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Progesterone Regulation of Endometrial Vascular Remodelling

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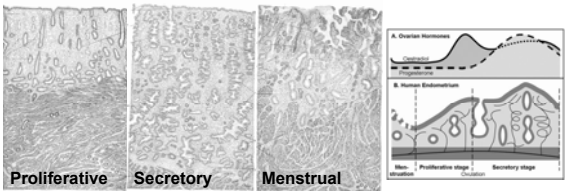
Endometrium through the Menstrual Cycle



From: Di Fiore MSH. 'Atlas of Human Histology' 4th Edition, 1974.

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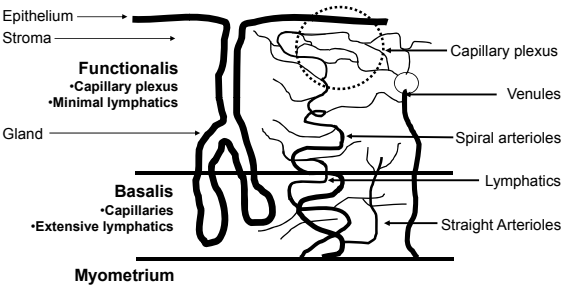
Endometrial Vasculature



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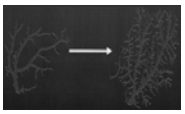
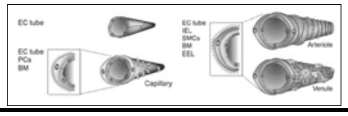
Vascular Remodelling

- **Angiogenesis**: growth of new blood vessels from pre-existing vessels
- **Lymphangiogenesis**: growth of new lymphatic vessels from pre-existing vessels



- **Vascular maturation**: addition of pericytes or vascular smooth muscle cells (VSMC).



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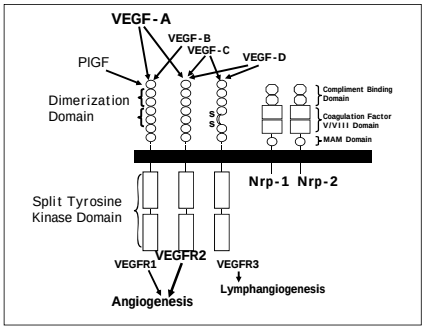
VEGF and its Receptors



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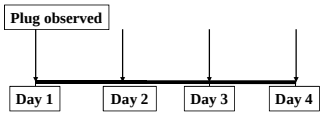
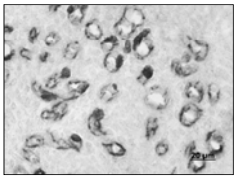
Pregnancy

- To quantify **proliferating** endothelial cells during the early stages of pregnancy in mice





Blue: endothelial cells, CD31
Brown: proliferating cells, BrdU

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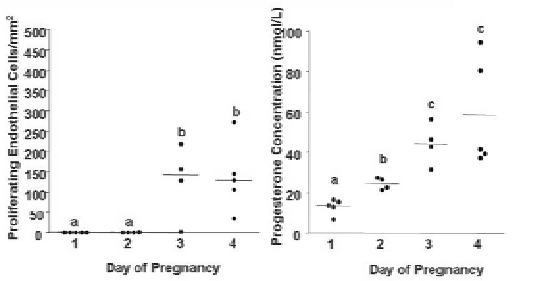
Proliferating endothelial cells and plasma progesterone concentrations



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Walter et al. (2005) Reprod. 129: 765-77

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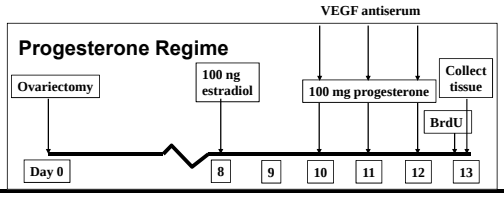
Progesterone

- To quantify **proliferating** endothelial cells in progesterone treated ovariectomised mice
  - Oestrogen priming required?
  - VEGF required?



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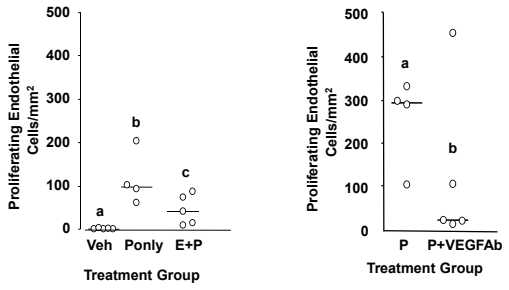
Endometrial Endothelial Cell Proliferation



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Walter et al. (2005) Reprod. 129: 765-77

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Vascular Maturation

To determine whether progesterone stimulates **vascular maturation** in the mouse endometrium

- To quantify **proliferating** mural cells
- To quantify changes in the proportion of vessels covered by **α-smooth muscle actin**

(**α-smooth muscle actin** = marker of vascular smooth muscle cells and pericytes)

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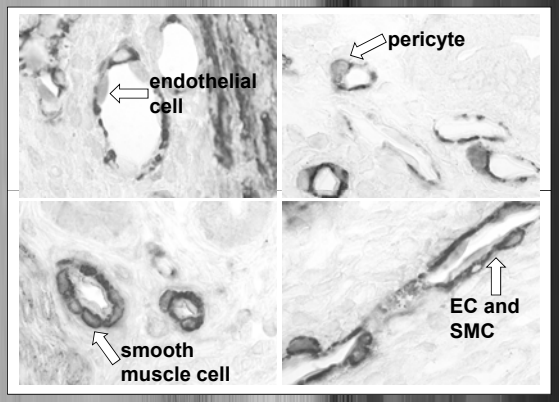
Mural cells (brown)/Proliferating cells (blue)



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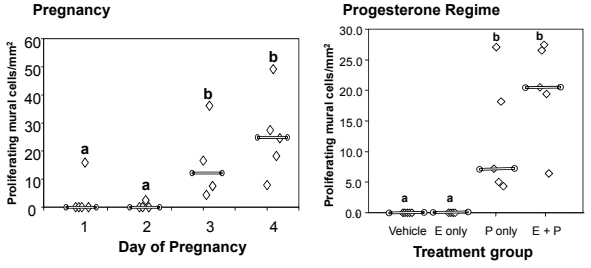
Proliferating Mural cells



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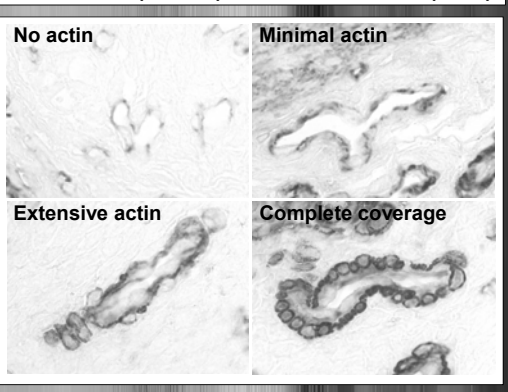
Girling et al. (2007) Endocrinology 148: 5433-5441

Mural cells (brown)/Endothelial Cells (blue)



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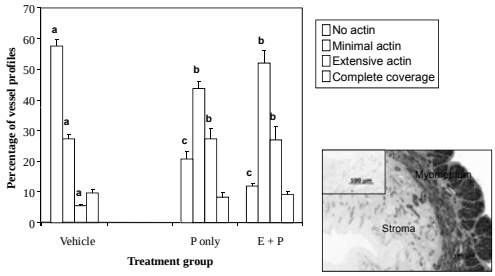
Percentage of mouse endometrial vessel profiles covered by mural cells



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Girling et al. (2007) Endocrinology 148: 5433-5441

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Summary

- **Progesterone** stimulates angiogenesis and vascular maturation in mouse endometrium
- **Oestrogen** moderates progesterone-induced angiogenesis
- **VEGF** has a role in progesterone-induced endothelial cell proliferation
- Progesterone-induced mural cell recruitment and proliferation are not affected by **oestrogen priming** or by **VEGF antiserum**

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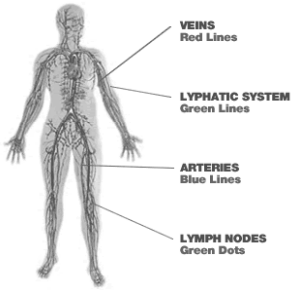
Lymphatic vessels

- Fluid balance: drain tissues, via lymph nodes, and ultimately return it to the blood
- Immune survellience



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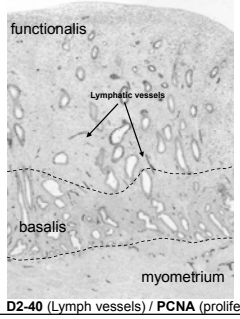
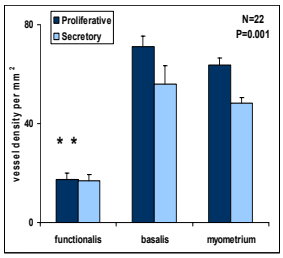
Human endometrial lymphatics





Lymphatic vessel density

D2-40 (Lymph vessels) / **PCNA** (proliferating cells)

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Donoghue *et al*, Hum Reprod, 2007

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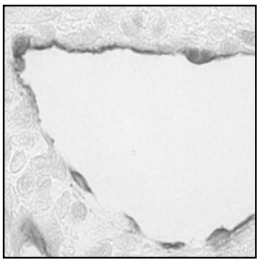
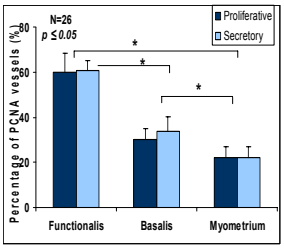
Proliferating endometrial lymphatic endothelial cells





D2-40 (blue) **and PCNA** (brown)

Regional proliferation

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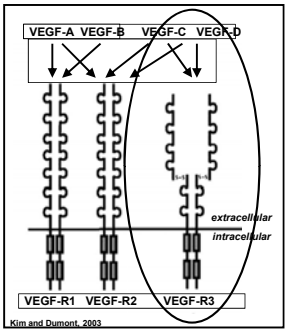
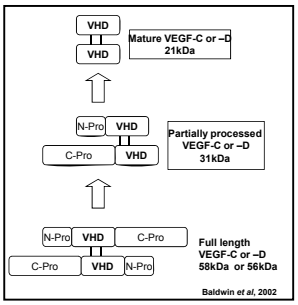
Lymphangiogenic growth factors and receptors



Kim and Dumont, 2003



Baldwin *et al*, 2002

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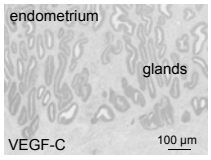
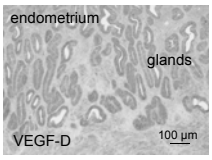
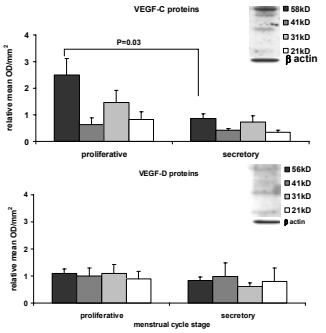
Endometrial VEGF-C and VEGF-D







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Donoghue *et al*, Hum Reprod, 2007

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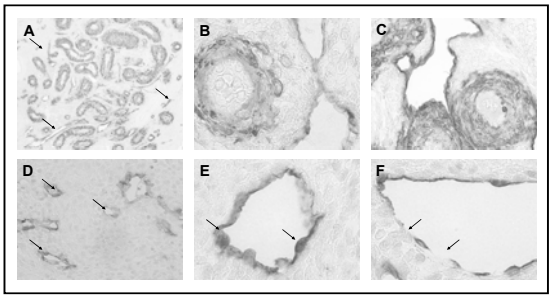
Human endometrial Lymphatics



(A-C) **Blue**: Lymphatics (D240), **Brown**: spiral arterioles (αSMA)
(D-F) **Blue**: Lymphatics (D240), **Brown**: proliferating cells (PCNA)

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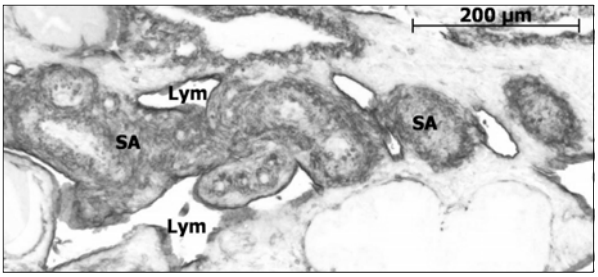
Interaction of Spiral Arterioles with Lymphatics



D2-40 – **Blue**, lymphatics, αSMA – **Brown**, spiral arteriole

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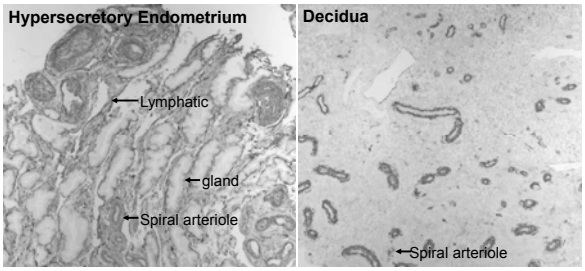
Placental bed, 8-10 weeks



Blue: αSMA (mural cells) **Brown**: D2-40 (Lymphatic vessels)

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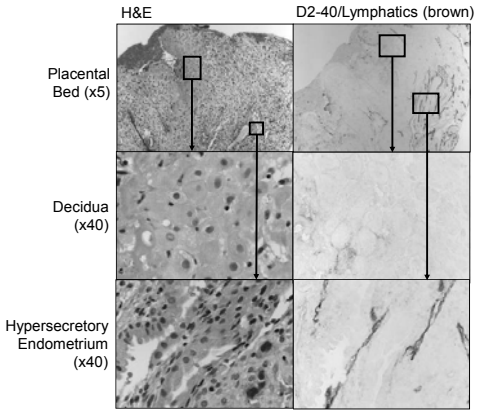
Placental Bed Biopsies 13 weeks



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Progestin treatment in Xenograft Model

- **Human Endometrium implanted subcutaneously into NOD SCID mice**
- Mice treated with: - Oestradiol valerate for 2 weeks
  - Medroxprogesterone acetate for 4 weeks.

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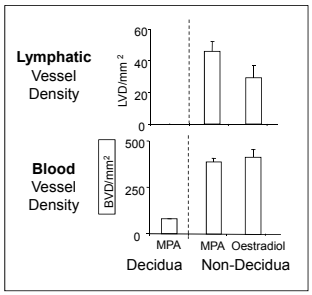
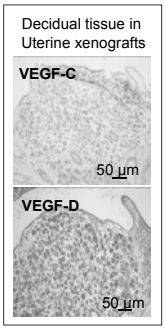
Progestin treatment in Xenograft Model





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Summary

- The endometrial functionalis provides a rare site of physiological lymphangiogenesis in the adult
  - Fewer lymphatic vessels in the functionalis
  - Greater proliferation in the functionalis
- Reduced lymphatics in functionalis – consequences?
  - Oedema
  - Immune surveillance and privilege
- Reduced lymphatics in functionalis – mechanisms?
  - Processed forms of VEGF-C and D present in endometrium
  - Is there an inhibitor present?

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Summary

- Interactions with Spiral arterioles?
  - How does interaction change as arterioles develop in secretory phase?
- What is happening during decidualisation?
  - Early pregnancy
  - Progestin treatment

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Conclusions

- Progesterone has differential effects on endometrial vasculature
  - Different regulatory mechanisms on:
    - Straight and spiral arterioles, blood and lymphatic capillaries, venules
  - How does progesterone act to stimulate vascular maturation?
- How do Progesterone and Progestins regulate lymphatics?
  - Effect on growth factor and receptor expression
  - Interactions with spiral arterioles
  - Interactions between decidualisation and lymphatics

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Acknowledgments

- CWHR
  - Leonie Cann
  - Fiona Lederman
  - Nancy Taylor
- Monash Medical Centre
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  - Steve Stacker
  - Marc Achen

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Progesterone and Blood Vessels

- Branching angiogenesis during secretory phase in human endometrium (Gambino et al. 2002 Hum. Reprod. 17:1199-1206.)
  - But, no change in endothelial cell proliferation during menstrual cycle, and
  - No peak in proliferation during artificial secretory phase in macaques (Nayak and Brenner 2002 J Clin Endocrinol Metab. 87:1845-55)
- Growth and coiling of spiral arterioles during secretory phase in human endometrium
- Variable results *in vitro*.

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