. 2000)
- Uterine NK cells express mRNA for VEGF-C, PlGF and ANG2 in secretory phase endometrium



Uterine NK cells and vascular remodelling

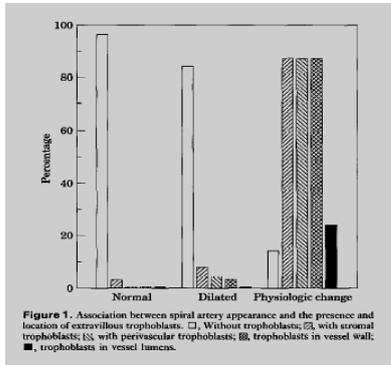
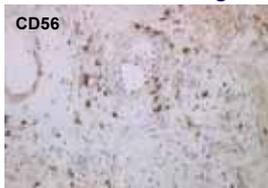
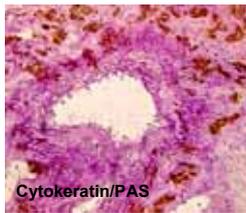


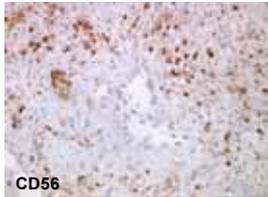
Figure 1. Association between spiral artery appearance and the presence and location of extravillous trophoblasts. □, Without trophoblasts; ▨, with arterial trophoblasts; ▩, with perivascular trophoblasts; ▧, trophoblasts in vessel wall; ■, trophoblasts in vessel lumen.

From Craven et al 1998

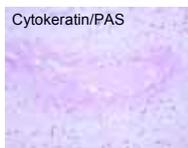
Uterine NK cells and vascular remodelling



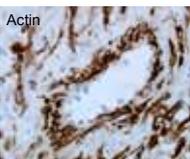
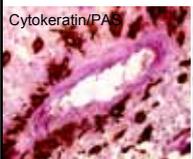
- Spiral artery remodelling is essential for normal pregnancy
- Studies of angiogenesis have focused on placental angiogenesis and menstrual cycle changes
- Little known about angiogenesis in the placental bed



Spiral artery remodeling without trophoblast

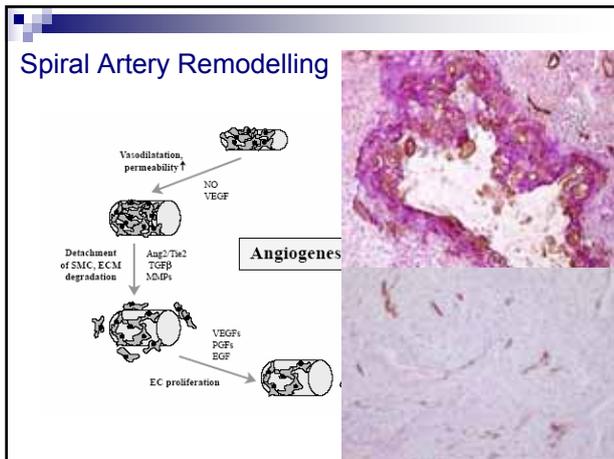


Pathological pregnancy



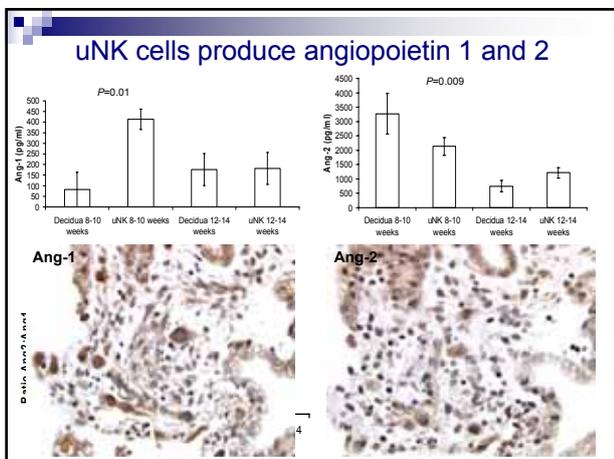
Late miscarriage

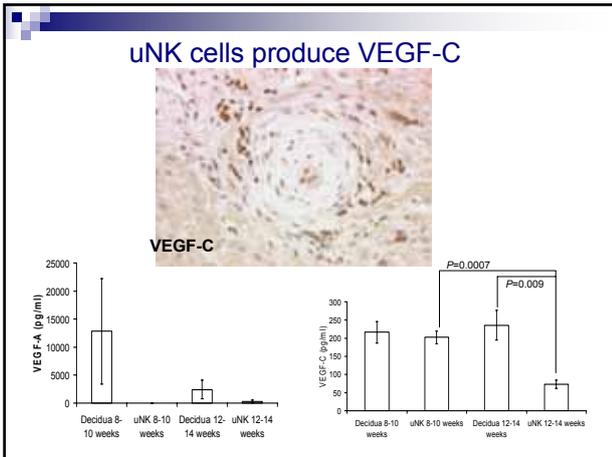
Fetal growth restriction



Do uNK cells produce angiogenic growth factors?

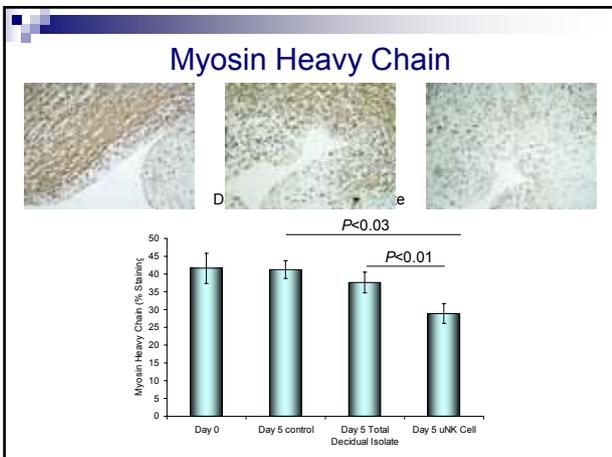
- Enzyme disaggregation of decidual tissue and CD56 Microarray separation - total decidual and uNK fractions 8-10 and 12-14 weeks gestation (n=5 each group).
- Cultured for 48 hours; supernatant measured by ELISA or FASTQuant multicytokine assay
- Total decidual fraction produced significant amounts of Angiogenin, Ang1, Ang2, FGF-B, ICAM-1, PlGF, TIMP-1, VEGF-A, VEGF-C; secretion of Ang2, ICAM-1, KGF and TIMP-1 higher at 8-10 weeks gestation than at 12-14 weeks
- uNK cells major producer of Ang1, Ang2 and VEGF-C

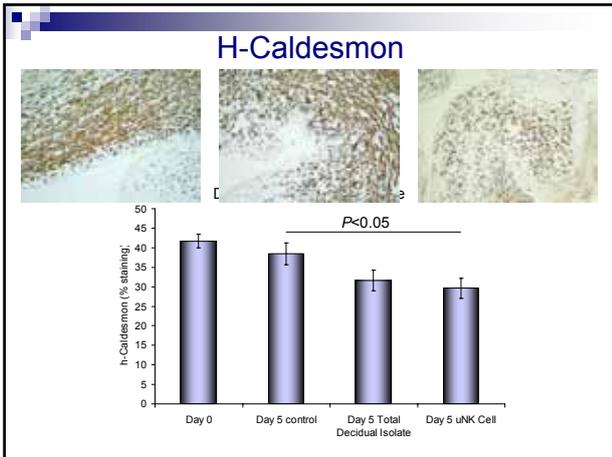


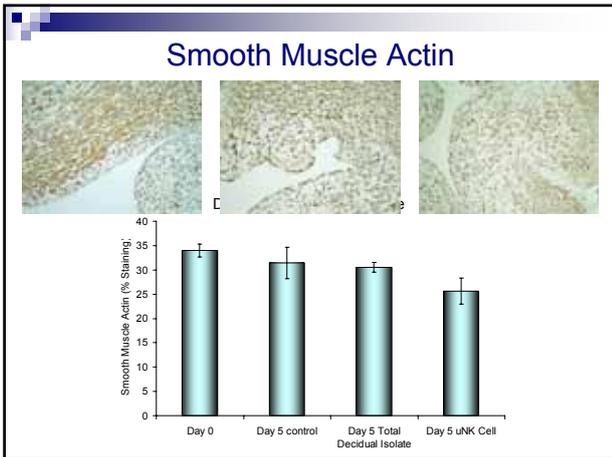


Uterine NK cells and vascular remodelling – an in vitro model

- Chorionic artery dissected from term placenta
- Intact rings 5mm length incubated in 20% uterine NK cell or 'total' decidual cells culture supernatant (medium changed daily) for 5 days
- Samples fixed and embedded in paraffin wax for or frozen for immunohistochemistry or Western blot analysis

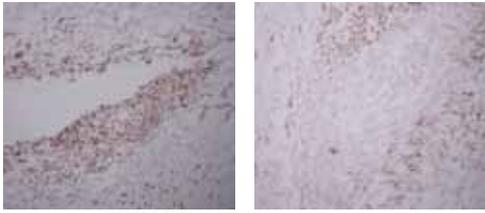






- ### Vascular smooth muscle cell differentiation
- Alpha smooth muscle actin (α SMA)
 - Calponin
 - h-caldesmon (HCD)
 - Smooth muscle alpha tropomyosin
 - Gamma smooth muscle actin
 - Myosin heavy chain (MHC)
 - Desmin

Connexin 43



Control Total Decidua uNK

Evidence for uNK cell involvement in spiral artery remodelling

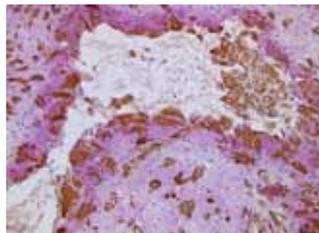
- Close association between uNK cells and partially remodelled vessels in the absence of trophoblast
- High numbers of uNK cells during the period of maximal spiral artery remodelling
- uNK cells produce key angiogenic growth factors
- uNK cell supernatants disrupt vascular smooth muscle in an in vitro model

Spiral artery remodelling in miscarriage

Punch biopsies of human placental bed

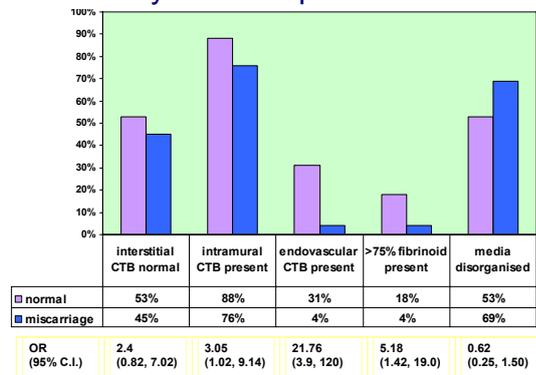
Gestational age 6 - 21 weeks (LMP and scan)

152 normal pregnancies
112 decidual SA
220 myometrial SA



76 miscarriages
225 decidual SA
197 myometrial SA

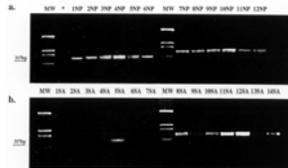
Spiral artery remodelling in miscarriage Myometrial spiral arteries



IFN- γ in human decidua in miscarriage

No significant difference in IFN- γ levels in non-purified decidual cell suspensions between normal early pregnancy (5/15; 215 pg/ml \pm 15) and sporadic miscarriage (5/19; 287 pg/ml \pm 61)

IFN- γ mRNA detected in total decidual mRNA from all normal pregnancies but only 6/14 miscarriages



Uterine NK cells and normal pregnancy

- Minor gestational age differences may have profound effect on function – 8-10 vs. 12-14 weeks
- Do not need trophoblast to be present - large numbers in progesterone treated endometrium
- Consistently present in areas of ectopic decidua eg ovarian surface, cervix, peritoneum, endometriosis etc
- Usually not seen at implantation site in ectopic pregnancy – though present in endometrium
- Present in wide range of species, including those with no/minimal trophoblast invasion
- Are vascular changes required for pregnancy the common factor?

Uterine NK cells and miscarriage

- Lots of questions and few answers so far
 - Increased CD56+ uNK cells in luteal phase endometrium in recurrent miscarriage
 - Inconsistent results for decidual and peripheral blood NK cells in sporadic and recurrent miscarriage
 - Th1/Th2 cytokine balance – but IFN- γ may be important for normal pregnancy

