Effect of placental growth factor on trophoblast integration into endothelial cell networks in the presence of inflammation

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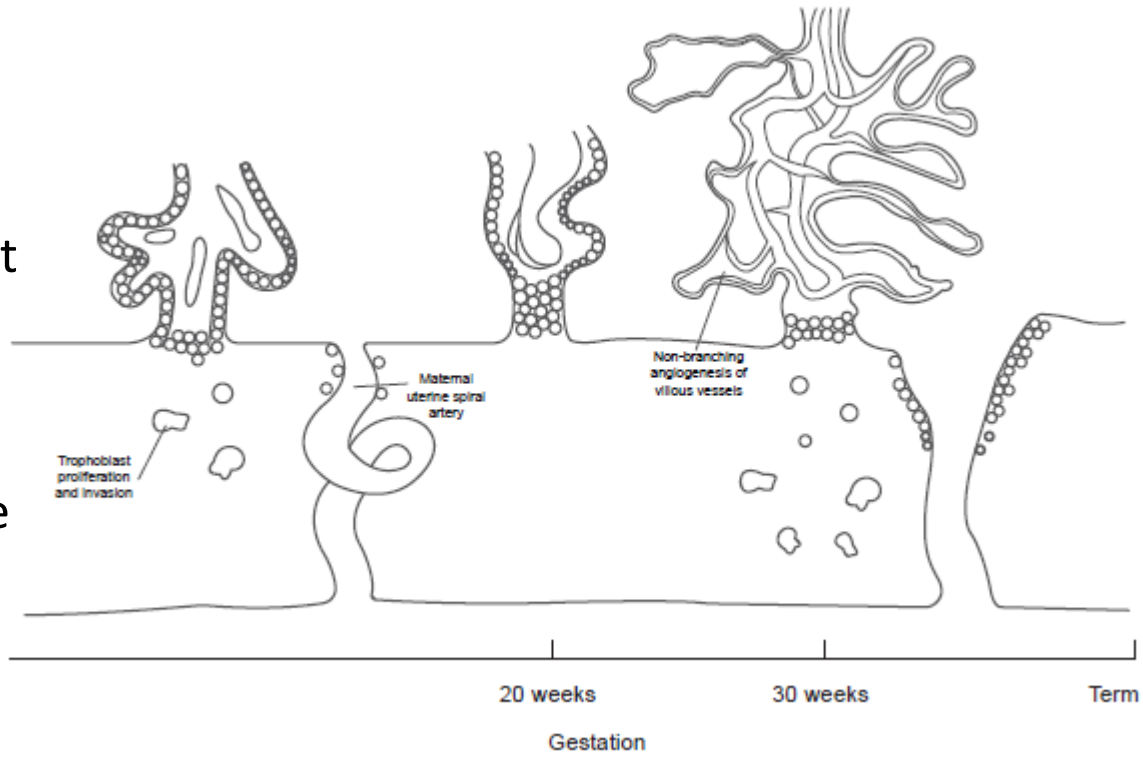
Background

- Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality, affecting 3 - 5% of pregnancies.
- The pathogenesis of preeclampsia is a ‘two stage’ process

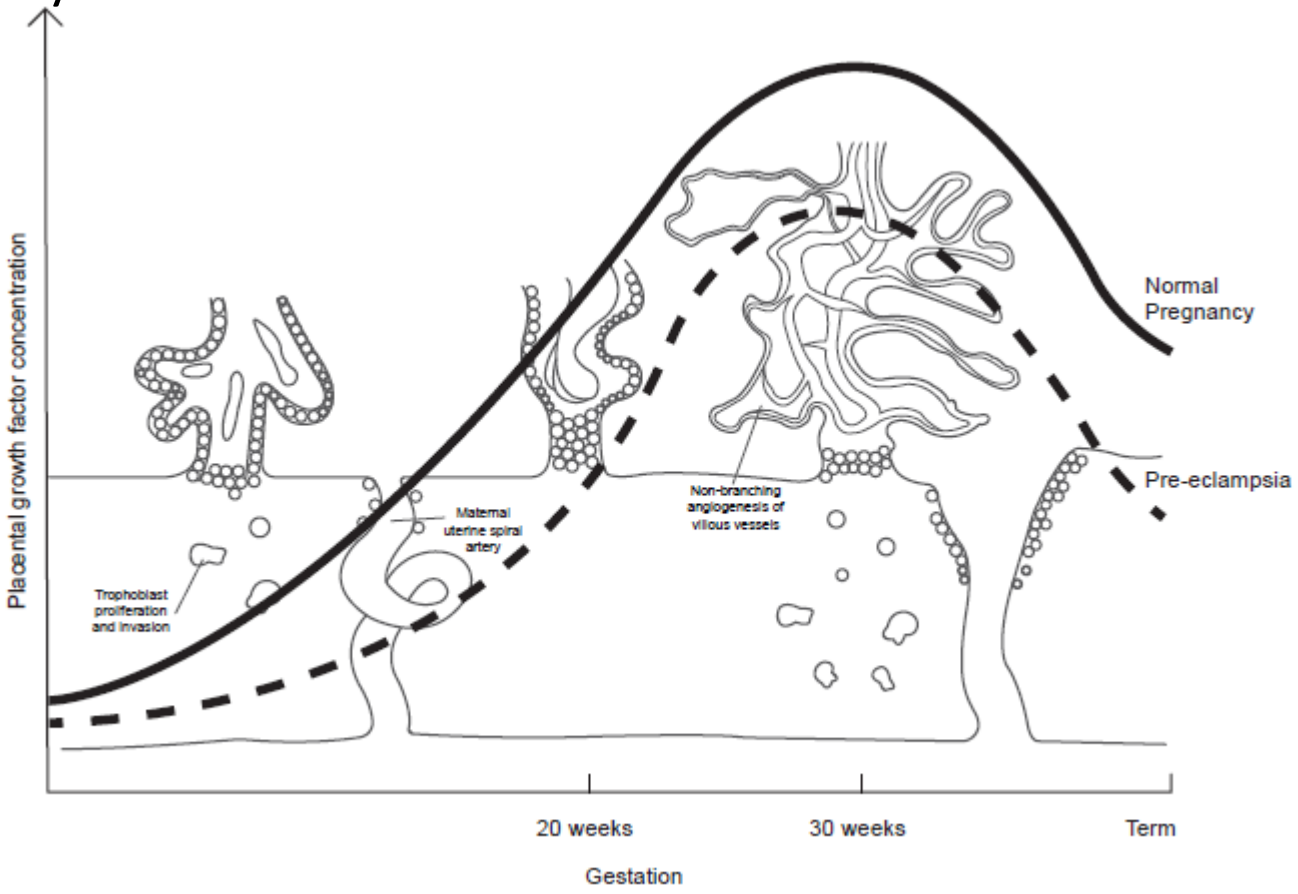
Stage 1: Abnormal placental development with incomplete remodelling of uterine spiral arterioles by trophoblast cells

Stage 2: The abnormal placenta then produces factors which contribute to the development of the maternal syndrome

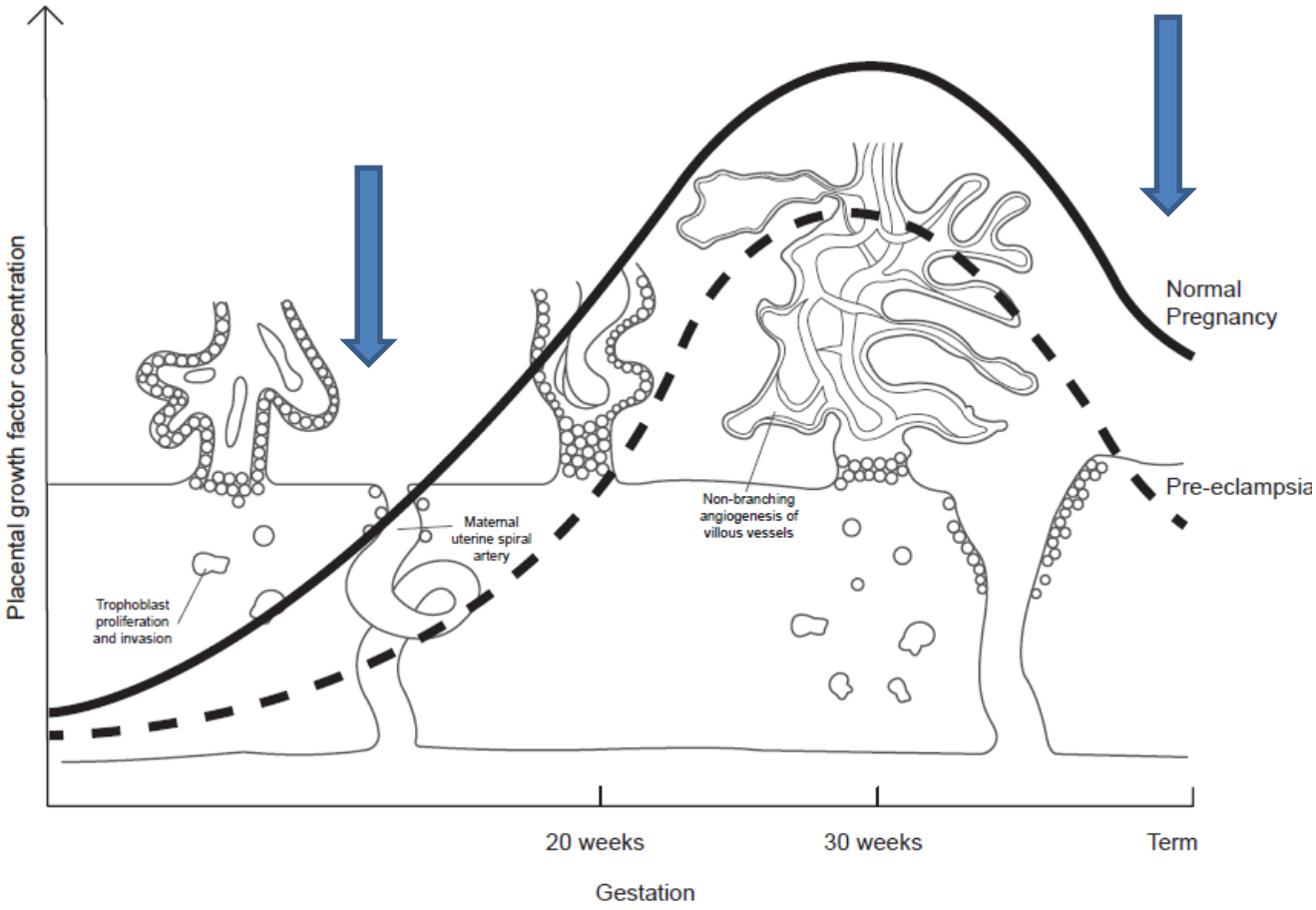


Background

Placental growth factor (PLGF) is a pro-angiogenic factor. PlGF rises to a peak at approximately 30 weeks' gestation and then decreases. In women who develop preeclampsia, PlGF is lower in early pregnancy.



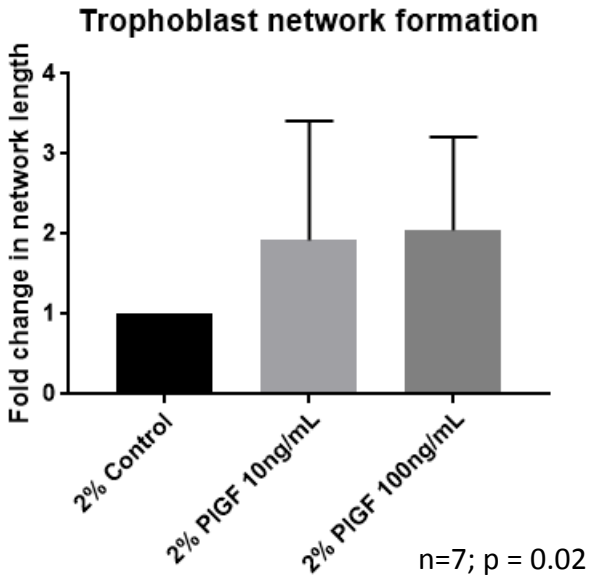
Background



- PlGF has been used as a treatment in experimental models of preeclampsia with promising results
- As circulating PlGF is low in advance of clinical symptoms, replacement of PlGF is hypothesised as a preventative treatment. However, the role of PlGF in early placental development remains unclear.

Effect of PlGF on trophoblast and endothelial cell networks

- Single culture of HTR8/SV neo cells on matrigel showed trophoblast network formation was improved 2-fold in 2% oxygen with high dose (100 ng/mL) rhPlGF ($p = 0.02$)
- Integration between trophoblast and endothelial cells was unaffected by rhPlGF in both 2% oxygen and 21% oxygen conditions compared to control
- Integration was also no different when comparing 2% to 21% oxygen conditions



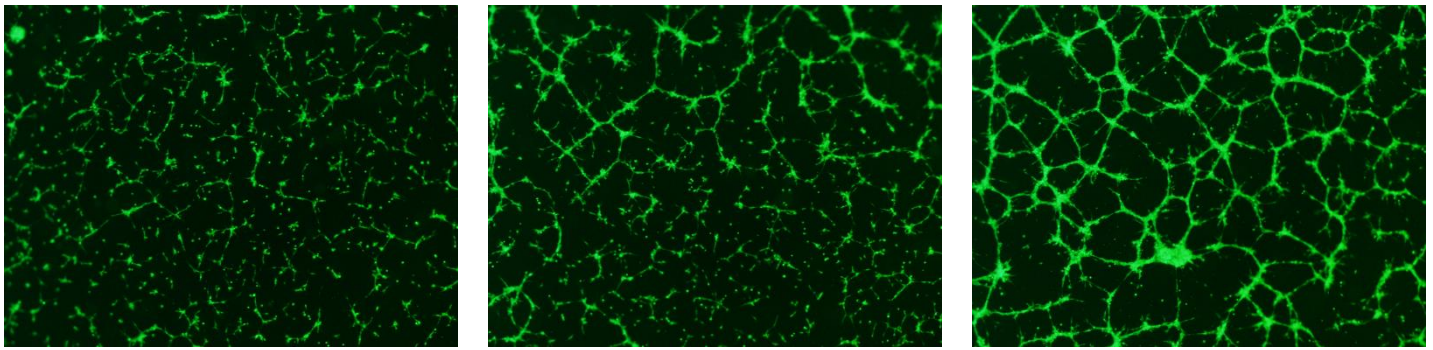


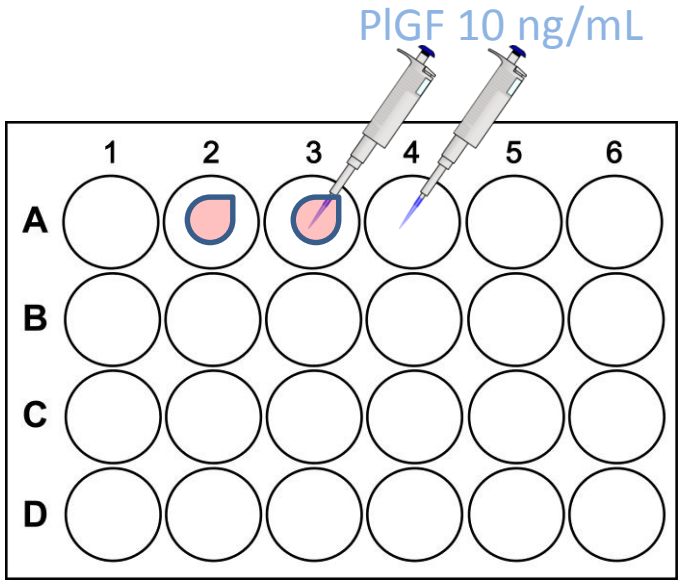
Figure: HTR8/SVneo on matrigel. Length of trophoblast network structures measured using Angiogenesis Analyzer on Image J. i) Untreated ii) PlGF 10 ng/mL iii) PlGF 100 ng/mL

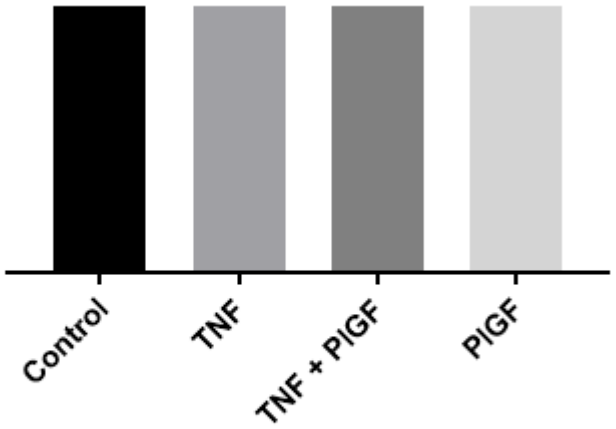
Aim

- To investigate the effect of supplemental placental growth factor in an *in vitro* model of early maternal blood vessel remodelling in the presence of TNF-α
- TNF-α is elevated in women with preeclampsia and induces an experimental model of preeclampsia in animals

Methods

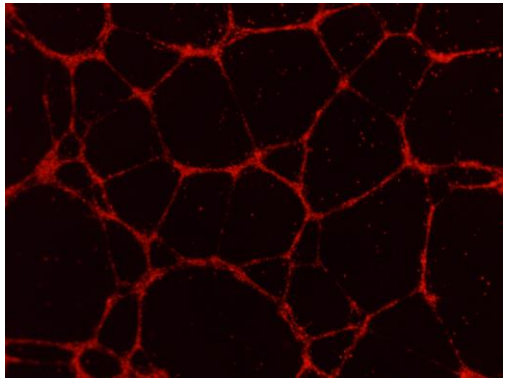
- Tissue culture plates were coated with undiluted Matrigel
- Fluorescent labelled (RED) uterine myometrial microvascular endothelial cells (1 X 10^5/well) were seeded into each well.
- Capillary networks formed, then fluorescent (GREEN) labelled trophoblast HTR8/SVneo cells (1 X 10^5/well) were added.
- Wells were exposed to TNF-α and treated with recombinant human PlGF at concentration of 10 ng/mL

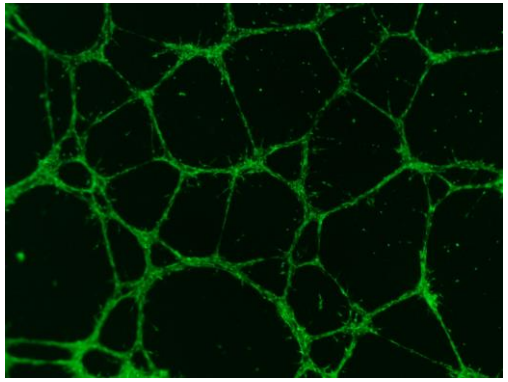




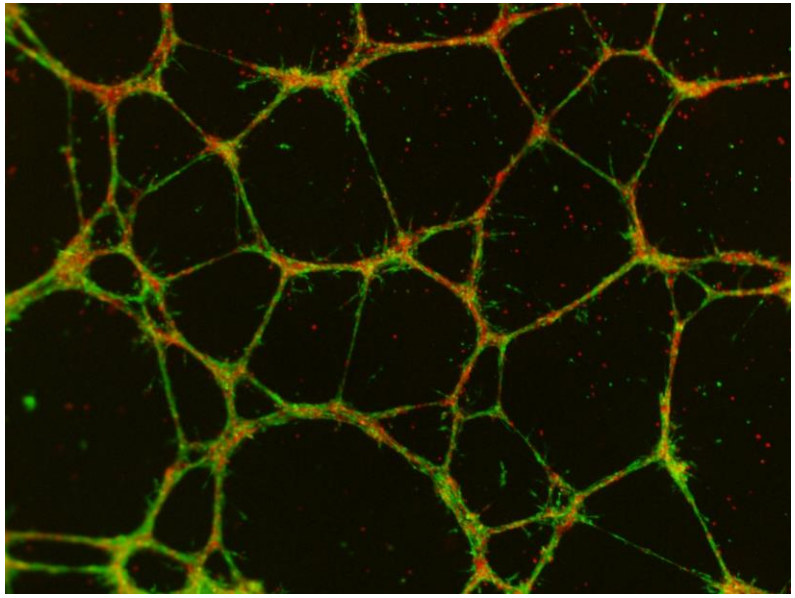
Methods

Uterine myometrial microvascular endothelial cell networks



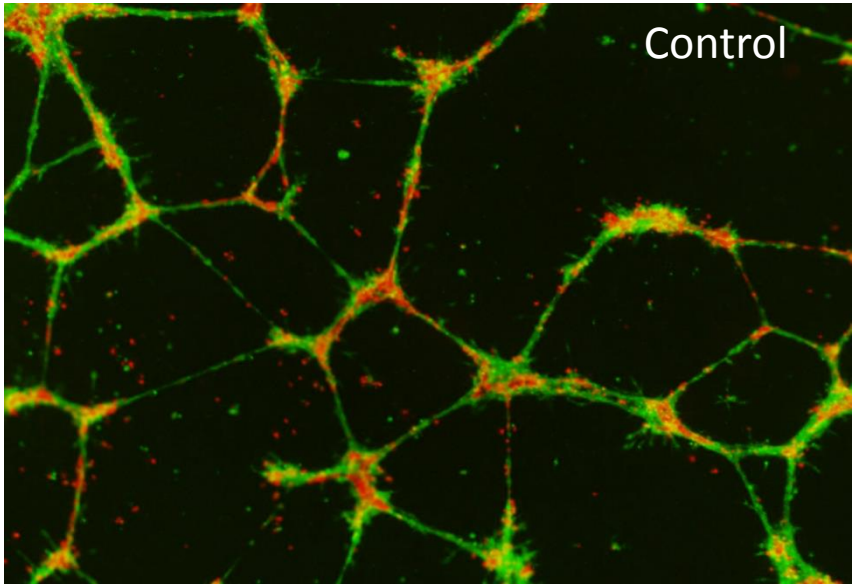


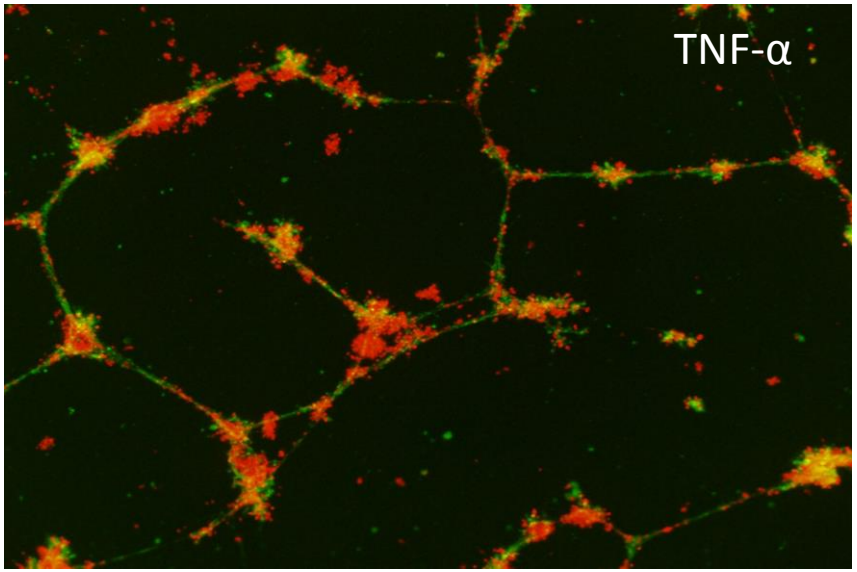
HTR8/SVneo trophoblast integrate into endothelial cell network

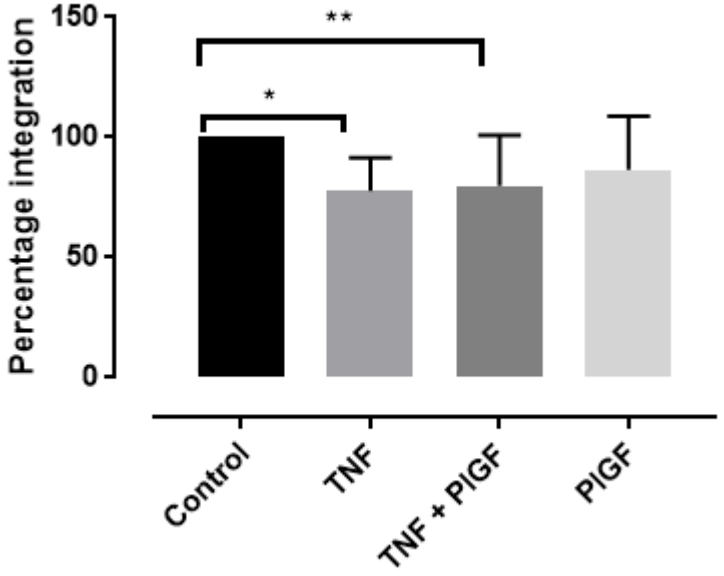


% integration = Trophoblast area (μm^2)/Endothelial area (μm^2)

Cell integration





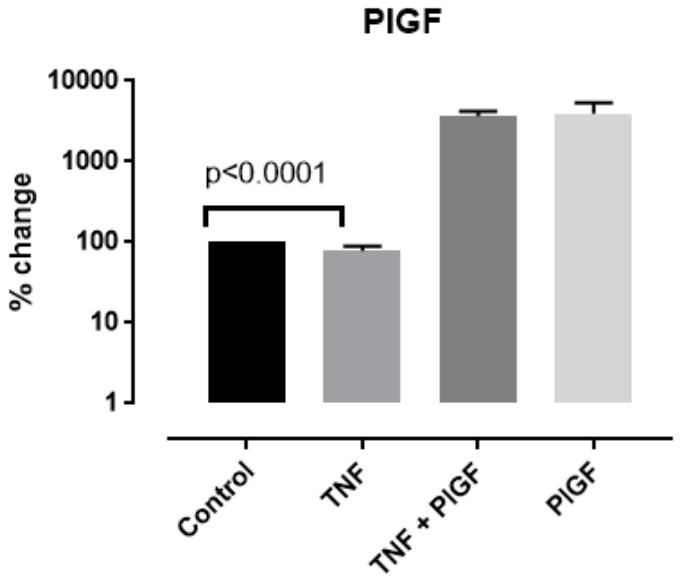


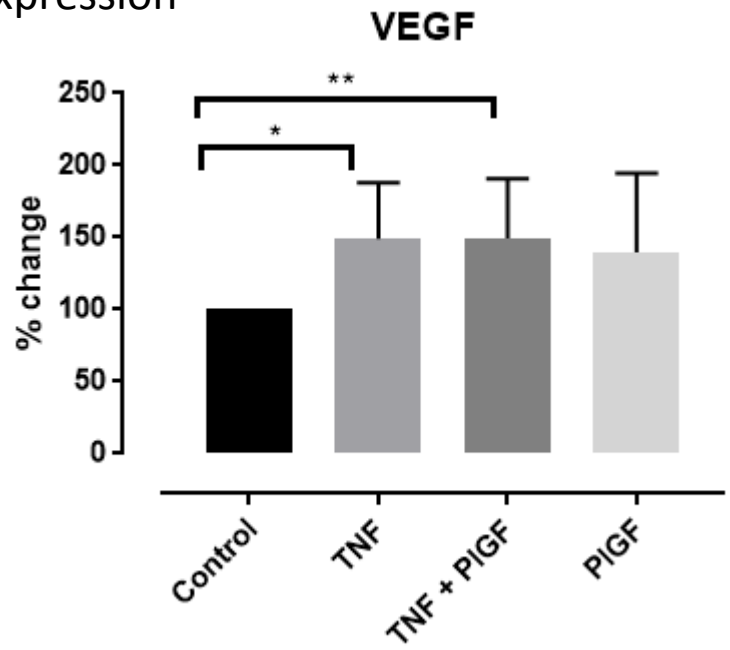
* Control vs TNF p = 0.01

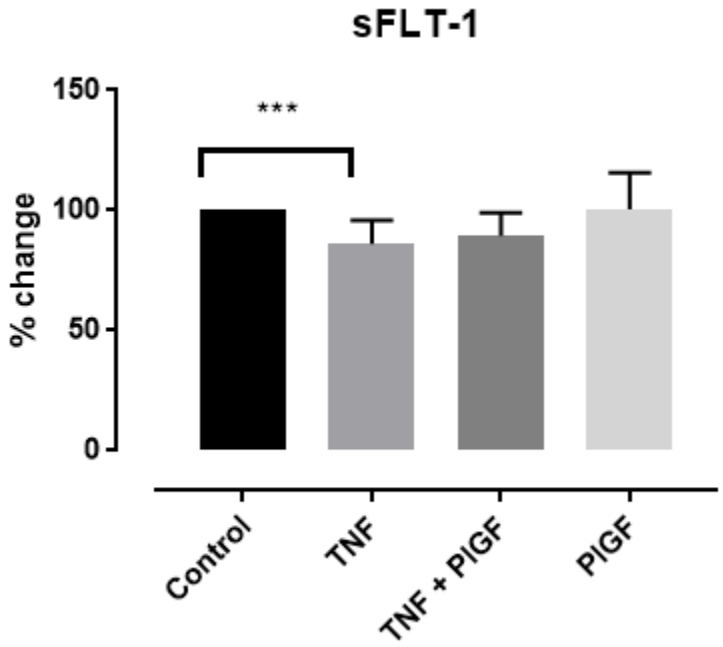
** Control vs TNF + PlGF p = 0.03

Angiogenic factors

- Addition of TNF-α decreases PlGF, increases VEGF and reduces sFLT-1 expression in conditioned media measured by ELISA
- PlGF did not reverse the effect of TNF-α upon VEGF or sFLT-1 protein expression
- PlGF alone does not VEGF or sFLT-1 protein expression



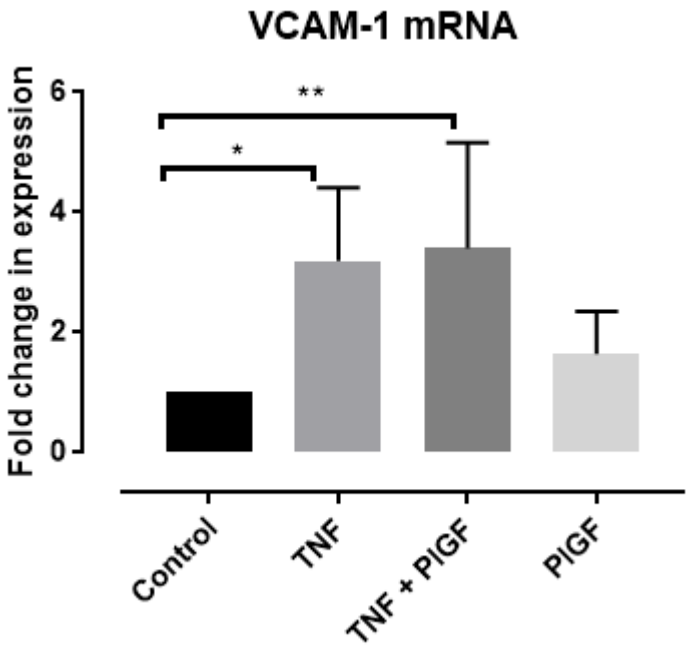




*control vs TNF p = 0.02; ** control vs TNF + PlGF p = 0.02; *** control vs TNF p = 0.01

mRNA expression of cell surface molecules

- Expression of integrin α1β1 and toll-like receptor 3 was unchanged across treatment wells
- Vascular cell adhesion molecule-1 (VCAM-1) expression was increased by TNF-α exposure but this was not changed by the presence of PlGF
- PlGF alone does not affect VCAM-1 expression



*Control vs TNF p = 0.0004

** Control vs TNF + PlGF p = 0.0003

Conclusion

- Supplemental PlGF does not abrogate the inhibitory effects of TNF-α on first trimester trophoblast and uterine myometrial microvascular endothelial cell interactions
- Clinical implication – Replacement of PlGF early in pregnancy may not improve placentation

Acknowledgements

- NHMRC postgraduate scholarship
- PEARLS Preeclampsia Research Laboratories