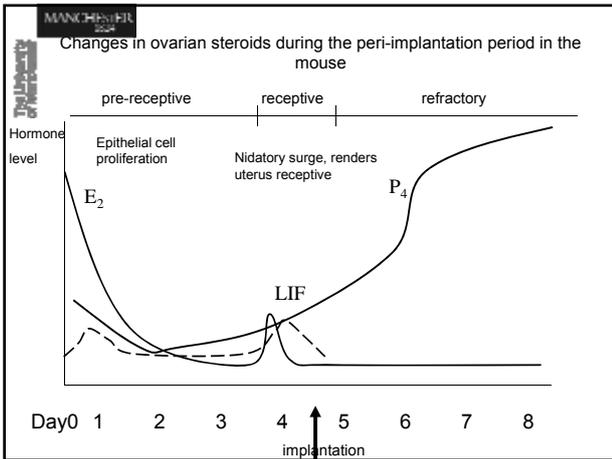
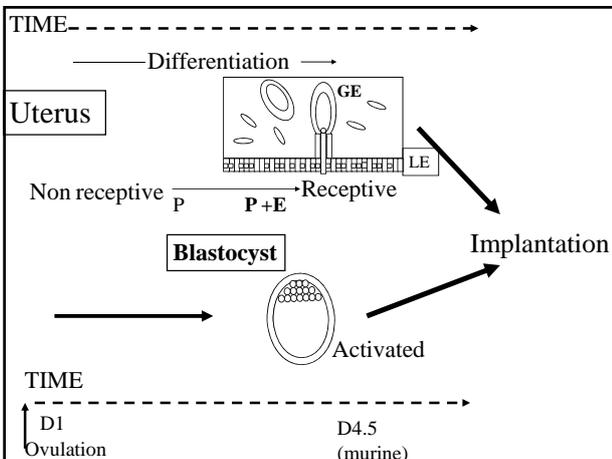


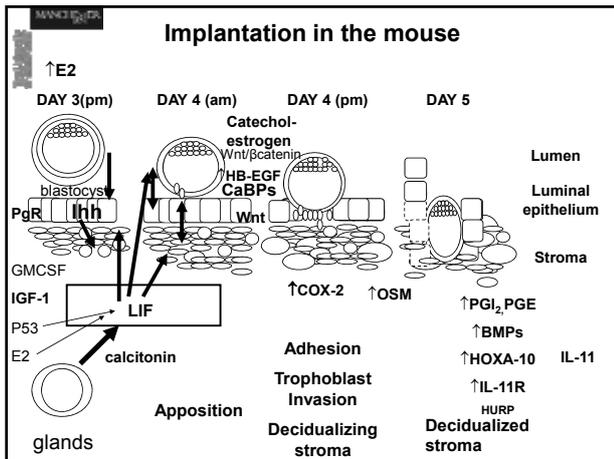
MANCHESTER 2004

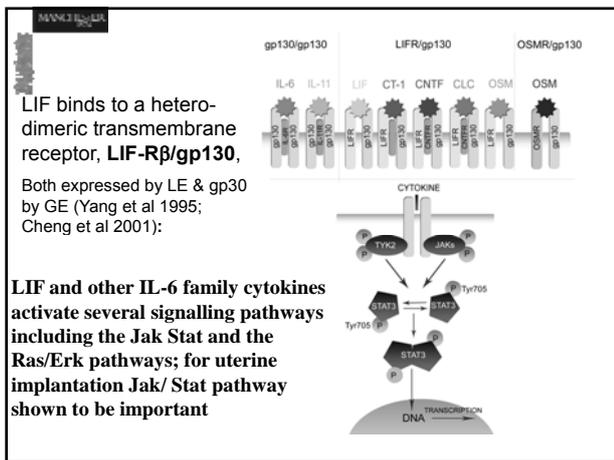
Cytokines and implantation in the mouse

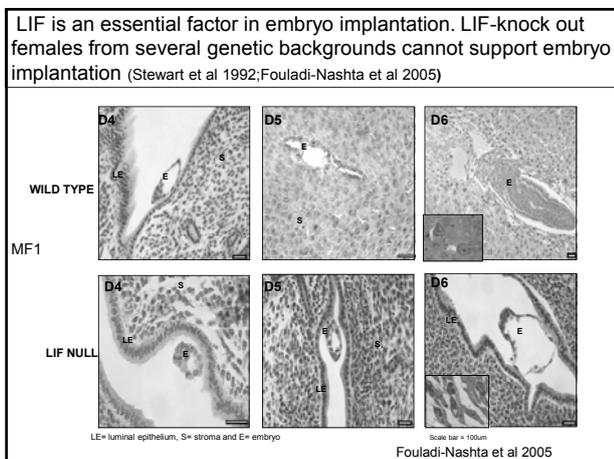
Dr Sue Kimber
Faculty of Life Sciences, University of Manchester UK











LIF protein is strongly expressed in the GE on day 4 a.m. of pregnancy. It is not expressed around the implantation site

	Glands d4 a.m.	Implantation site d4 22.00h.	Implantation site day 5 a.m.
Anti LIF			
DAPI			

Glands are still slightly positive for LIF at 22.00h on day 4 but peri-implantation site is unstained

MANCHESTER 2004

How does LIF affect the stromal and epithelial compartments of the uterus?

Strategy:

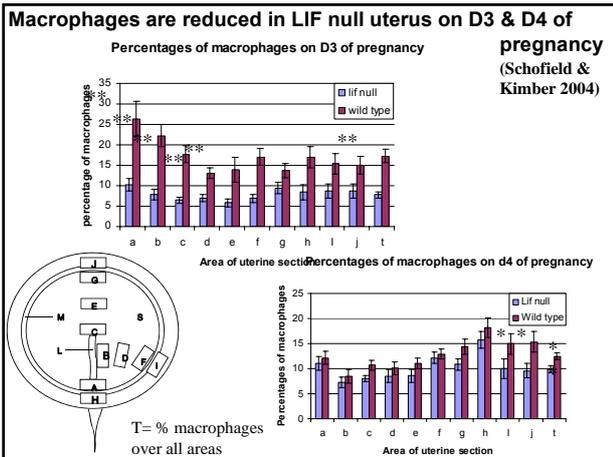
- Examine the stromal and epithelial phenotype of LIF-null mice
- Determine effect of inhibiting LIF signalling
- How do murine uterine LE and stromal cells in vitro respond to LIF?

Day 5

Uterine phenotype of LIF null female

- Changes are initiated in LIF null uterus well before day 4 of pregnancy e.g cell density is increased; glandular density and NK cells increase; macrophages decrease.
- By day 4 uterine cell division is reduced (Schofield & Kimber 2004)

←



Day 5

Uterine phenotype of LIF null female

Over peri-implantation period misregulation of:

Luminal Epithelial molecules

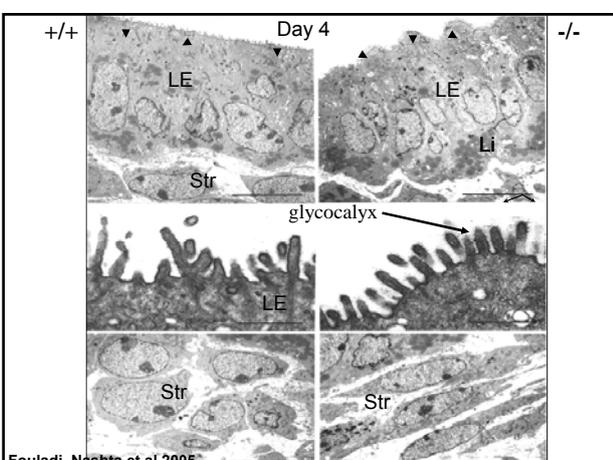
1° targets? e.g. Amphiregulin, Epiregulin, H-type-1, HB-EGF, Cochl, Msx-1

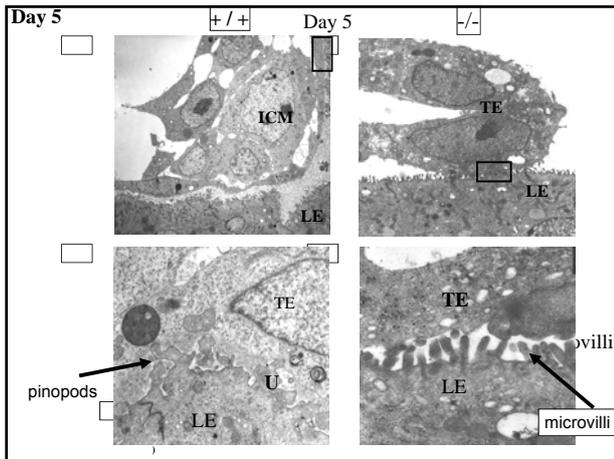
Stromal (decidual-related) molecules

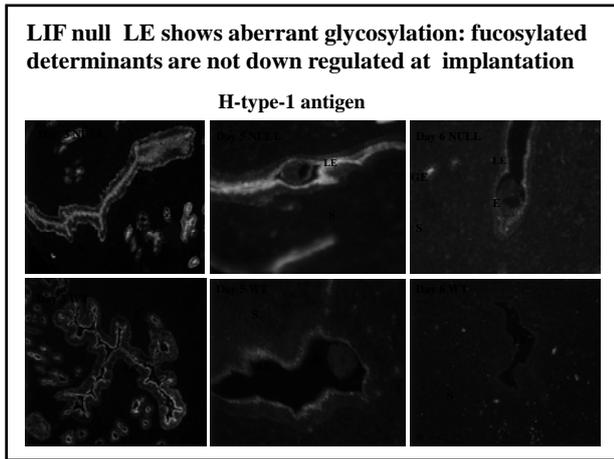
2° targets e.g. desmin, BMP-2 & -7, tenascin, Cox-2, Osm

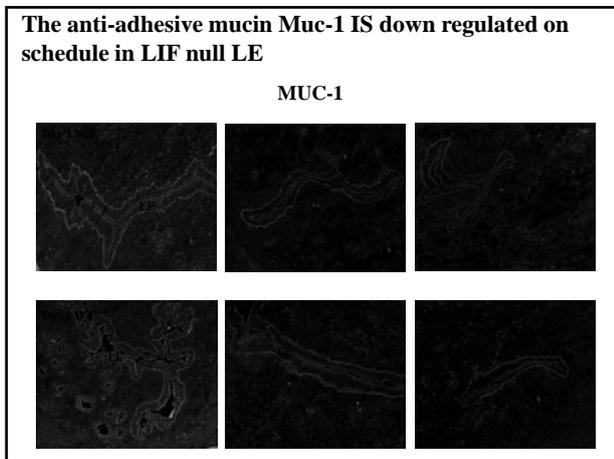
At the implantation site: Retention of abundant glycocalyx and absence of pinopods.

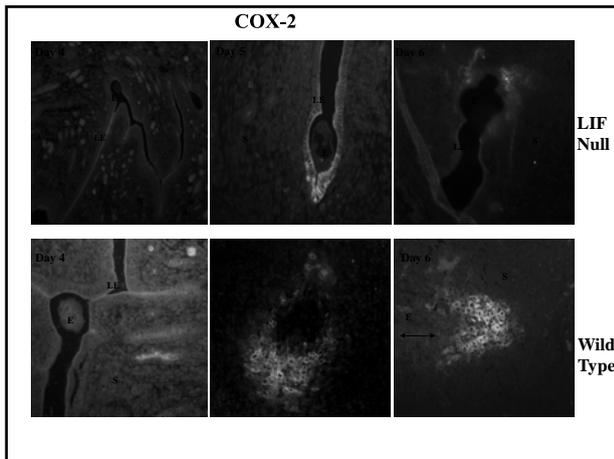
Song et al 2000; Dakaku et al 2004; Sherwin et al 2004; Rodriguez et al 2004; Fouladi-Nashta et al 2005;

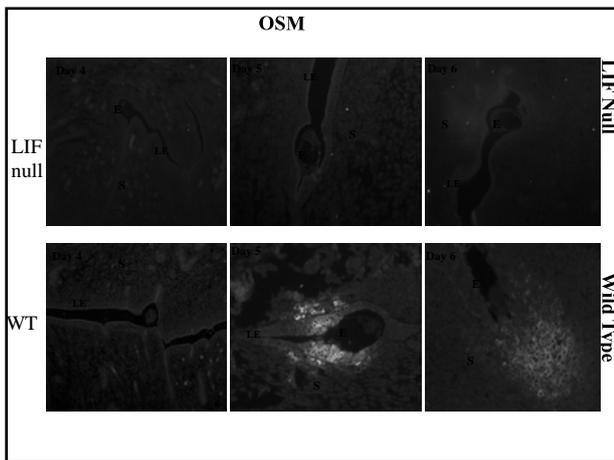












Disadvantages of knockout

- Homozygous null animals lack protein during development and throughout life.
- Compensation by other family members?
- Possible cis effects on other genes (Olsen et al 1996)?
- Time *limited* loss of expression requires conditional knock outs with inherent induction systems

Alternative approaches useful to confirm or modify conclusions

Biochemical 'knock out'

LIF-05: competitive inhibitor of LIF (Hudson et al 1996)

- Binds to the LIF receptor (not IL-6-R or Osm R)

- Does not activate signalling

Advantages

- No cis or trans influence on other genes

- Lack of LIF signalling in females which **normally express LIF**

- Can precisely time effect

- Potential dosage effects possible

Small molecules have potential use in human reproductive medicine

LIF inhibition (LIF05) *in vivo*: experimental procedure

D4 of pregnancy



Injections of 5µl LIF05 into the uterine lumen of one horn; Vehicle into the contralateral horn

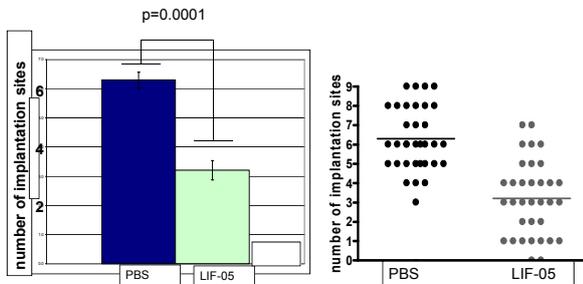


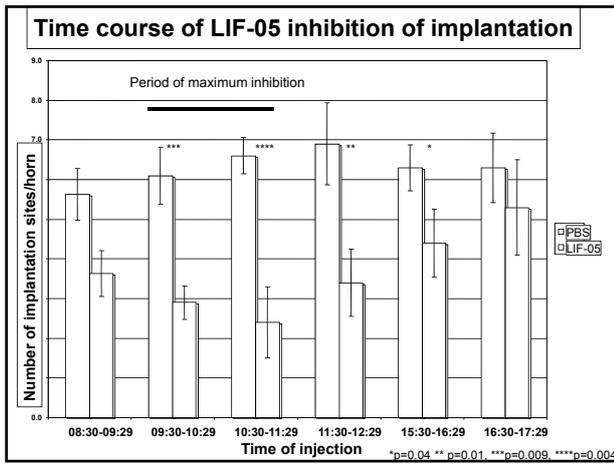
D5 or 6 of pregnancy

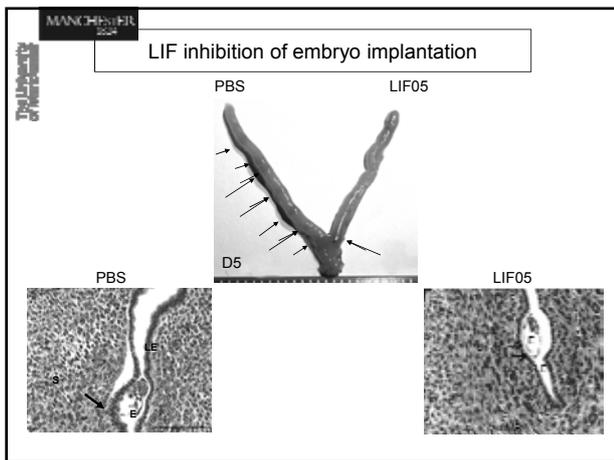
Uterine horns dissected after pontamine blue tail-vein injection to visualised implantation sites

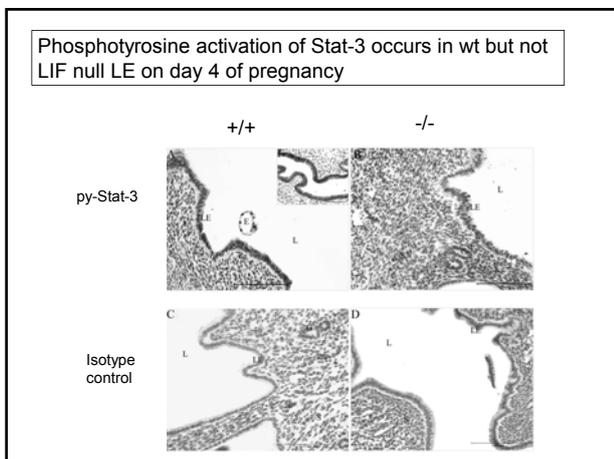
Uterine horns processed for histological and immunohistochemical analysis

Effect of LIF-R inhibition by LIF-05 on implantation

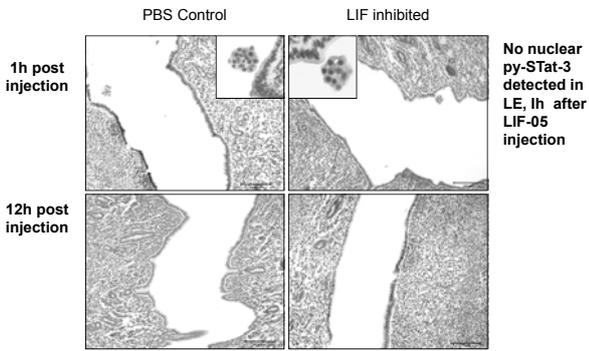




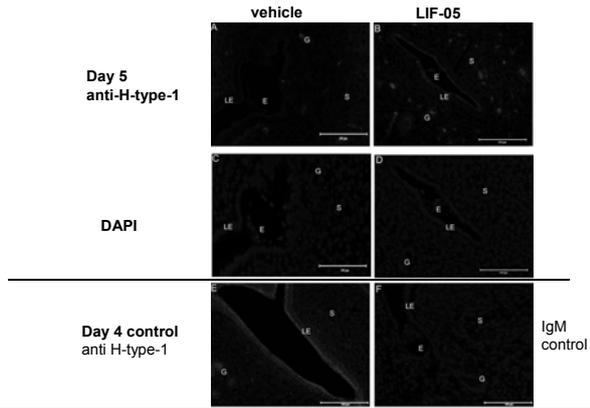




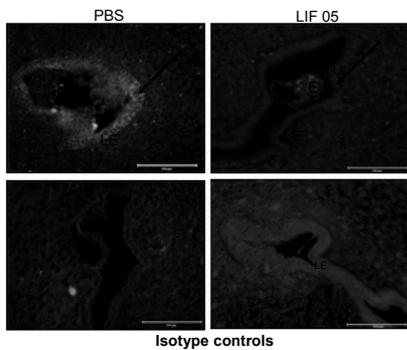
LIF 05 prevents phosphotyrosine mediated activation of Stat-3 on day 4 of pregnancy



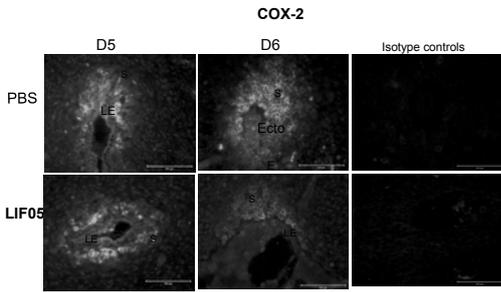
H-type-1 antigen is retained in LE on day 5 after LIF inhibition



Amphiregulin expression in the LE is severely reduced on evening of D4 after LIF-05 injection

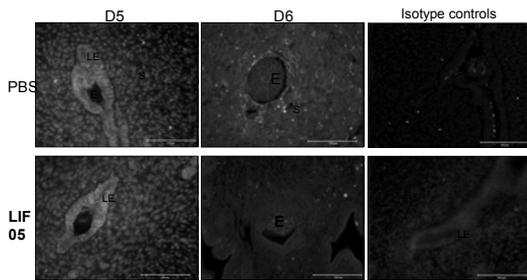


Stromal response to LIF-05 at the 'implantation site'



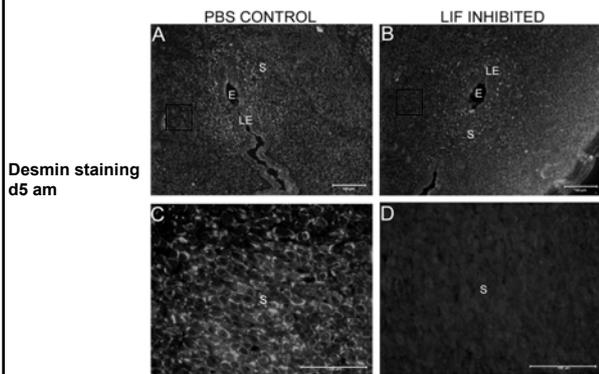
COX-2 expression is attenuated by day 6 but not ablated in response to LIF-05 inhibition which blocks implantation

Inhibition of Oncostatin M in stroma but not LE by LIF-05



OSM is not expressed in stroma on D6 after LIF-05

Decidualisation is defective after LIF 05 treatment



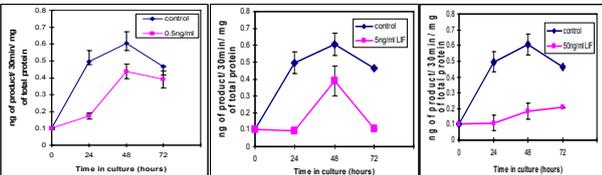
Conclusion

- A competitive inhibitor of LIF (LIF-05) can block implantation through inhibition of Stat-3 signalling
- Maximum sensitivity occurs between 9.30 and 11.30 am day 4.
- LIF-05 mimics many but not all of the phenotypic changes seen after LIF knock out
- This reagent will be invaluable in determining the key targets of LIF required for implantation
- Commercially, similar competitive inhibitors may be developable for human contraception or other reproductive strategies (for instance pegylated LIF White et al 2007)

Role of LIF in Decidualisation

- In LIF-null female mice decidualisation is reported to be deficient (Stewart; Fouladi-Nashta et al; others)
 - 1) Can LIF DIRECTLY affect stromal decidual differentiation?
- or**
- 2) Does LIF just influence the transduction of decidual inducing signals (from the embryo) through the LE?

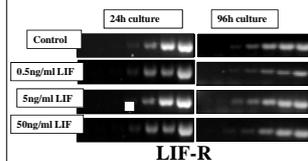
At least one **direct effect** of LIF on stroma



LIF directly inhibits alkaline phosphatase production by uterine stromal cells

No effect on PGE₂

Fouladi-Nashta et al. 2004



What happens when LIF binds to LE?

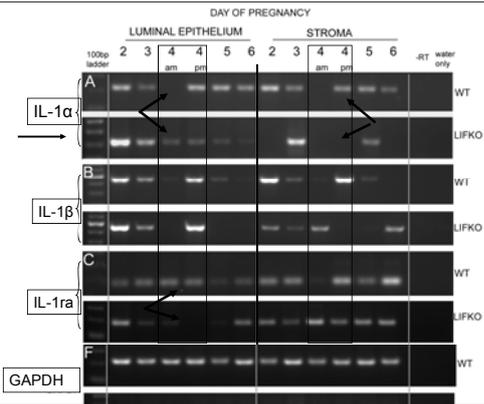
IL-1 as a down stream target of LIF

- LE cells secrete IL-1
- IL-1 α has been shown to induce stromal Cox-2 and PGE2 secretion in vitro in mice (e.g Jacobs & Carson 1993)
- Repeated IL-1ra injection reduced implantation (*Simon et al 1994*) although not *IL-1 R1 Ko* (*Abbondanzo et al 1996*)

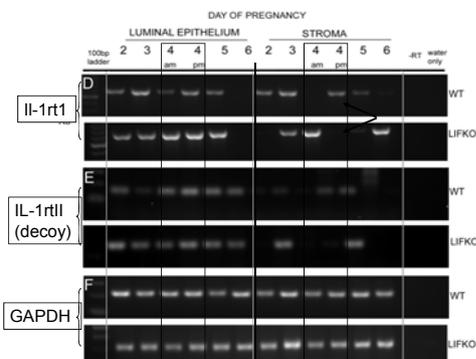
Is expression of IL-1 and its associated molecules affected in LIF null mice?

Fouladi-Nashta Mohamet et al 2008

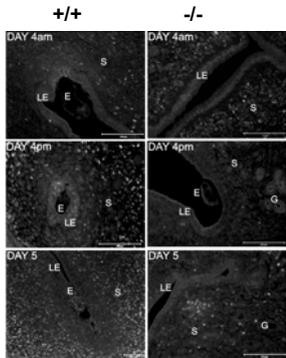
In LIF null uterus IL-1 α and IL-1ra are mis-regulated in LE during the peri-implantation period and IL-1 α and β are misregulated in stroma



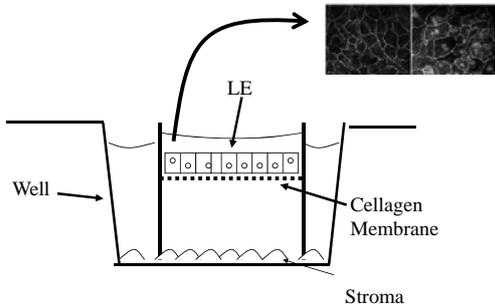
IL-1rt1, signalling receptor for IL-1 is mis-regulated in stroma of LIF-null mice



IL-1 α is lower in LE and stroma on day 4 -6 of pregnancy in LIF null uterus



Does LIF directly influence secretion of IL-1 by LE cells in vitro?



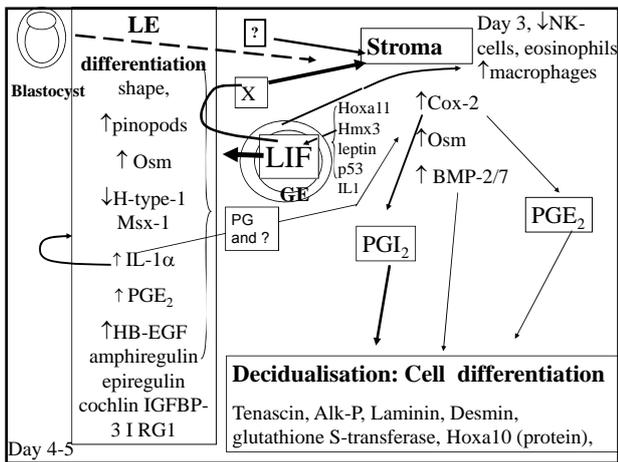
Set up for luminal epithelial cell culture and epithelial-stromal co-culture

Summary: influence of LIF on LE secretion

- LIF induces apical IL-1 α secretion by co-cultured LE cells
- LIF stimulates apical and some basal PGE₂ secretion by LE cells in co-culture.
- In absence of stroma little effect of LIF on IL-1 α emphasising the importance of cross talk between LE and stroma....

For decidualisation, LIF may act

- Via upregulation of stromal IL-1 to induce decidualisation (e.g. via cox2)
- via LE [autocrine] to **stimulate** decidualisation inducing signals; one of which is PGE₂ from LE
- Later away from implantation site, directly on stroma to **inhibit** decidualisation (Fouladi-Nashta et al 2004)



Acknowledgements

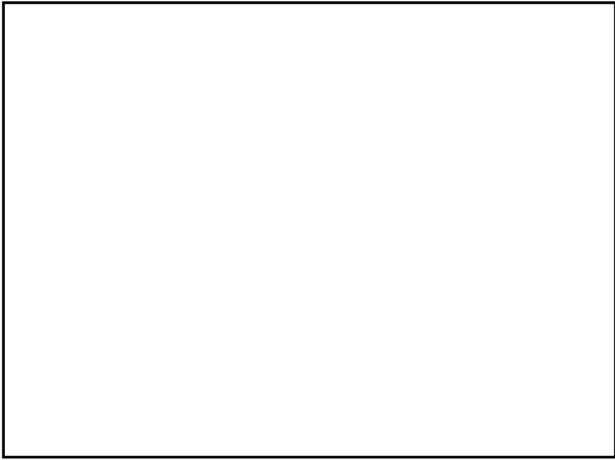
Who did the work?

- Lisa Mohamet
- Ali Fouladi-Nashta
- Claudia Andreu
- Gemma Schofield
- Nahida Nijjar
- Carolyn Jones, Dept. Obs & Gynae Univ. Manchester

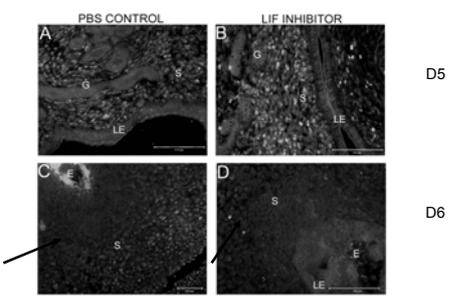
Collaborators

- John Heath
University of Birmingham
- Anne Vernallis
University of Birmingham/Aston

AND... We thank the John Pinto Fund UK, BBSRC and MRC UK for funding

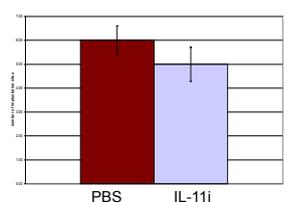


LIF-05 injected on day 4 also causes a reduction in IL-1 α but only by day 6



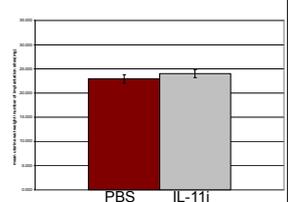
IL-11 inhibition on implantation rate and uterine wet weight

Implantation rates were unaffected by IL-11 inhibition (end pt D6&D8)



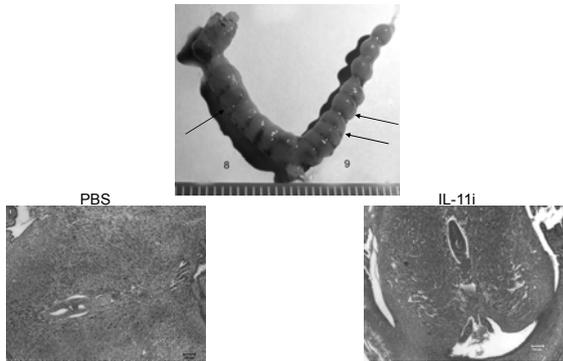
n = 12
p = .172

Uterine wet weights were unaffected by IL-11 inhibition (D8)

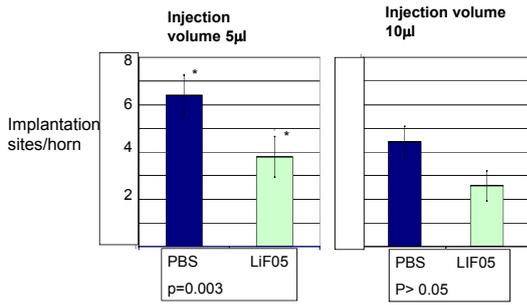


n = 6
p = 0.761

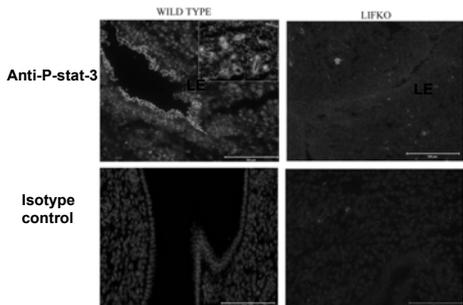
IL-11 inhibition on embryo development



Implantation sites on day 5 of pregnancy after injection of LIF05 on day 4 am: Pilot Study



P-serine Stat-3 is also detected in LE and GE in wild type mice but not LIF-nulls on Day 4 of pregnancy



LIF in the murine uterus

- Synthesised in two bursts: from uterine LE on d1 and GE on **morning of d4 of pregnancy** (Bhatt et al 1991)
 - Expressed under control of estrogen in mouse and independent of the blastocyst
 - LIF signalling in LE occurs through phosphorylation of Stat-3 (Cheng et al 2001)
- LIF-R is expressed strongly by LE (Cheng et al 2001) but also possibly by GE (Yang et al 1995) and in stroma (Fouladi-Nashta et al 2004)
- Gp 130 mRNA is expressed by GE and LE (Cheng et al 2001)
 - Both mRNA & protein for gp130 are expressed in stroma from d5/6 (Yang et al 1995; Ni et al 2000)

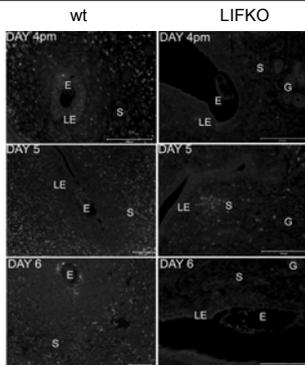
Evidence for Involvement of Leukemia Inhibitory Factor (LIF) in implantation

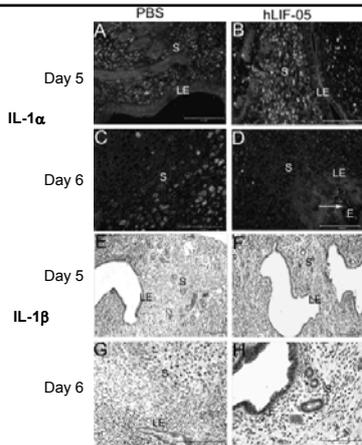
- In LIF-null female mice, embryos develop to blastocysts but do not implant (Stewart et al 1992): implantation is rescued by injection of LIF.
- LIF-null embryos implant after transfer to WT females
- After E and P priming LIF-null mice fail to respond to a decidualising stimulus, unlike WT mice
- LIF can substitute for E to restore implantation and decidual response in ovex P-treated WT mice (Chen et al 2000)
- In LIF null mice mRNAs for Amphiregulin, Epiregulin, blastocyst-dependent tm-HBEGF & stromal Cox-2 are not induced (Song et al 2000) in the peri-implantation period

Involvement of Leukemia Inhibitory Factor (LIF) in implantation in human

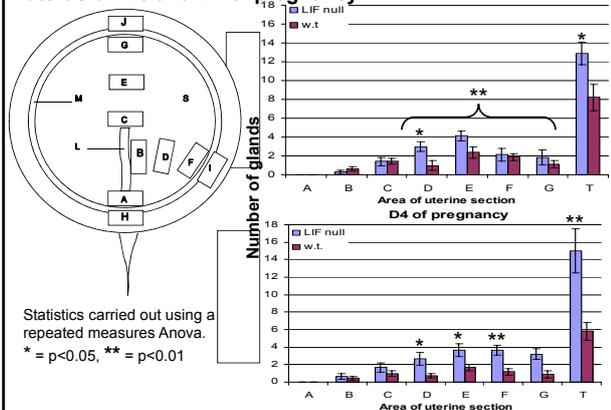
- LIF has been associated in some studies with recurrent human miscarriage and unexplained infertility (Lass et al 2001; Hambartsoumian 1998) but still conflicting evidence.

IL-1 α is lower in LE and stroma on day 4 -6 of pregnancy in LIF null uterus

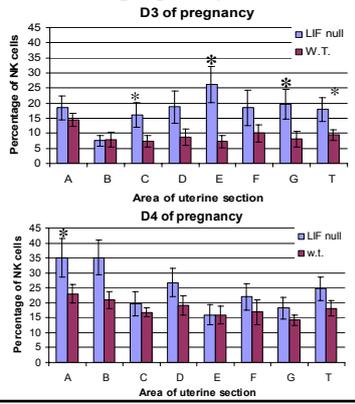
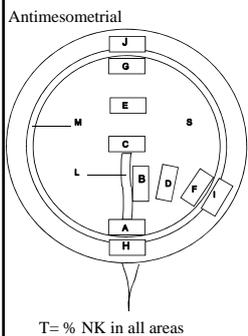




Glands are increased in the LIF null uterus on D3 and D4 of pregnancy



Natural Killer cells are increased in the antimesometrial region of the null uterus on D3 of pregnancy



Schofield and Kimber 2004
