

NUMBER 1

Authors:

Sara van Boeckel MBChB^{1*}, Heather MacPherson PhD¹, Donald J Davidson PhD², Jane E Norman MD¹, Sarah J Stock PhD¹

1 Tommy's Centre for Maternal and Fetal Health at the MRC Centre for Reproductive Health, University of Edinburgh, QMRI, Edinburgh, United Kingdom

2 Centre for Inflammatory Research, University of Edinburgh, QMRI, Edinburgh, United Kingdom

*Contact author: svanboe@exseed.ed.ac.uk

Abstract Title:

Cell-free Fetal DNA Does Not Trigger Either Inflammation Induced Preterm Birth *In-vivo* or Inflammation *Ex-vivo*

Abstract Body:

Background: Preterm Birth (PTB) is the leading cause of neonatal mortality, but the pathogenesis of it remains unclear. Cell-free Fetal DNA (Cff-DNA) originates from the placenta, increases throughout gestation and is increased in women who develop PTB. Furthermore, fetal DNA can be pro-inflammatory through the Toll-like Receptor 9 (TLR9) and it has been proposed that it may have a role in stimulating preterm birth through the inflammation-parturition cascade. In this study, we test the hypothesis that Cff-DNA is pro-inflammatory and can initiate PTB.

Methods: Human placentas collected from uncomplicated pregnancies after elective cesarean section were cultured using a placental explant method. Supernatants were harvested for Cff-DNA extraction at 24 hours. Peripheral Blood Mononuclear cells (PBMCs) were acquired using histopaque density gradient from 15 healthy pregnant women between 38 and 40 weeks of gestation. PBMCs were stimulated with different dosages of Cff-DNA, and positive controls Lipopolysaccharide (LPS), the TLR9 ligand CpG Oligonucleotide (ODN) or vehicle (negative control) for 18 hours. Supernatants were harvested for quantification of cytokines by ELISA analysis.

At gestational day 17, C57BL/6 pregnant mice were given ultrasound-guided intra-uterine mouse placental DNA in different dosages (N=11) or saline (N=4). Time to delivery was assessed using CCTV cameras.

Results: PBMCs stimulated with different dosages of cff-DNA showed no increase in IL-8 (N=3), TNF- α (N=3), IL-6 (N=9) or CXCL10 (N=15) production compared to vehicle controls. PBMCs stimulated with LPS showed a robust IL-6 ($p=0.0178$), IL-8 ($p=0.0001$) and TNF- α ($p=0.0035$) response. ODN showed a significant increase in CXCL10 production ($p=0.0002$). PTB (defined as delivery within 36hours of treatment) was not observed in any mice treated with mouse placental DNA.

Conclusion: Cff-DNA alone is not sufficient to elicit an inflammatory response in *ex-vivo* human PBMC cultures. Furthermore, high dosages of mouse placental DNA failed to cause PTB *in-vivo*. Further studies are needed to unravel the role of Cff-DNA and the potential pro-inflammatory role it may play during pregnancy.

Session preference: Preterm labour/miscarriage/implantation

Oral or Poster: no preference

In training presenter: Sara van Boeckel is an investigator in training.

NUMBER 2

Abstract Title: Preeclampsia is Associated with Reduced Endothelial GATA2 - Implications for Endothelial Dysfunction.

Abstract body: Preeclampsia (PE) is associated with widespread endothelial dysfunction. GATA2 plays a key role in maintaining normal endothelial cell function via regulating micro-RNAs (miRs) 126 and 221.

We aimed to assess:

- 1) circulating GATA2, miR126 and miR221 expression in preeclampsia and determine if their source is endothelial or placental
- 2) if endothelial GATA2 is regulated by placental factors, and can be therapeutically manipulated by anti-oxidant resveratrol.

Circulating RNA coding GATA2, miR126 and miR221 was assessed in maternal blood at 28 weeks gestation in 35 women destined to develop PE (and 244 controls). Expression was assessed in blood and placentas from patients with established early onset (<34wks) preeclampsia (n=34) and controls (n=23). For functional studies, primary HUVECs were administered primary trophoblast conditioned media to induce endothelial dysfunction and GATA2 expression measured. Finally, primary HUVECs were exposed to TNF α +/- resveratrol and GATA2 expression measured.

Circulating mRNA coding GATA2 was significantly reduced (p=0.0082) at 28 weeks in those destined to develop PE and significantly reduced (p=0.0002) in patients with established preterm disease. In established PE, circulating pro-angiogenic miR126 was reduced by 74% (p<0.0001) and anti-angiogenic miR221 significantly increased (p=0.0449). GATA2 expression was unchanged in preeclamptic placentas versus controls, suggesting it is not the source of circulating GATA2 mRNA.

Trophoblast conditioned media administered to HUVECs significantly increased VCAM-1, a marker of endothelial dysfunction, and significantly decreased GATA2 expression. TNF α induced HUVEC VCAM-1, and significantly reduced GATA2.

These effects were dose-dependently reversed by resveratrol.

Circulating GATA2 and miRs 126, 221 are dysregulated in PE and their likely source in the circulation is endothelial, not placental. Functional studies demonstrate placental factors reduce endothelial GATA2, which may contribute to endothelial dysfunction, and that endothelial GATA2 can be therapeutically increased with small molecules such as resveratrol.

Authors/institutions:

Presenter: Carole-Anne Whigham MbChB^{1,2} PhD Candidate

cwhigham@student.unimelb.edu.au

Teresa MacDonald^{1,2} MBBS (hons)

Sue Walker^{1,2} MD

Lisa Hui^{1,2} PhD

Natalie Hannan^{1,2} PhD

Ping Cannon^{1,2} BSci

Tuong Vi Nguyen^{1,2} BSci

Stephen Tong^{1,2} PhD

Tu'uhevaha Kaitu'u-Lino^{1,2} PhD

1. Department of O&G, University of Melbourne, Victoria, Australia
2. Mercy Perinatal, Mercy Hospital for Women. Melbourne, Victoria, Australia

NUMBER 3

Macrophage Phenotypes during Ripening of the Prepartum Murine Cervix.

Anne C. Heuerman, B.S.¹, Megan Keys, B.S.¹ and Lara Campana, Ph.D.³, Steven M Yellon, Ph.D.^{1,2}

¹Longo Center for Perinatal Biology, Department of Basic Sciences, Division of Physiology, and Pediatrics, ²Loma Linda University School of Medicine, Loma Linda, CA, USA, ³MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, Scotland, UK

As the gatekeeper for parturition, successful labor requires sufficient prepartum cervix remodeling. In various mammals, inflammation is a common theme among relevant reproductive tissues as pregnancy nears term and in the pathophysiology of preterm birth. In the cervix of mice, the decreased density of cells, degradation of cross-linked collagen, and increased presence of mature macrophages (Mφs) occur between days 15 and 18 post-breeding (dpb), in advance of term birth (~19 dpb). Whether a particular Mφ phenotype is associated with this inflammation during ripening was the focus of the present study. The cervix from perfused nonpregnant (NP) and pregnant CD-1 mice (15 and 17 dpb) was dispersed and live cells (7AAD⁻) analyzed by flow cytometry with a sequence of gates for leukocytes (CD45⁺) that excluded neutrophils, eosinophils, T cells, B cells, dendritic cells, and NK cells (lin⁻). Initial efforts determined the census of monocyte precursors (Ly6C, MHCII, CD11b), mature Mφs (F4/80), and phagocytic inclination (CD206). Consistent with our previous results in the cervix, F4/80 Mφs increased with pregnancy, constituted nearly half of leukocytes on 15dpb and more were present on 17dpb. Analyses of multiple markers indicate a unique population of resident Mφs (F4/80⁺, CD11b⁺, Ly6C⁻) in pregnant cervix (15=17dpb), half of which expressed MHCII⁺. Phagocytic proclivity (CD206⁺ MFI) of this population was equivalent at both dpb, but greater compared to the NP group. Also present in cervix irrespective of pregnancy were non-phagocytic Mφs (<20% in 15 and 17 dpb groups and ~50% in NP mice) derived from infiltrated monocytes (CD11b⁺, F4/80⁻, Ly6C⁻, MHCII⁺). These findings suggest that both resident and infiltrated precursors contribute to the increased census of Mφs in the prepartum cervix with differing aptitudes for phagocytosis. This initial foray into Mφ populations in the cervix raises the possibility that inflammation associated with degradation of collagen structure during ripening may be further characterized by other phenotypes in preparation for birth. Understanding the role of Mφ phenotypes in the mechanism for cervix ripening may be useful to assess progress of remodeling, as well as have therapeutic applications to regulate local differentiation or activities by specific phenotypes to induce or suppress ripening in women at risk for preterm birth. (Supported in part NIH HD054931)

Are Macrophages Important for Prepartum Cervix Remodeling?

Steven M. Yellon, Ph.D.¹, Erin Greaves Ph.D.², Anne C. Heuerman, B.S.¹, Victoria S. Magloire, B.A.¹, Abigail E. Dobyns, B.A.¹, and Jane E. Norman, M.D.²

¹Center for Perinatal Biology, Departments of Basic Sciences, Division of Physiology, and Pediatrics, Loma Linda University School of Medicine, Loma Linda, CA, USA, ²MRC Centre for Reproductive Health, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Scotland, UK

Inflammation is associated with prepartum changes in multiple reproductive tissues and especially with the transition from a soft to ripening cervix well before labor at term or with preterm birth. In the prepartum cervix, morphological and structural remodeling processes focus attention on macrophages (Mφs) due in part to their increased presence several days before birth in rodents. The approach to selectively induce apoptosis of Mφs has been used to study remodeling processes in non-reproductive tissues. For the novel focus of the present investigation, this model was used to test the hypothesis that Mφs are essential for ripening of the cervix. Pregnant homozygous transgenic *dtr* mice (human diphtheria toxin receptor HB-EGF under the control of the CD11b promoter) were injected with diphtheria toxin (DT; 20 ng/g BW, i.p., n=11 on day 14 postbreeding, dpb) to induce apoptosis of CD11b Mφs. Controls included pregnant *dtr* mice injected with saline (n=7) or DT-injected pregnant wild-type mice (WT, n=7). Blood was taken for serum progesterone and various tissues obtained 24h later on 15 dpb, to stain for collagen, F4/80 Mφs, and cell nuclei. Results indicate that serum progesterone (~30ng/ml) and gross appearance of the cervix did not differ among groups. However uterine morphology raised concerns in as many as 5 of 11 DT-treated *dtr* mice about the continuation of pregnancy. Analyses of cervix sections indicated reduced cell nuclei density and decreased cross-linked collagen, typical of an advance in ripening (p<0.05). For Mφs, degenerate morphology and diminished presence was evident with DT treatment versus controls (p<0.05). As in other studies of nonpregnant *dtr* mice, DT depleted Mφs in the kidney, but not liver. By contrast, Mφs appeared unaffected in the ovary across all groups of DT-treated pregnant mice. These findings suggest ripening result from an impaired population of CD11b Mφs independent of a change in systemic progesterone. However, this model is complicated by concerns that DT-induced apoptosis of Mφs may promote local inflammation with detrimental effects on pregnancy in *dtr* mice concentrations. (Supported in part NIH HD054931)

NUMBER 6

Abstract title:

DNA Methylation Variation In Lean and Obese Placenta

Authors/Institutions:

Liu Yang MSc, Jessy Cartier PhD, Jon Manning PhD, Amanda J Drake PhD, Rebecca M Reynolds PhD

University of Edinburgh Centre for Cardiovascular Science and Tommy's Centre for Maternal and Fetal Health

Abstract body:

Obesity in pregnancy is associated with risks of complications for mother and child.

Mechanisms linking maternal obesity with adverse outcomes for the offspring are not

entirely understood though recent studies have suggested epigenetic modifications

may be important. As the placenta plays a key role in fetal nutrition, metabolism and

protection we hypothesized there would be DNA methylation changes between lean

and obese placenta. DNA methylation array (Infinium HD Assay) was carried out on

placentas collected from n=31 obese (BMI>40 kg/m²) and n=29 lean (BMI<25 kg/m²)

women. Two genes miR-411 and *FABP1*, with false discovery rate (FDR) adjusted

P<0.05, were selected for validation of DNA methylation by pyrosequencing and

measurement of mRNA levels by RT-qPCR. The mean (sd) miR-411 and FABP1 DNA

methylation percentage was significantly higher in obese placenta vs lean (68.9 (13.4)%

vs 58.9 (15.8)%, P=0.01 and 89.7(2.76)% vs 85.8(7.16)%, P=0.01, respectively), in

accord with the array findings. MiR-411 DNA methylation was significantly higher in

samples from non-smoking obese vs non-smoking lean (70.42(9.6)% vs 58.63(17.4)%,

P=0.02). MiR-411 DNA methylation percentage was highest in placentas from male babies born to obese mothers. There were no significant differences in mRNA levels of miR-411 between obese and lean groups and there were no correlations between methylation levels and mRNA levels of miR-411 in either obese or lean placentas (miR-411 obese $r=0.21$, $P=0.32$; lean $r=-0.32$, $P=0.14$). MiR-411 mRNA levels were significantly higher in current smokers vs non-smokers and ex-smokers (3.61(3.2) vs 1.3(1.2) vs 1.13(0.7), $P\leq 0.05$). Infant BMI was also positively correlated with mRNA levels of miR-411 in lean but not obese group (lean $r=0.636$, $P=0.003$; obese $r=0.021$, $P=0.931$). In conclusion DNA methylation and gene expression differ between lean and obese placenta, but are also influenced by maternal environment, fetal sex and infant BMI. The explanation for the lack of association of DNA methylation changes and gene expression changes is not known but may be due to the small magnitude of the DNA methylation changes. Further studies are needed to understand the functional outcomes of these DNA methylation changes. Exploration of the regulation pathway, down-stream genes and function of miR-411 is a potential avenue for future work.

NUMBER 7

Contact Author: Laura Stirrat

Email: Laura.Stirrat@ed.ac.uk

Submission for oral presentation

In training presenter – Laura Stirrat is Clinician in Training

Hypothalamic Pituitary Adrenal Axis Dysregulation in Obese Pregnancy: Clinical Implications and Underlying Mechanisms

Laura Stirrat¹ MBChB, Ksenia Stryjakowska¹ MBChB, Sarah Barr¹ PhD, Ruth Andrew² PhD, Stafford Lightman³ PhD, Jane Norman¹ MD and Rebecca Reynolds² PhD

1. MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, Scotland
2. BHF Centre for Cardiovascular Sciences, University of Edinburgh, Edinburgh, Scotland
3. MRC Centre for Synaptic Plasticity, University of Bristol, Bristol, England

Introduction: Offspring of obese are at risk of increased fetal size and cardio-metabolic disease later in life. These outcomes may be mediated by *in utero* glucocorticoid exposure. We hypothesized that maternal obesity is associated with decreased hypothalamic pituitary adrenal axis activity (HPAA) and underlies increased fetal size and longer gestation in obese. We also studied the effects of pregnancy on cortisol release, metabolism and excretion.

Methods: We measured fasting circulating cortisol and CRH at 16wk, 28wk and 36wk (obese n=276, lean n=135); serum cortisol at delivery (n=259; BMI 18-55kg/m²), and urinary glucocorticoid metabolites at 19wk and 36wk (obese n=6, lean n=5) and non-pregnant (obese n=7, lean n=7) subjects. Cortisol pulsatility was studied from 10-minute serum sampling between 0800-1100h and 1600-1900h, and 20-minute interstitial fluid sampling over 24-hours, at 16-24wk and 30-36wk (obese n=11, lean n=8,) and non-pregnant controls (obese n=4, lean n=3,). Placental cortisol metabolism was examined using an *ex vivo* placental perfusion model (n=5) perfused with D4-cortisol which is metabolised by 11 β -HSD2 enzyme to D3-cortisone.

Results: In obese, circulating cortisol was lower throughout pregnancy, higher BMI predicted lower cortisol at delivery ($\beta_a=-0.011$, $p_a=0.007$) and lower CRH predicted longer gestation ($\beta_a=-0.39$, $p_a=0.05$). Urinary glucocorticoid metabolites increased significantly in lean pregnancy, but not in obese. Higher BMI at 30-36wk correlated with lower serum pulse frequency ($r=-0.908$, $p=0.005$); predicted lower interstitial fluid pulse amplitude ($\beta_a=-1.512$, $p_a=0.016$), and increased offspring birthweight ($\beta_a=-1.043$, $p_a=0.025$). In *ex vivo* studies higher maternal D4-cortisol perfusion associated with increased transplacental cortisol passage and metabolism to D3-cortisone in the fetal circuit ($p<0.0001$). Regeneration of D3-cortisol from D3-cortisone by 11β -HSD1 was minimal ($<0.1\text{ng/g}$ tissue). Inhibition of 11β -HSD enzymes increased transplacental passage of D4-cortisol and prevented cortisol metabolism ($p<0.0001$).

Conclusions: In obese pregnancy, lower maternal cortisol and urinary clearance suggests reduced HPAA, which may underlie increased fetal size and prolonged pregnancy. Underlying mechanisms may include altered glucocorticoid pulsatility and urinary excretion. The placenta was confirmed as a mechanism of cortisol clearance while protecting the fetus from the high circulating cortisol levels during pregnancy.

NUMBER 8

Authors: Higgins Lucy^{1,2}, Ingram Emma^{1,2}, Ormesher Laura^{1,2}, Heazell Alexander^{1,2}, Johnstone Edward^{1,2}, Myers Jenny^{1,2}

¹ Maternal & Fetal Health Research Centre, Faculty of Biology, Medicine & Health, University of Manchester. ² St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, M13 9WL.

Contact: Lucy.Higgins@manchester.ac.uk

Title: Understanding maternal PIGF & sFlt1 concentrations: do they reflect placental structure/function?

Introduction: Measurement of placental growth factor (PIGF) & soluble fms-like tyrosine kinase-1 (sFlt1) is increasingly common. However, their clinical utility is limited by poor understanding of the biologically relevant information they provide. We hypothesised that maternal serum PIGF & sFlt-1 concentrations reflect placental size, structure & function.

Methods: Maternal serum PIGF & sFlt1 concentrations/ratio were compared by factor analysis to placental size (placental weight, villus area, trophoblast area/ratio), placental vascularity (vessel number/density, luminal area/ratio), placental endocrine function (relative mRNA expression, lysate/explant conditioned media hormone content) & placental arterial function (EC₅₀ U46619/SNP, passive tension accumulation (Tau) & diameter at peak active tension, DPAT). Eigen values >1 were investigated further by regression analysis.

Results: PIGF & sFlt1 were poorly correlated (Adj R²=0.0083, p=0.24). PIGF demonstrated a trend to positive correlation with placental weight (Adj R²=0.05, p=0.054) & trophoblast area (Adj R²=0.080, p=0.059). There was no relationship between PIGF & villous area or trophoblast ratio or between sFlt1 & PIGF/sFlt1 and placental/trophoblast size measures (p≥0.25). Serum PIGF, sFlt1 & PIGF/sFlt1 were independent of endocrine function measures (Eigen values <1). A trend to correlation between PIGF & luminal area was seen (Adj R²=0.098, p=0.097), with no relationship to vessel number/density or luminal ratio (p≥0.22) & no relationship between sFlt1 or PIGF/sFlt1 & villous vascularity (p≥0.13). Trends to positive correlation between PIGF & EC₅₀ U46619 (Adj R²=0.22, p=0.11) & DPAT (Adj R²=0.19, p=0.13), & between sFlt1 & Tau (Adj R²=0.30, p=0.058) are seen. No other significant associations were detected between PIGF, sFlt1 or PIGF/sFlt1 & vascular function measures.

Conclusions:

Our data suggest links between placental size, vascularity & vascular function in keeping with published phenotypes of *ex vivo* placentas from FGR/PET pregnancies. However the majority of variation in circulating PIGF & sFlt1 concentrations/ratio remains unexplained.

NUMBER 9

Abstract Title: Elevated DAMP and Pro-inflammatory Cytokine Release in an *In Vitro* Model of Early Placental Insult.

Abstract Body:

Objectives: Stillbirth is frequently attributed to placental dysfunction but the underlying mechanisms are poorly understood. Recently elevated maternal circulating levels of damage-associated molecular patterns (DAMPs), mediators of sterile inflammation (uric acid, high-mobility group box 1 (HMGB1) and cell-free fetal (cff)DNA) were identified in pregnancies at high risk of stillbirth, along with a pro-inflammatory placental profile^[1]. The origin of the DAMPs and their relationship to placental inflammation remain unknown. We hypothesised that a prior placental insult such as oxidative stress or subclinical infection results in release of DAMPs, which subsequently promotes placental inflammation.

Methods: Placental explants from normal term pregnancies (n=8) were treated with H₂O₂ (1mM) and LPS (1ng/ml) for 72hrs to mimic oxidative stress and subclinical infection respectively. Release of DAMPs and cytokines into media was assessed at 96hrs by chromogenic assay or ELISA. Intracellular cytokine production was measured in tissue lysates by ELISA and trophoblast apoptosis was assessed by immunohistochemistry for cytokeratin M30.

Results: Both H₂O₂ and LPS treatment increased release of uric acid and HMGB1 into media (p<0.05). H₂O₂ exposure elicited an additional increase in release of the DAMP S100A8 (p<0.01). Tissue content of pro-inflammatory cytokines (IL-1 α , IL-1 β , IL-6 and TNF α) and secretion into culture medium was elevated in H₂O₂ and LPS treated explants (p<0.05). H₂O₂ and LPS exposure significantly increased trophoblast apoptosis (p<0.05-0.01).

Conclusion: These *in vitro* data support the hypothesis that DAMPs are released by the placenta following oxidative stress or low level infection, both of which have been implicated in early origins of placental dysfunction. These insults also result in trophoblast apoptosis and placental inflammation, which may exacerbate placental dysfunction and predispose to stillbirth. The profile of inflammatory cytokines released, particularly IL-1, suggests activation of the NLRP3 inflammasome as a potential underlying mechanism.

[1] Girard et al, 2014, AJRI; 72: 422-434

Authors/Institutions: Bernadette Baker¹ (PhD), Helen Bischof¹ (BSc), Frances Beards¹(MPhil), Alexander Heazell¹ (PhD, MRCOG), Colin Sibley¹ (PhD), Sylvie Girard² (PhD), Rebecca Jones¹ (PhD)

1. Maternal and Fetal Health Research Centre, University of Manchester, Manchester, UK.

2. Universite de Montreal, Montreal, Canada

Contact author: Bernadette Baker email: bernadette.baker@manchester.ac.uk

NUMBER 12

Mid Trimester Ultrasound Screening for Women with Abnormal Maternal Serum Biomarkers

Laura Ormesher (MBChB)¹, Emma Ingram (MBChB PhD MCROG)¹, Lucy Higgins (MBChB PhD MCROG)¹, Jenny Myers (BMBS PhD MCROG)¹, Ed Johnstone (MBChB PhD MRCOG)¹

¹Division of Developmental Biology and Medicine, Maternal and Fetal Health Research Centre, The University of Manchester, Manchester, UK

Contact author: laura.ormesher@postgrad.manchester.ac.uk

Background: Maternal serum biomarkers, including free-beta human chorionic gonadotrophin (β hCG), pregnancy associated plasma protein (PAPP-A), inhibin A and alpha-fetoprotein (AFP) are routinely used to screen for fetal chromosomal abnormalities. In the absence of aneuploidy, abnormal maternal serum biomarkers (aMSBMs) are associated with adverse pregnancy outcomes, including fetal growth restriction (FGR). However, aMSBMs alone have a limited predictive value for adverse outcomes, necessitating an adjunct test to stratify risk.

Methods: This retrospective observational study was performed in single large tertiary referral centre between June 2009 and April 2017. As per local guidance, 680 women with aMSBMs and singleton euploid pregnancies underwent a placental screen at 22-24 weeks' gestation. This consisted of: fetal size, 2D placental biometry, liquor volume, uterine and umbilical artery Dopplers. Logistic regression models were used to determine the accuracy of prediction for preterm (<34 weeks') FGR (birthweight <3rd centile).

Results: There was an increased incidence of FGR (<3rd centile) and small for gestational age (SGA; <10th centile) in this cohort (11.3% and 25.0% respectively). Despite this, the risk of preterm FGR in this cohort was low (2.1%, n=14). The best model for exclusion of preterm FGR included estimated fetal weight (efw), uterine artery PI and placental biometry (width x width/depth=PEC). This model included 659 women (13 with preterm FGR, 1.97%) with complete datasets and had a positive likelihood ratio (LR) of 5.47, negative LR of 0.18 (table 1) and AUC of 0.8625. PEC improved the model (figure 1). The strongest predictor for preterm FGR within the model was uterine artery PI (p=0.002) > efw (p=0.014) > PEC (p=0.092). The negative and positive placental screen groups differed significantly in birthweight centile (p=<0.0001, figure 2) and gestation at delivery (p<0.0001).

Conclusion: Women with a negative placental screen had a very low risk of preterm FGR (0.4%), allowing a 22-24 week placental screen to effectively stratify women and reduce scan frequency. The inclusion of placental biometry improved the model's sensitivity. By replacing serial scans with a single 34 week scan in the negative screen group, 1096 scans (41.6%) could be avoided in this cohort. This could provide significant cost savings to the NHS.

Table 1:

	With PEC		
	True +ve	True -ve	
Test +ve	11	100	PPV=9.91%
Test -ve	2	546	NPV=99.64%
	Sensitivity=84.62%	Specificity=84.52%	
	Without PEC		
	+ve	True -ve	
Test +ve	9	97	PPV=8.49%
Test -ve	4	564	NPV=99.30%
	Sensitivity=69.23%	Specificity=85.33%	

Figure 1:

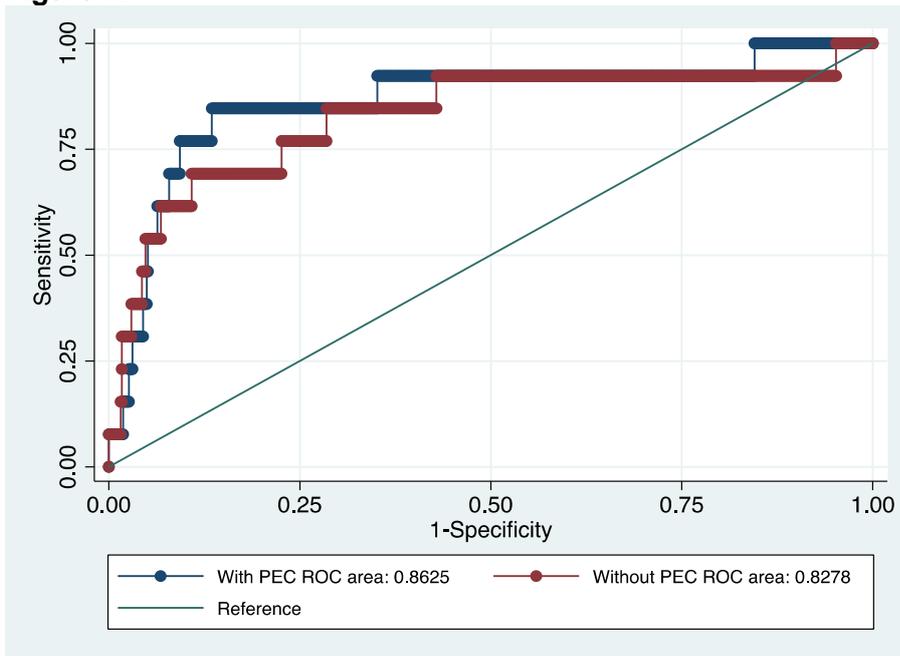
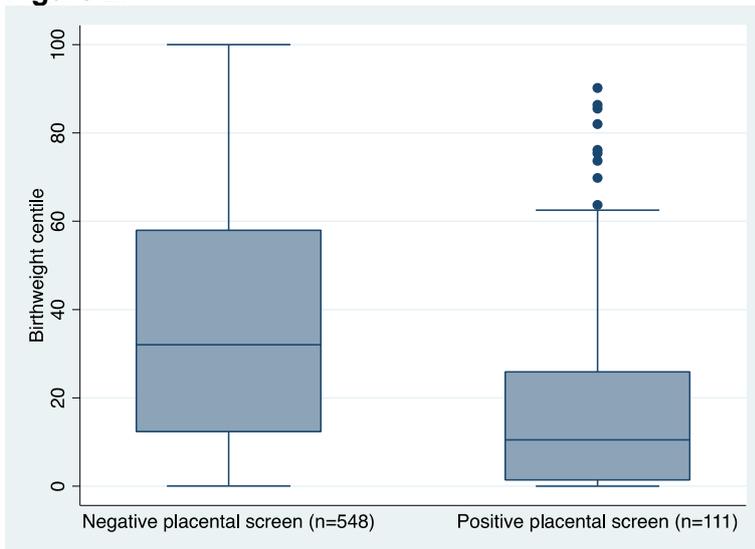


Figure 2:



Character count: 2698 (including title, abstract body, headings, tables and figures)

NUMBER 13

Measuring Simvastatin, Simvastatin Hydroxy Acid and Progesterone in Maternal Serum from a Mouse Model of Preterm Birth.

Ashley K Boyle MSc^{1*}, George Just BSc², Adrian J Thomson MSc², Sara F Rinaldi PhD¹, Adriano G Rossi PhD³, Philippa T Saunders PhD³, Jane E Norman MD¹

¹Tommy's Centre for Maternal and Fetal Health, MRC Centre for Reproductive Health, University of Edinburgh, Queen's Medical Research Institute, Edinburgh, UK.

²University/BHF Centre for Cardiovascular Science, University of Edinburgh, Queen's Medical Research Institute, Edinburgh, UK.

³MRC Centre for Inflammation Research, University of Edinburgh, Queen's Medical Research Institute, Edinburgh, UK.

*a.boyle-5@sms.ed.ac.uk

Background

We have previously shown that simvastatin treatment can prevent preterm birth (PTB) in a mouse model. We aimed to determine if simvastatin and its metabolite simvastatin hydroxy acid can be measured in the maternal serum of pregnant mice and whether progesterone levels are affected by this treatment.

Methods

On gestational day (D)17, mice received an intraperitoneal (IP) treatment of PBS or simvastatin (20, 40µg). Maternal serum was collected 1 hour (h) (n=6) or 2h (n=3) later. For the PTB mouse model, a separate cohort received an IP injection of PBS or simvastatin (20, 40µg) on D16, ultrasound guided, intrauterine injection of LPS (1µg)/PBS on D17, then PBS or simvastatin (20, 40µg) treatment 2h after. Maternal serum was collected 4h later. Simvastatin, simvastatin hydroxy acid and progesterone were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results

Simvastatin levels were very low or undetectable. Simvastatin hydroxy acid levels after 1h were 1.2±0.4ng in mice treated with 20µg simvastatin and 2.5±0.6ng for mice given 40µg simvastatin. After 2h this reduced to 0.4±0.2ng and 1.4±0.1ng, respectively. Progesterone levels were unaffected by simvastatin treatment. In a mouse model of PTB, 4h after simvastatin treatment, the hydroxy acid levels were very low (20µg: 0.068±0.009ng, 40µg: 0.2±0.03ng). LPS treatment did not affect these levels. LPS significantly reduced progesterone levels (p<0.0001 vs PBS). Serum progesterone levels were also reduced in mice given simvastatin and LPS (20µg p<0.0001, 40µg p=0.0039). However, there was a dose dependent trend for simvastatin to inhibit this effect of LPS.

Conclusion

It is possible to measure simvastatin and its hydroxy acid in mouse serum. Simvastatin levels were very low and simvastatin hydroxy acid levels reduced rapidly from a peak at 1h, to a

lower level at 4h. Simvastatin treatment did not affect progesterone levels. As expected, LPS significantly reduced progesterone levels in a PTB mouse model.

NUMBER 14

Sedentary Behaviours in Morbidly Obese Pregnant Women.

Caterina Fazzi MSc, David H Saunders PhD, Jane E Norman MD, Rebeca M Reynolds PhD
Tommy's Centre for Maternal & Fetal Health (CF, JEN, RMR), Institute for Sport, Physical Education & Health Sciences (DHS), University of Edinburgh.

Adverse outcomes of obese pregnancy are thought to be mediated through obesity-associated inflammation. Novel interventions to reduce inflammation in obese pregnancy are needed. We hypothesise that reducing time spent in sedentary behaviours, defined as awake activities that expend very low energy, may be one such strategy, particularly among morbidly obese (BMI>40) women. In the adult population 55% of awake time is spent sedentary. This is associated with numerous adverse effects for health as well as higher levels of inflammatory markers such as C-reactive protein (CRP).

We aim to test the hypothesis that reducing time spent in sedentary behaviour is a feasible approach to reduce pregnancy risks among morbidly obese pregnant women.

We first conducted a systematic review (SR). The n=26 papers meeting the inclusion criteria showed that pregnant women spend at least 50% of time awake on sedentary activities. Time spent in sedentary activities was linked to higher levels of LDL Cholesterol and CRP, larger new born abdominal circumference, and higher risk of macrosomic babies.

We then compared total energy expenditure and energy expended on sedentary activities between lean (n=173) and morbidly obese (n=244) pregnant women using the Pregnancy Physical Activity Questionnaire (subjective), and the Actical accelerometer (objective). Morbidly obese expended significantly less energy on total expenditure per kilogram and on sedentary behaviours per kilogram than lean, though no differences were found on time sedentary, suggesting they may not be the best tools to assess sedentary behaviours in pregnancy.

With the knowledge from our SR that pregnant women spend significant time sedentary and our study observation that they expend significantly less energy than lean pregnant women we are designing an intervention to increase energy expenditure during sedentary time in morbidly obese pregnant women. We carried out a focus group administering questionnaires (n=43) and interviews (ongoing) with morbidly obese pregnant women to find out about their knowledge of sedentary behaviours and interest in exercising whilst watching television. Though most women had little knowledge of the health consequences of sedentary behaviour most (n=42; 97.7%) were interested in exercising whilst sitting. We are conducting a pilot study to assess feasibility of such an approach.

Session preference: Any of the above.

Contact/Presenter author: Caterina Fazzi, caterina.fazzi@ed.ac.uk.

First author is an investigator in training (full-time PhD student).

NUMBER 15

Association between Vaginal Microbiota Dysbiosis and Miscarriage (50words)

Maya Al-Memar BSc MRCOG^{1,2}, Shabnam Bobdiwala BSc MBBS^{1,2}, Yun Lee PhD², Ann Smith PhD³, Julian R Marchesi PhD⁴, Dirk Timmerman PhD FRCOG⁵, Phillip Bennett PhD FRCOG^{1,2}, Tom Bourne PhD FRCOG^{1,2,5}, David A MacIntyre PhD^{1,2}

1. Tommy's National Early Miscarriage Research Centre, Queen Charlotte's & Chelsea Hospital, Imperial College, Du Cane Road, London, W12 0HS, UK
2. Institute of Reproductive & Developmental Biology, Department of Surgery and Cancer, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK
3. School of Biosciences, Cardiff University, CF103AX, UK, UK
4. Centre for Digestive and Gut Health, Imperial College London, W2 1NY
5. Department of Development and Regeneration, KU Leuven, Belgium

Corresponding Author:

Dr Maya Al-Memar

Early Pregnancy & Acute Gynaecology Unit

Queen Charlotte's & Chelsea Hospital, Du Cane Road, London, W12 0HS

Email: mayaal-memar@hotmail.co.uk

Tel: +447799601442

Abstract

Introduction

While aneuploidy is a known cause of miscarriage, evidence supports an infectious aetiology in some cases. In this study, we aimed to assess vaginal microbiota composition in the first trimester of women subsequently experiencing miscarriage or viable pregnancy.

Methods

Pregnant women were recruited from the early pregnancy unit with ultrasound scan confirmation of an intrauterine pregnancy. Participants were followed with serial ultrasound scans every two weeks between 6 and 14 weeks gestation until final pregnancy outcome was known: miscarriage or viable pregnancy. Vaginal swabs were collected at each visit. Swabs from miscarriage pregnancies were selected and

Figure 1. (A) Hierarchical clustering (Ward linkage) of genus level data of samples collected from patients with first trimester miscarriages (n=41) or viable pregnancies (n=112) diagnosed at the time of sampling (excluding patients with bleeding scores > 1). **(B)** Atypical communities characterised by low levels of *Lactobacillus* and high diversity were overrepresented in miscarriages compared to viable controls (47.6% vs 25.6%; $P=0.020$).

NUMBER 16

SRI Satellite 2017 Abstract

Session Preference: Preterm Labour

Long Term Cognitive Outcomes of Early Term (37-38 weeks) and Late Preterm (34-36 weeks) Births: A Systematic Review

Authors

Sarah Murray MBChB^{1*}, Susan Shenkin PhD², Kirsten McIntosh BSc¹, Jane Lim MBChB¹, Benjamin Grove³, Jill Pell PhD⁴, Jane Norman MD¹, Sarah Stock PhD¹.

¹MRC Centre for Reproductive Health, University of Edinburgh

²Geriatric Medicine Unit, University Of Edinburgh

³Department of Psychology, University of Edinburgh

⁴Section of Public Health, University of Glasgow

*Corresponding author Smurray8@staffmail.ed.ac.uk; Investigator in training

Abstract Body

Objective: There is a paucity of evidence regarding long-term outcomes of late preterm (34-36 weeks) and early term (37-38 weeks) delivery. This systematic review was performed to assess long-term cognitive outcomes of these gestations.

Data sources: Three electronic databases (Medline, Embase and PsycINFO) and reference lists of included studies were searched and a forward citation search performed of included studies. Last search was 5th August 2016.

Methods of study selection: Studies were included if they reported the range of gestational age, a validated IQ measure and the ages assessed. The protocol was registered with the Centre for Reviews and Dissemination International prospective register of systematic reviews (PROSPERO Record CRD42015015472, <http://www.crd.york.ac.uk/PROSPERO/>)

Tabulation, Integration and Results: Two independent reviewers assessed the studies. Of 11,905 potential articles, six studies and one conference abstract reporting on 41,344 children were included. For early term births, three studies and one conference abstract (n = 35,711) showed an increase in cognitive scores for infants born at full term (39-41 weeks) compared to those born at early term (37-38 weeks) with statistically significant increases for each week of term, despite differences in age of testing (one, four and six years) and method of cognitive/IQ testing (Bayley Scales of Infant Development (BSID), Stanford-Binet general IQ test and the Wechsler Abbreviated Scale of Intelligence). Three studies (n = 5644) reporting childhood cognitive outcomes of late preterm births (34 – 36 weeks) also differed in study design (cohort and case control); age of testing (2 years and 13-14 years); and the method of IQ testing (BSID and the Wechsler Intelligence Scale) and found no differences in outcomes between late preterm and term births.

Conclusion: Children born at 39-41 weeks have higher cognitive outcome scores compared to those born at early term (37-38 weeks), but not early preterm. This should be considered when discussing long term outcomes of elective delivery with parents.

NUMBER 17

Session preference: endometriosis

Suitable for a talk or poster

Macrophage-Derived Insulin-Like Growth Factor-1 (IGF-1): a Key Player In Endometriosis?

Rachel Forster (MSc)¹, Brett McKinnon (PhD)², Andrew W Horne (PhD)³, Philippa TK Saunders (PhD)⁴, Erin Greaves (PhD)^{3*}

¹Usher Institute of Population Health Science and Informatics, University of Edinburgh, Edinburgh, UK

²Department of Obstetrics and Gynaecology, Inselspital, Berne University Hospital, Berne, Switzerland

³MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK

⁴MRC Centre for Inflammation Research, University of Edinburgh, Edinburgh, UK

* Corresponding Author (egreaves@ed.ac.uk)

Introduction: Endometriosis is a common inflammatory disorder affecting 6-10% of reproductive-age women. Endometriosis is associated with debilitating chronic pelvic pain mediated in part by direct innervation of endometriosis lesions. Macrophages are central to the pathophysiology of the disorder and are seen in close association with nerve fibres in lesions. In the current study we hypothesized that macrophages in endometriosis have a phenotype that promotes nerve growth and activation.

Methods: We modelled 'endometriosis-associated' macrophages (EAMs) *in vitro* by activating human peripheral blood monocyte derived macrophages (n=7 volunteers) with peritoneal fluid (PF; n=8 pain and no endometriosis, n=7 endometriosis). We explored the phenotype and function of these macrophages using QPCR, flow cytometry, ELISA and neuronal assays of nerve outgrowth and sensitization (rat dorsal root ganglia (DRGs) and hESC derived sensory neurons).

Results: Flow cytometry and QPCR revealed that *in vitro* generated EAMs exhibit a phenotype characterised by expression of both inflammatory and repair markers. EAMs exhibit a unique neurotrophic profile characterised by elevated neurotrophin-3 (*NT-3*), brain derived neurotrophic factor (*BDNF*; $p<0.05$) and insulin-like growth factor-1 (*IGF-1*; $p<0.001$; QPCR and ELISA). Neuronal outgrowth from DRG explants was stimulated by recombinant IGF-1, PF from women with endometriosis and conditioned media from EAMs ($p<0.05$). EAM conditioned media also induced an up-regulation of the nociceptive sodium-gated ion

channels *SCN9A* ($p < 0.05$), *SCN11A* and *TAC1* ($p < 0.001$) in hESC derived sensory neurons. IGF-1R inhibition using Picropodophyllin attenuated PF and macrophage-induced nerve outgrowth as well as nociceptive gene expression.

Conclusions: We have demonstrated a unique neurotrophic phenotype for the 'endometriosis-associated' macrophage and a role for macrophage-derived IGF-1 in nerve outgrowth and sensitization. Our data suggests that future immunotherapy targeted to macrophages may alleviate symptoms associated with endometriosis.

NUMBER 18

SRI Abstract 2017

Theme: Preterm labour

Title: Manipulating microglial functional phenotypes rescues white matter pathology following perinatal brain injury

Authors:

Graeme Ireland^{1*}, Rebecca Holloway¹, Julie-Clare Becher², David H. Rowitch³, Colin Smith⁴, Jane E. Norman¹, Pierre Gressens⁵, Veronique E. Miron¹

*presenting author

Affiliations:

1. MRC Centre for Reproductive Health, The Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, Scotland
2. Simpson Centre for Reproductive Health, Royal Infirmary of Edinburgh, Edinburgh, Scotland
3. Department of Neurological Surgery, University of California San Francisco, San Francisco, USA
4. Centre for Clinical Brain Sciences, The University of Edinburgh, Edinburgh, Scotland
5. Department of Perinatal Imaging and Health, Rayne's Institute, Kings College London, London, England

Perinatal brain injury, commonly in the form of inflammation and hypoxia, impairs oligodendrocyte differentiation and myelination leading to neurocognitive defects and development of cerebral palsy. Our previous work identified that, following adult brain injury, oligodendrocyte differentiation and myelin generation requires a transition in microglia activation from a pro-inflammatory (iNOS+) to a pro-regenerative (Arg-1+ mannose receptor+) functional phenotype. We hypothesized that pathology following perinatal brain injury results from impaired transition in microglia activation, and that manipulating this activation could rescue pathology. Analysis of human neonatal brains with brain injury revealed higher densities of iNOS+ microglia relative to mannose receptor+ microglia. Modelling perinatal brain injury in an explant model using lipopolysaccharide and hypoxia mirrored this imbalance in microglia activation. Using gadolinium chloride (GdCl₃) to deplete pro-inflammatory microglia, as previously done in adult *in vivo* lesions, we induced a compensatory increase in Arg-1+ microglia resulting in increased oligodendrocyte differentiation and myelination. To determine whether these Arg-1+ microglia were driving these effects, microglia were depleted in Cd11b-DTR-derived explants by diphtheria toxin and severe hypomyelination and axonal loss were observed. These findings have directly demonstrated for the first time a role for microglia activation in regulating perinatal brain injury and recovery, and postulate that manipulating microglia would represent an effective therapeutic strategy to promote normal white matter development following perinatal brain injury.

NUMBER 19

Associations Between Human Papillomavirus (HPV) Infection and Early Miscarriage in a Scottish Data-Linkage Study.

Marian C. Aldhous PhD¹, Ramya Bhatia PhD², Roz Pollock MSc³, Kate Cuschieri PhD⁴, Heather A. Cubie PhD², Jane E. Norman MD¹, Sarah J. Stock PhD¹.

¹Tommy's Centre for Maternal and Fetal Health, MRC Centre for Reproductive Health, QMRI, University of Edinburgh, Edinburgh, EH16 4TJ UK.

²HPV Research Group, Department of Pathology, University of Edinburgh, Edinburgh EH16 4TJ UK

³Research Coordinator, eDRIS, Information Services Division, Farr Institute Scotland, Nine Edinburgh Bioquarter, Little France Road, Edinburgh EH16 4UX UK.

⁴Scottish HPV Reference Laboratory, Division of Laboratory Medicine, Royal Infirmary of Edinburgh, Edinburgh EH16 4SA UK.

Human papillomavirus (HPV) infects cervical epithelial cells and specific high-risk (HR) types are known to cause cervical cancer. Some studies have suggested that HPV infection may be associated with early miscarriage, but the data are inconsistent.

We carried out a data-linkage study to investigate whether there was an association between infection with HPV and early miscarriage (<13 weeks gestation) compared with term live births (≥37 weeks gestation). Clinical HPV data (cytology, histology, HPV genotype) from women in the Scottish HPV Archive (N=31,320) were linked to their own pregnancy records. Pregnancy outcomes (post-HPV test results) from 5,212 women were early miscarriage (n=270) or term-live birth (n=4,942).

Binary Logistic regression analysis (unadjusted and adjusted models) showed that early miscarriage was associated with HR HPV infection and with mild cervical abnormalities, but not severe cervical disease (table).

HR HPV infection but not severe cervical disease is associated with early miscarriage. Further research is required to define the mechanisms.

HPV Parameter	Term live birth N=4,942 n (%)	Early miscarriage N=270 n (%)	Unadjusted Logistic Regression models			Adjusted Logistic Regression models		
			OR	(95% CI)	P value	OR	(95% CI)	P value
HR HPV genotype								
• Negative	2281 (95.7)	103 (4.3)	Reference			Reference		
• Positive	1302 (93.7)	88 (6.3)	1.497 (1.117–2.005)		0.007	1.469 (1.093–1.973)		0.011
HPV16/18 genotype								
• Negative	2281 (95.7)	103 (4.3)	Reference			Reference		
• HPV 16/18+ve	471 (94.6)	27 (5.4)	1.269 (0.822–1.962)		0.283	1.222 (0.788–1.895)		0.333
• Other HR HPV	369 (93.4)	26 (6.6)	1.560 (1.001–2.433)		0.050	1.522 (0.972–2.383)		0.066

HPV-associated disease							
• <i>Negative</i>	-	(95.6)	-	(4.4)	Reference	<0.001	Reference
• <i>HR positive no disease</i>	-	(91.5)	-	(8.5)	2.015 (1.355–2.997)	0.001	2.030 (1.359–3.033)
• <i>Mild</i>	-	(92.9)	-	(7.1)	1.653 (1.185–2.308)	0.003	1.590 (1.135–2.228)
• <i>Severe</i>	-	(97.9)	-	(2.1)	0.464 (0.145–1.483)	0.195	0.510 (0.159–1.639)

Section preference: Preterm labour / miscarriage / implantation

Dr Aldhous is an Investigator-in-training. M.Aldhous@ed.ac.uk

This abstract has not been presented elsewhere.

NUMBER 20

Increased Vaginal Bacterial Diversity and Reduced *Lactobacillus* Species Dominance in Women with an Ectopic Pregnancy

Shabnam Bobdiwala¹, Maya Al-Memar¹, Yun Lee², Ann Smith³, Julian Marchesi⁴, Phillip Bennett^{1, 2}, Tom Bourne^{1, 5}, David MacIntyre^{1, 2}

1. Tommys' National Centre for Miscarriage Research, London, United Kingdom
2. Department of Surgery and Cancer, Institute of Reproductive & Developmental Biology, London, United Kingdom
3. School of Biosciences, Cardiff University, United Kingdom
4. Centre for Digestive and Gut Health, Imperial College London, United Kingdom
5. Department of Development and Regeneration, KU Leuven, Belgium

INTRODUCTION

Ectopic pregnancy (EP) remains the leading early pregnancy-related cause of maternal mortality. Little is understood about its' aetiology but pathogenic bacterial species have been implicated. A vaginal bacterial composition abundant in *Lactobacillus* species can be associated with healthy pregnancy outcomes and a *Lactobacillus* deplete composition is more common in adverse pregnancy outcomes. We aimed to compare the vaginal bacterial community composition of women with an EP versus those with a viable intrauterine pregnancy (VIUP).

METHODS

Vaginal swabs were collected at 4-7 weeks gestation from women presenting to an Early Pregnancy Unit. EP status was confirmed at 12 weeks gestation (n=19). Samples from VIUP were used as controls (n=22). Bacteria composition was examined using MiSeq (Illumina) based sequencing of bacterial 16S rRNA genes (hypervariable regions V1-V2). Sequence data was processed using the Mothur pipeline and analysed in the Statistical Analysis of Metagenomic Profiles (STAMP) programme.

RESULTS

Hierarchical clustering of genera sequence data permitted samples to be classified as *Lactobacillus* dominant (>93% total sequence reads), intermediate (67-81% *Lactobacillus*) or atypical (<17% *Lactobacillus*). *Lactobacillus* dominance was higher in VIUP compared to EP (91% v 58%; P=0.027; Fishers exact). Reduced abundance of *Lactobacillus* spp. in EP (P=0.016, Welch's t-test) was accompanied by increased alpha-diversity (Shannon index, P=0.034). At a species level, EP was associated with an increased relative abundance of *L iners* (P=0.027), *Prevotella timonensis* (P=0.041) and decreased *L gasseri* (P=0.005).

CONCLUSIONS

This is the first evidence demonstrating an association between EP and increased vaginal bacterial diversity with reduced dominance of *Lactobacillus* species. Further work is being undertaken to establish if this association reflects an increased risk of ascending infection leading to tubal damage and subsequent EP, or is secondary to the presence of an EP and its' associated aberrant implantation.

Contact author: Shabnam Bobdiwala, sbobdiwala@gmail.com
Shabnam is an Investigator In-Training

Session preference: preterm labour/ miscarriage/ implantation

The abstract is suitable for both oral and poster presentation

This abstract has not previously been presented as written

NUMBER 21

SRI Satellite 2017 Abstract

Abstract Title: Characterisation of Uterine Macrophage Populations and Their Role in Endometrial Repair and Remodelling

Abstract Body:

The endometrium is a dynamic tissue that exhibits an extensive regenerative capacity as well as cyclical episodes of scarless repair in response to the 'injury' inflicted on it during menstruation. Inflammatory cells play an essential role in tissue breakdown during menses but their role in repair and restoration of tissue homeostasis remains poorly understood. Defective post-menstrual repair and remodelling has been implicated in the aetiology of several gynaecological disorders including heavy menstrual bleeding, endometriosis and Asherman's syndrome. In the current study we used a mouse model that recapitulates the key phases of endometrial repair and regeneration (simulated menses) to investigate and characterise immune cell populations during scarless tissue repair.

Menstruation was simulated in MacGreen[®] mice, in which cells of the mononuclear phagocyte system express GFP and uterine tissues collected prior to tissue breakdown and during endometrial repair and remodelling (0, 12, 24 48h after removal of progesterone pellet). Tissue distribution of GFP⁺ immune cells was determined by immunohistochemistry. Flow cytometry was used to quantify and characterise different immune cell populations at 24h following progesterone withdrawal. Q-PCR was used to analyse the expression of chemoattractants in uterine tissues at all time points.

We found that endometrial breakdown and repair was associated with a striking transient influx of GFP⁺ inflammatory monocytes which peaked 24 hours after withdrawal of progesterone. Immunohistochemistry and flow cytometry has shown distinct populations of GFP⁺ monocytes and GFP⁺F4/80⁺ monocyte-derived macrophages that were associated with discrete regions of tissue breakdown and repair. GFP⁺ cells underwent apoptosis by 48 hours following restoration of tissue integrity and completion of endometrial repair. A stable, tissue-resident F480⁺ macrophage population was detected that was unchanged throughout the time course dispersed throughout the repairing endometrial tissue and tissue that had undergone full restoration. The expression of chemoattractants varied in uterine tissues throughout endometrial repair.

These data provide the first compelling evidence to support a dynamic role for inflammatory monocytes in regulating endometrial repair and provide the platform for future studies on the role of these cells in the regulation of endometrial function in health and disease.

(Supported by MRC programme grant to PTKS and an MRC Doctoral Training Grant to PMK).
(2532 characters)

Authors/Institutions: Phoebe Kirkwood, Fiona Cousins*, Olympia Kelepouri, Philippa Saunders, Douglas Gibson

Medical Research Council Centre for Inflammation Research, The University of Edinburgh, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ. UK

*Current address: The Ritchie Centre, Hudson Institute of Medical Research, 27-31 Wright Street, Clayton, Victoria, 3168, Australia

Email: P.Kirkwood@ed.ac.uk

Phoebe Kirkwood is an Investigator in training

This abstract has NOT previously been presented as written

Section: Heavy Menstrual Bleeding/fibroids as an oral OR poster presentation

NUMBER 22

Circulating Monocytes Differentiate into Immune Suppressive Precursors of Metastasis-Associated Macrophages at The Metastatic Site in Mouse Models of Breast Cancer.

Takanori Kitamura^{1*} (PhD, DVM), Dahlia D. Shenton² (PhD), Luca Cassetta¹ (PhD), Stamatina Fragkogianni¹ (PhD), Demi Brownlie¹ (MSc), Yu Kato³ (PhD), Neil Carragher² (PhD), Jeffrey W. Pollard¹ (PhD).

¹ MRC Centre for Reproductive Health, The University of Edinburgh, Edinburgh, UK.

² Edinburgh Phenotypic Assay Centre, The University of Edinburgh, Edinburgh, UK.

³ Oncology Product Creation Unit, Eisai Co. Ltd., Ibaraki, Japan.

* Contact: tkitamura@exseed.ed.ac.uk

Metastasis-associated macrophages (MAMs) play pivotal roles in breast cancer metastasis. In a metastatic breast cancer model in mouse, we previously reported that circulating inflammatory monocytes (IMs) preferentially migrated into the tumor-challenged lung where they differentiated into MAMs. However, the fate and characteristics of IMs in the metastatic site has not been clear. In this study we identified that adoptively transferred IMs (F4/80^{low}CD11b⁺Ly6C⁺) differentiated into a distinct myeloid cell population that is characterized as F4/80^{high}CD11b^{high}Ly6C^{high} and gives rise to MAMs (F4/80^{low}CD11b^{high}Ly6C^{low}) within 18 hours after the transfer. In mouse models of breast cancer, the CD11b^{high}Ly6C^{high} MAM precursor cells (MAMPCs) were commonly found in the metastatic lung, and their accumulation was increased during the metastatic tumor growth. We further found that morphology and gene expression profile of MAMPCs were distinct from IMs but similar with MAMs, and that the cells expressed mature macrophage markers such as CD14, CD36, CD64, and CD206 at comparable levels with MAMs, suggesting that MAMPCs have committed to macrophage lineage in the tumor microenvironment. Consistently, MAMPCs expressed high level of signature genes characteristic to tumor-associated macrophages. Expression of these genes in MAMPCs was reduced by genetic deletion of colony-stimulating factor 1 receptor (CSF-1R) gene. On the other hand, CSF-1R blockade did not reduce the number of MAMPCs in the metastatic site, suggesting that CSF-1 signaling is active in MAMPCs but is not required for the MAMPC accumulation. We further revealed that MAMPCs suppressed cytotoxicity of CD8⁺ T cells *in vitro*, which was partly reversed by inhibition of superoxide production but not by checkpoint receptor blocking antibodies. Overall our results indicate that once migrate into the metastatic tumors IMs immediately differentiate into immunosuppressive MAM precursor cells that might be a novel target to enhance efficacy of immunotherapy for metastatic breast cancer.

Session preference: Reproductive cancer, either oral or poster presentation will be fine.

NUMBER 23

MAP4K4 and its Role in the Ectopic Growth of Endometrial Epithelial and Stromal Cells in Endometriotic Lesions.

Helen Clarke, MBChB; ¹ helenclarke@doctors.org.uk

Jo Drury, BSc (Hons), MPhil;¹

Nicola Tempest, MRCOG;¹

Krina Zondervan, PhD;²

Dharani Hapangama, MD,¹

¹Department of Women's and Children's Health, University of Liverpool; Liverpool Women's Hospital; ²University of Oxford.

Endometriosis is a common chronic inflammatory condition defined by the presence of ectopic endometrial tissue outside of the endometrial cavity. Its pathogenesis is poorly understood. MAP4K4 is a pro-inflammatory, pro-migratory and cell regulatory kinase with a strong evidence base in malignancy, particularly in ovarian carcinoma.

In this study we have used in silico analysis of published endometrial microarray data to evaluate the regulation of MAP4K4 throughout the menstrual cycle in the context of endometriosis; progressing to wet lab experiments using eutopic endometrial samples from healthy and women with endometriosis (n=116) as well as ectopic lesions (n=10), using immunohistochemistry (IHC). We also used laser capture micro dissection and qPCR (n=17) to further identify issues associated with analyzing ectopic endometriotic samples in research.

In silico analysis showed down regulation of MAP4K4 in mid-secretory endometrium in women with mild to moderately severe endometriosis compared with healthy controls. However, the ectopic endometrium from these women showed an upregulation of MAP4K4 when compared with the eutopic endometrium. IHC data confirmed that MAP4K4 protein expression to be up regulated in mid-secretory phase (MSP) endometrium compared to proliferative phase, in both healthy and endometriosis groups; and the expression scores to be higher in women with endometriosis when compared with the healthy controls particularly in MSP ($p = <0.0001$) [figure 1]. Matched eutopic and ectopic MAP4K4 expression scores showed no statistically significant difference ($p = 0.5380$). We also examined the MAP4K4 expression by qPCR on LCM isolated ectopic and eutopic endometrial epithelial cells and identified reasons for the observed differences.

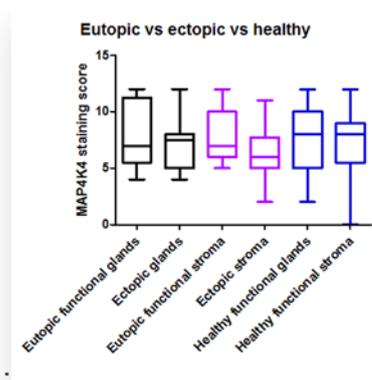


Figure 1:

Figure 1: Box and whisker plots showing the variation in MAP4K4 staining of mid secretory phase tissue.

MAP4K4 may have a role in the aberrant endometrial cell function associated with endometriosis both in eutopic and ectopic sites. There are particular concerns when examining the ectopic lesions such as lack of ectopic endometrial tissue surviving extraction laparoscopically, degradation of lesions during frozen section processing and uptake of LCM stains to facilitate laser capture of specific cells prior to RNA extraction.

Word count: 2699 characters inc spaces. This abstract has not previously been presented as written. Session: endometriosis, poster presentation. Presenter in-training.

NUMBER 24

Metastasis-Associated Macrophages Promote Breast Cancer Metastasis Through Secreting Hepatocyte Growth Factor.

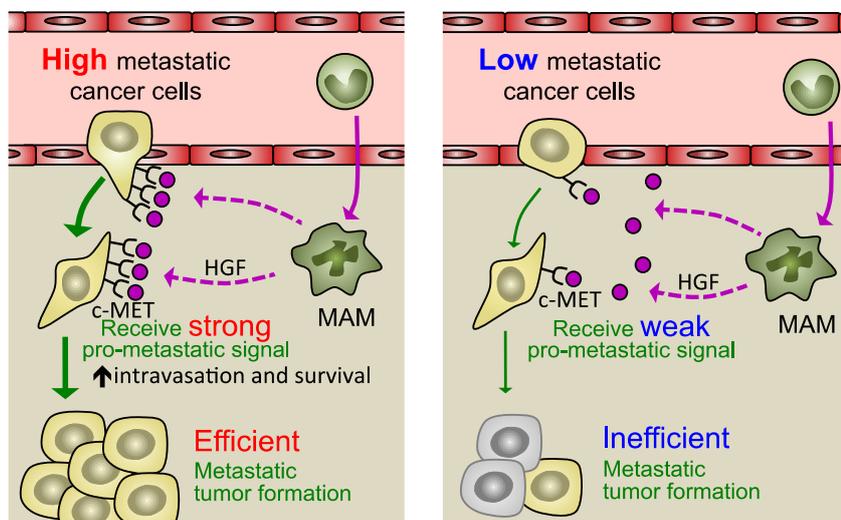
Kitamura Takanori^{1*} (PhD), Kato Yu² (PhD), Brownlie Demi¹ (MSci), Kippen Nicolle¹ (MSc), Sugano Gael¹ (PhD), Soong Daniel¹ (PhD), Pollard Jeffrey^{1*} (FMedSci FRSE)

¹MRC Centre for Reproductive Health, Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, EH16 4TJ, Scotland, UK

²Oncology Product Creation Unit, Eisai Co. Ltd., Tsukuba, Ibaraki, Japan.

Metastatic breast cancer is the leading cause of cancer-associated death in females worldwide. We have reported that tumour-associated macrophages (TAMs) promote extravasation and persistent growth of disseminated cancer cells in the metastatic site, although precise mechanisms have not been clarified. Since the interaction with TAMs is essential for metastatic cancer cells to establish lethal metastatic tumors, we hypothesized that highly metastatic cancer cells might be more sensitive to TAM-derived factors than low metastatic cells. To investigate this hypothesis, we developed a highly metastatic derivative from the E0771 mouse breast cancer cell line (HML2) by standard in vivo selection. Compared with parental E0771 cells, 1) HML2 expressed higher levels of hepatocyte growth factor (HGF) signaling molecules including its receptor c-Met, 2) activation of HGF signaling in HML2 was significantly increased and prolonged, 3) in vitro extravasation induced by macrophage-conditioned medium was enhanced in HML2, which was suppressed by blockade of c-Met. By Q-PCR and immunostaining, we also identified that TAMs are the major source of HGF in the metastatic tumors developed by E0771 cells. We further found that conditional knock down of c-MET in E0771 cells before and after intravenous injection both significantly decreased metastatic tumor establishment in the lung, and that knock-down of c-MET increased apoptosis within these foci. Importantly, pulmonary metastasis of MDA-MB-231:4175 human breast cancer cells was enhanced in human HGF knock in mice compared with control NOD/SCID mice, and was significantly suppressed by knock-down of c-MET in cancer cells.

Together these results indicate that TAMs promote extravasation and survival of disseminated breast cancer cells at least in part through secretion of HGF, and suggest



*Contact Authors

Dr .Takanori Kitamura
Chancellor's Fellow,
MRC Centre for Reproductive Health,
Queen's Medical Research Institute,
University of Edinburgh,
47 Little France Crescent,
Edinburgh, EH16 4TJ
E-mail tkitamura@exseed.ed.ac.uk

Prof. Jeffrey W. Pollard, FMedSci FRSE
Director, MRC Centre for Reproductive
Health,
Wellcome Senior Investigator,
Professor of Resilience Biology
College of Medicine and Veterinary
Medicine,
Queen's Medical Research Institute,
University of Edinburgh,
47 Little France Crescent,
Edinburgh EH16 4TJ
Email Jeff.Pollard@ed.ac.uk

Session preference: Reproductive cancer

In training presenter

Poster presentation only

NUMBER 25

Administration of Selective Progesterone Receptor Modulator (SPRM) Ulipristal Acetate (UPA) Modifies TGF β Family Members

Heavy menstrual bleeding (HMB) is common, debilitating and associated with presence of uterine fibroids. Fibroids are associated with defective implantation. Implicated is an increase of TGF β 3 and decreased BMP2 expression with consequent decreased HOXA10 expression. HOXA10 regulates FOXM1, implicated in cell proliferation. The SPRM, UPA is licensed for the treatment of fibroids.

UPA administration alters many progesterone (P) regulated genes, consistent with UPA acting with low P-agonism. However despite circulating oestradiol levels consistent with mid-follicular phase concentrations, treatment with UPA significantly reduces cell proliferation in human endometrium. A previous microarray of endometrium from women with fibroids undergoing endometrial biopsy in the proliferative phase of the menstrual cycle then following treatment with UPA identified TGF β family member *GREM2* as the most significantly down regulated gene. We hypothesised that reduction in *GREM2* might be implicated in the anti-proliferative effect.

Nine women with fibroids underwent hysterectomy following treatment with UPA for up to 12 weeks. Endometrial biopsies were obtained at the time of surgery and RNA extracted for qPCR. Control biopsies from women with fibroids in the proliferative & secretory phases of cycle were obtained from archival resources. Other genes implicated downstream of *GREM2* signalling were assessed using qPCR including *BMP2*, *TGF β 3*, *TGF β receptor 2*, *HOXA10* and *FOXM1*.

Consistent with our previous microarray *GREM2* was reduced relative to proliferative phase (Figure 1A). *BMP2* was not significantly altered by UPA administration although there was a trend to increased levels relative to secretory phase (1B). *TGF β 3* (1C), *TGF β R2* (1D), *HOXA10* (1E) and *FOXM1* (1F) mRNA levels were all altered by UPA administration.

Administration of UPA results in increased *TGF β 3* but the decrease in *GREM2* may prevent further reduction in *BMP2*. Decreased *TGF β R2* may also blunt the effect of raised *TGF β 3*, thus potentiate *BMP2* and contribute to the increase in *HOXA10*. The increase in *HOXA10* may be responsible for the decrease in *FOXM1* and thus contribute to the anti-proliferative effect observed following UPA administration.

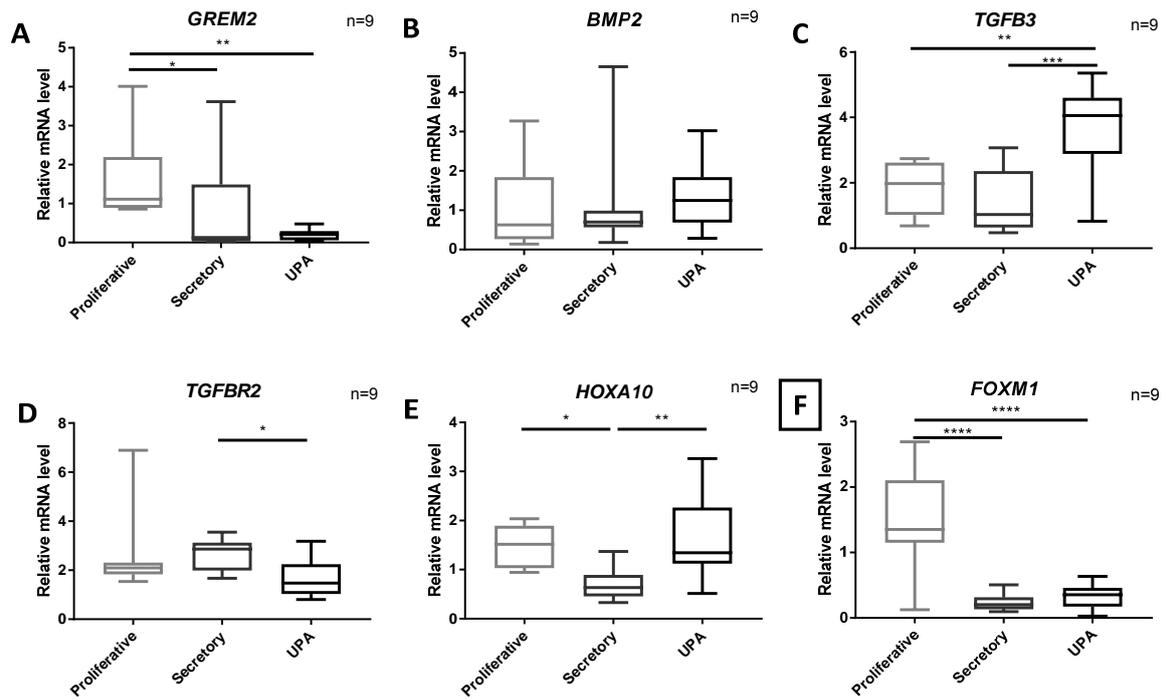


Figure 1 Effect of UPA administration on mRNA levels

Lucy Whitaker MBChB¹, Alison Murray PhD¹, Hui Wei Leow², Alistair Williams MBChB;MD³, Hilary Critchley MBChB;MD¹

¹ MRC Centre for Reproductive Health University of Edinburgh, Edinburgh UK

² University of Edinburgh Medical School, Edinburgh UK

³ Department of Pathology University of Edinburgh, Edinburgh UK

NUMBER 26

Peritoneal Macrophage Dynamics In a Mouse Model Of Endometriosis.

Chloe Hogg (BSc)*, Andrew W Horne (PhD)*, Jeffrey Pollard (PhD)*, Erin Greaves (PhD)*

*MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, Scotland

Introduction: Endometriosis is a chronic inflammatory disorder characterised by the presence of endometrial-like tissue (lesions) outside the uterus, most commonly in the peritoneal cavity. Macrophages are critical for growth and angiogenesis in endometriosis lesions, however little is known about the origins or activation status of these cells. In the peritoneal cavity macrophages exist as two populations; large peritoneal macrophages (LPM; high in number and associated with immunosurveillance and homeostasis) and small peritoneal macrophages (SPM; usually low in number but increase with inflammation). We aimed to discern whether mice with induced endometriosis have altered numbers of peritoneal macrophages and whether these subsets are incorporated into endometriosis lesions.

Methods: Using our established mouse model of endometriosis (including naïve (n=8) and sham+E2 (n=6) controls for comparison), peritoneal lavage using 7ml DMEM was performed and peritoneal macrophage subsets were analysed by flow cytometry using F4/80 (LPM) and MHC II (SPM) expression, to delineate macrophage populations. Dual immunofluorescence was performed on mouse lesions using large and small peritoneal macrophage markers, GATA6 and F4/80, and RELM α and MHC II, respectively.

Results: Flow cytometry revealed that there was no significant difference in LPM between sham+E2 (10554 cells/ μ l) compared to naïve mice (5382 cells/ μ l; p=0.097). Mice with endometriosis had higher numbers of LPM than naïve mice but lower numbers than sham mice (7299 cells/ μ l). A similar non-significant trend was observed with SPM. Heterogeneity in numbers and ratios of LPM and SPM in endometriosis mice was seen with some animals exhibiting an increased SPM: LPM ratio. Immunofluorescence identified that 48% (\pm SEM 4%) of cells in endometriosis lesions express F4/80 (n=10 lesions from n=7 animals), and of these 2% (\pm SEM 1%) co-express GATA6.

Conclusions: Heterogeneity in the numbers and ratios of LPM and SPM was observed in endometriosis mice. We speculate that lesion number, distribution and relative activity of lesions could contribute to this heterogeneity. Our data also suggests that large peritoneal macrophages do not significantly contribute to the lesion-resident macrophage population.

Contact author: Chloe Hogg (BSc), Chloe.hogg@ed.ac.uk, Investigator-In-Training.

Most suitable section: Endometriosis

Suitable for: Oral and poster presentation

Abstract has not been previously presented as written

A Monocyte Signature for Diagnosis of Breast and Endometrial Cancer

Fragkogianni Stamatina¹, Cassetta Luca¹, Swierczak Agnieszka¹, Dixon Michael²,
Wiechmann Lisa³, Smith Harriet⁴, Zhang Hui⁵, Sims Andrew², Pollard Jeffrey¹

¹MRC Centre for Reproductive Health, QMRI, The University of Edinburgh, Edinburgh EH16 4TJ, UK;

²CRUK Edinburgh Cancer Centre Crewe Rd S, Edinburgh EH4 2XU, UK; ³Northeast Medical Group, Yale New Haven Health, Ct, USA; ⁴Department of Obstetrics & Gynecology and Women's Health, Albert Einstein College of Medicine, ⁵Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, New York, NY, USA;

Breast and endometrial cancers are the most common gynaecological cancers in women in the UK. Early detection of tumours is crucial for improving patient survival. In breast cancer, mammography is the most reliable screening method; however, its sensitivity is limited by breast density. Currently, there are no early screening assays for endometrial cancer. Thus, there is an urgent need to improve diagnosis of breast and endometrial cancer. Monocytes are precursors of macrophages and recent studies show an association with pro-tumoral functions. The aim of this study was to examine the transcriptional profiles of human circulating monocytes in order to investigate their biological relevance and potential as biomarkers for cancer detection. RNA-seq was performed on purified monocytes from 59 samples (22 healthy, 21 breast cancer, 16 endometrial cancer). A gene-signature was extracted using a combination of Chi-square feature selection (X^2) and Random Forest (RF) machine learning methods. The signature was externally validated on an independent cohort of 19 samples (5 healthy and 13 breast and 1 endometrial cancer). We observed compared to control, a shift in the transcriptional profile of monocytes that we have termed Tumour Educated Monocytes (TEMo). We identified a total of 2,169 differentially expressed genes (1946 up and 223 down, FDR <0.05, Log₂FC 1.5/-1.5) when comparing cancer and healthy samples. A TEMo-derived 13-gene signature yielded an accuracy of 94%, positive predictive value (PPV) of 92% and, negative predictive value (NPV) of 97%. External validation confirmed the ability of the signature to accurately identify cancer patients with 100% accuracy. Transcriptional profiling of breast and endometrial cancer monocytes shows evidence of an altered expression

profile in the presence of cancer. These changes in gene expression were used to extract biomarkers for diagnosis of breast and endometrial cancer. In conclusion, our study shows that monocytes can offer a powerful non-invasive tool for diagnosis of cancer.

Contact authors:

Fragkogianni Stamatina, PhD
MRC Centre for Reproductive Health
The Queen's Medical Research Institute
University of Edinburgh
47 Little France Crescent, EH16 4TJ
E-mail: sfragkog@exseed.ed.ac.uk

Luca Cassetta, PhD
MRC Centre for Reproductive Health,
Queen's Medical Research Institute,
University of Edinburgh,
47 Little France Crescent, EH16 4TJ
E-mail: Luca.Cassetta@ed.ac.uk

Session preference: Reproductive cancer

In training presenter: Yes

Poster presentation only

NUMBER 28

Abstract Title: Nodal Regulates Placental Macrophages in a Mouse Model of Preterm Birth

Abstract Body: Nodal, a morphogen of the TGF- β superfamily, is involved in embryogenesis and was previously shown to be important during pregnancy, as uterine-specific Nodal knockout mice have reduced fertility, impaired placental development, and preterm birth. Recently, we demonstrated a novel role for uterine Nodal in the regulation of inflammation during pregnancy. However, the exact mechanism by which Nodal regulates immune responses was unknown. Using a uterine-specific heterozygous Nodal knockout mouse model, we assessed the role of Nodal in regulating uterine macrophages just prior to parturition (d16.5 of pregnancy). Multiplex immunoassays of placental cytokines showed that heterozygous Nodal knockout mice had elevated basal levels of IL-6 ($P = 0.001$), IL-1 β ($P = 0.02$), and IL-10 ($P = 0.04$). Immunofluorescence staining of the macrophage marker F4/80 in placental sections showed that heterozygous mice had a greater number of macrophages in the maternal decidua (Figure 1) and these results were confirmed using flow cytometry. Sequencing of Nodal from 768 patients, 207 of which had preterm birth, from a nested case-control prospective birth cohort study, revealed that Nodal SNP rs10999338 was associated with increased serum concentrations of MCP-1 ($P = 0.0208$), suggesting that Nodal may play a similar role during human pregnancy. Our results suggest that abnormal macrophage infiltration of the placenta caused by dysregulation of Nodal may be a mechanism underpinning preterm birth.

Figure 1. Immunofluorescence images of macrophages (F4/80; green) in the maternal portion of the placenta from d16.5 floxed control mice (A) and uterine-specific heterozygous Nodal knockout mice (B). Original magnification: 200 X.

Authors/Institutions:

Contact and presenting author: Lisa Starr, Ph.D., Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada, lisa.starr@mail.mcgill.ca

Taghreed Heba, M.Sc., Division of Experimental Medicine, McGill University, Montreal, Quebec, Canada

Daniel Dufort, Ph.D., Department of Obstetrics and Gynecology and Division of Experimental Medicine, McGill University, Montreal, Quebec, Canada

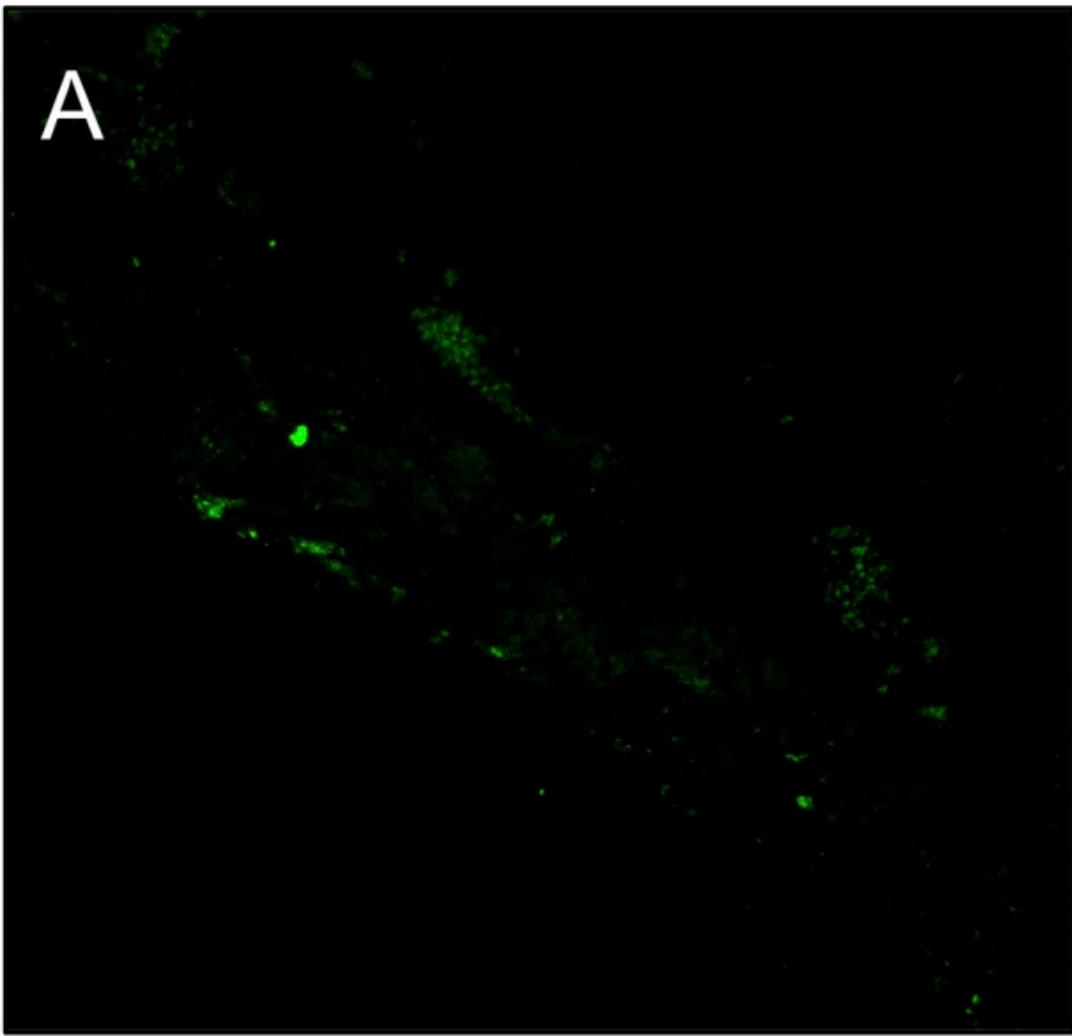
Session preference: Preterm labour/miscarriage/implantation

Oral or poster preference: No preference.

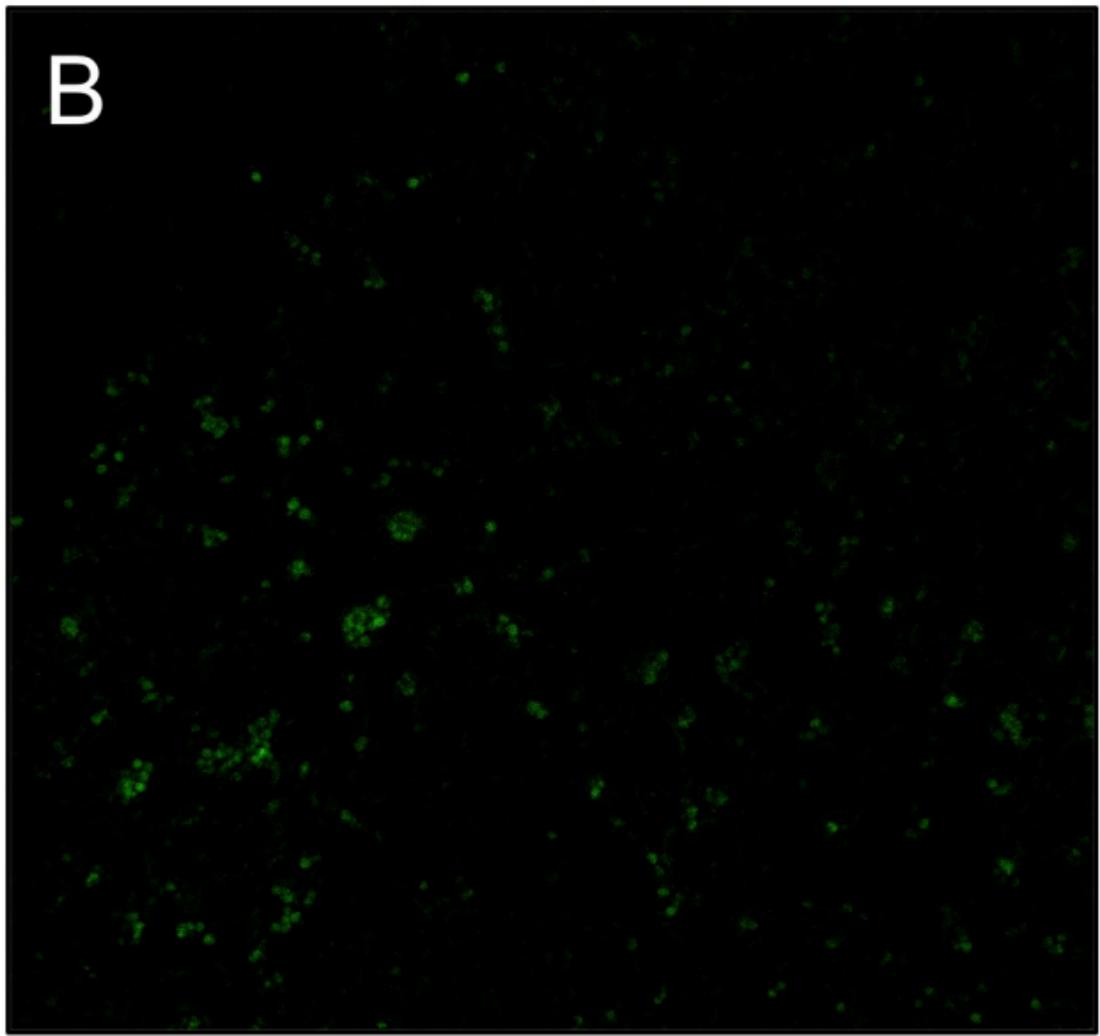
In-training presenter: Yes. First/presenting author is a post-doctoral fellow.

Abstract eligibility: This abstract has not been previously presented.

A



B



NUMBER 29

Macrophage Depletion Alters Endometrial Neuroinflammatory Gene Expression; Implications for Endometriosis

Najoua Ryane (BSc)^{1*}, Rachel Forster (MSc)², Kathryn Newton (BSc)¹, Andrew W Horne (PhD)¹, Erin Greaves (PhD)¹.

¹MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK

²Usher Institute of Population Health Science and Informatics, University of Edinburgh, Edinburgh, UK

*N.ryane@students.uu.nl

Introduction: Endometriosis affects 6-10% of reproductive age women and is characterised by the presence of endometrial-like tissue outside the uterus (lesions), commonly found on the pelvic peritoneum. The causes of endometriosis are unknown but endometrial tissue is thought to be disseminated in the peritoneal cavity by retrograde menstruation. Endometriosis is associated with debilitating pelvic pain, which is likely mediated by direct innervation of endometriosis lesions. Macrophages are present in high numbers in lesions and are observed in close proximity to nerve fibres. In our endometriosis mouse model, a proportion of macrophages in lesions appear to originate from the endometrium. We hypothesized that endometrial macrophages have an important role in generating an environment that may encourage nerve growth during establishment of endometriosis lesions.

Methods: In our mouse model of endometriosis, donor mice are induced to undergo a 'menses-like' event and this endometrial tissue is collected and injected into the peritoneal cavity of recipient mice. In the current study we aimed to achieve macrophage depletion of donor mouse endometrium and to assess neuroinflammatory gene expression. Two depletion strategies were explored: 1) Liposomal clodronate (liposome n=20, saline n=23) in wild-type C57BL/6 mice and 2) diphtheria toxin (DT) administered to homozygous CD11b-DTR mice (DT n=16, saline n=14) that express the human diphtheria toxin receptor (HB-EGF) under the control of the CD11b promoter. Flow cytometry was used to assess macrophage depletion of endometrial tissue and QPCR determined mRNA expression of neuroinflammatory genes.

Results: Endometrial macrophage depletion with liposomal clodronate was highly variable, however we did identify an increase in *Igf-1* ($p < 0.05$) and a decrease in *Il1- β* expression ($p < 0.0001$). Depletion using DT in Cd11b-DTR mice resulted in a 50% reduction in endometrial mononuclear cells. We found a corresponding up-regulation in *Ccl2* expression ($p < 0.05$). Both depletion strategies led to a non-significant shift in the expression of the neuroinflammatory genes *Ngf*, *Nt-3* and *Tnfa*.

Conclusions: Liposomal clodronate and DT administration in Cd11b-DTR mice had different macrophage depletion efficiencies in the endometrium. Endometrial macrophage depletion induced changes in neuroinflammatory gene expression. We propose that this has implications for nerve infiltration of lesions in endometriosis.

Session preference: Endometriosis

Presentation type: Poster

First and presenting author is an investigator in training

NUMBER 30

AUTHORS: Evan Chiswick PhD^{1,2}, Manu Kumar¹, Keith Isaacson MD^{2,3}, Douglas Lauffenburger PhD^{1,2}, Linda Griffith PhD^{1,2}. ¹Department of Biological Engineering, ²Center for Gynecopathology Research, Massachusetts Institute of Technology, Cambridge, MA. USA. ³Harvard Medical School and Center for Minimally Invasive Gynecologic Surgery, Newton, MA. USA

ABSTRACT TITLE: A High Throughput Multiplexed Approach to Characterize Intra vs. Inter-Uterine Heterogeneity of Tissue Lysates.

INTRODUCTION: The human endometrium undergoes extensive remodeling that is guided by proteolytic and inflammatory networks in a spatially asynchronous yet temporally resolved manner, culminating in implantation or menses. Aberrant proteolytic and inflammatory activity is known to facilitate metastasis in cancer, and numerous studies have detailed their aberrations in gynecopathologies such as endometriosis.

Uterine pathologies such as adenomyosis and fibroids have foci of disease often surrounded by healthy tissue. Whether this seemingly healthy tissue that is proximal to disease facilitates pathogenesis more so than distal unaffected tissue is unclear. This preliminary study addresses whether the location (i.e. anterior vs posterior) of grossed tissue blocks impacts the molecular signature observed, and whether sub-grossing (i.e. endometrium vs myometrium) enhances or obscures features compared to full thickness sections.

METHODS: Fresh tissue was excised from several regions of the uterus from patients post-hysterectomy and flash-frozen. 50mg of tissue was sectioned per block, and lysed with RIPA buffer + protease inhibitors. Total protein was normalized and assayed for 70+ inflammatory mediators and MMPs. Analyte concentrations were used for hierarchical clustering using correlation as a distance metric (i.e. samples cluster together if closely correlated).

RESULTS: Unsupervised hierarchical clustering showed samples from the same patient (intra-uterine) tend to cluster together irrespective of sample location, and intra-uterine samples are more closely related than inter-uterine thus suggesting relative intra-uterine homogeneity.

CONCLUSION: Although several analytes differed dependent upon location of the sample within the uterus, intra-uterine samples still clustered more closely than inter-uterine samples. This highlights the importance of multiplexed data acquisition and analyses in which networks or clusters of mediators are suggestive of shared physiological processes that are robust to false positives/negatives that occur in univariate analyses due to natural biological variation.

NUMBER 31

Does Omega 3 and Vitamin D Supplementation in the Six Weeks Prior to *In Vitro* Fertilisation Improve Embryo Quality?

Alexandra J. Kermack MBBS, BSc^{1,2,3}, Philippa K. Lowen BSc, MSc, PhD³, Susan J. Wellstead DipHE RN, BSc, RM^{2,3}, Markus Montag BSc, PhD⁴, Franchesca D. Houghton BSc, DPhil², Philip C. Calder BSc, PhD, DPhil, RNutr, FSB, FSfN^{1,2}, Nicholas S. Macklon MBChB, MD, FRCOG^{1,2,3}.

¹NIHR Southampton Biomedical Research Centre, ²Unit of Human Development & Health, Faculty of Medicine, University of Southampton, Southampton, SO16 6YD, UK. ³Complete Fertility Centre, Department of Obstetrics & Gynaecology, Princess Anne Hospital, Southampton, SO16 6YD, UK, ⁴International Reprolab Consulting, St Augustin, Germany.

Contact: Alexandra.kermack@uhs.nhs.uk

Introduction

There is a growing body of evidence that demonstrates that a major determinant of fecundity and success of IVF is the patient's preconceptional health and lifestyle. However, little is known about which diet may have the greatest benefit. Recently, a 'Mediterranean' diet, high in vegetable oils and fish, was reported to increase pregnancy rates by up to 40%. The PREPARE trial aimed to investigate whether a six week dietary supplementation with Omega 3 and Vitamin D for couples undergoing IVF improved embryo quality.

Method

One hundred and eleven couples were recruited to PREPARE and given the trial drink, containing 2g of DHA and EPA and 10micrograms of vitamin D or placebo, to consume daily for six weeks prior to their oocyte retrieval. Dietary questionnaires and samples of blood were taken before and after the intervention. Day 3 and 5 KIDScores (Known Implantation Data Score) were calculated from morphokinetic markers for individual embryos. Statistical analysis was performed using SPSS.

Results

No difference was observed in the day 3 or day 5 KIDScores (mean taken per couple) between the groups ($p=0.074$ and $p=0.178$, respectively). However, when those fertilised using IVF were analysed independently, couples in the treatment arm produced significantly higher mean embryo KIDScores on day 5 when compared to those on placebo ($p=0.032$). Furthermore, embryo quality positively correlated with the women's red blood cell DHA and EPA levels: d3 KIDScores with EPA (coefficient=0.243, $p=0.018$) and DHA (coefficient=0.201, $p=0.050$) and d5 KIDScores with DHA (coefficient=0.258, $p=0.012$).

Conclusion

This data suggests that a diet high in EPA and DHA improves embryo quality in couples who undergo conventional IVF, but not in those utilising IVF-ICSI. This implies that the improvements are mediated through the sperm and further investigation into which specific male parameters are enhanced is required.

NUMBER 32

Authors:

Daniel Ying Hon Soong^{1,2}, Luca Cassetta², Stamatina Fragkogianni², Jeffrey William Pollard²

¹ Corresponding Author

² MRC Centre for Reproductive Health, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom.

Abstract Title:

Machine Learning and Classification of Cells in Complex Heterogeneous Breast Cancer Tissues

Abstract Body:

Accurate segmentation, detection, and classification of cells exhibiting highly polarised, dendritic, or complex 3D morphologies in tissue sections is hindered by the punctate, broken, and discontinuous nature of cross-sectional signal used to stain such cells in tissues and is further compounded by natural expression heterogeneity of cell populations. Simple intensity thresholding methods are inadequate and result in misclassification due to fragmented but often intense signal colocalising with neighbour cells of different (and incorrect) classes. We have implemented cell simulation, machine learning, and k-nearest neighbour classification of immunofluorescence-labelled and slide-scanned whole tumour tissues and needle biopsies using morphological, textural, statistical, and intensity metrics constituting a 19-dimensional feature space to automatically detect, classify, and quantify tumour-associated macrophage subpopulations in breast cancer.

Session preference: Reproductive Cancer

Oral or Poster presentation: Poster only

In training presenter: No

NUMBER 33

Authors: Sonia Whyte¹, Lorraine Adamson¹, Neil Marlow², Andrew Shennan³, Phillip R Bennet⁴, Steven Thornton⁵, Stephen C Robson⁶, Mark Kilby⁷, Jane Denton⁸, Joel Smith⁹ Sarah Cunningham-Burley¹, John Norrie¹, Sarah Stock¹, Jane E. Norman¹ for the STOPPIT2 study group

Institution: ¹University of Edinburgh, Edinburgh, UK, ²University College London, London, UK, ³King's College London, London, UK, ⁴Imperial College London, London, UK, ⁵Queen Mary University of London, London, UK ⁶Medical School, University of Newcastle, Newcastle UK ⁷ University of Birmingham, Birmingham, UK ⁸Multiple Births Foundation, London, UK ⁹ HERC University of Oxford, Oxford UK

Objectives: STOPPIT2, An open randomised trial of the Arabin pessary to prevent preterm birth in twin pregnancy including health economics and treatment acceptability.

Introduction/Background: There is a clear need for treatments to prevent preterm birth in twins, with neonatal and infant mortality rates of 14.2 and 18.8 per 1,000 live births respectively: there are currently no effective treatments. A subgroup analysis of the ProTwin study suggests that the Arabin pessary is effective in preventing preterm birth in women with a twin pregnancy and a short cervix (Liem 2013).

Materials & methods: STOPPIT 2 is a multicentre open randomised trial to evaluate the Arabin pessary compared with standard treatment. Women with a twin pregnancy attending a participating hospital (n=50) in the UK/Belgium are recruited to undergo screening with cervical length ultrasound (n=1850). Those with a short cervix ≤ 35 mm (≤ 30 th centile) are eligible for randomisation (n=500). Randomisation is conducted centrally via a web portal. Quality control systems for cervical length measurements are integral to the study as are the cost effectiveness and maternal acceptability.

Results: Results are anticipated 2020 for the primary obstetric outcome; spontaneous onset of labour leading to delivery before 34 weeks gestation, and neonatal outcome composite of specific adverse outcomes or death occurring up to the end of the first four weeks after birth to either or both babies.

In year one, an internal pilot was performed to assess recruitment rates and feasibility. A meta-analysis published during this time suggested that the 30th centile cervical length in twins at 18-21 weeks gestation is 35mm, hence the cervical length cut off was modified from ≤ 30 mm to ≤ 35 mm (considered to be ≤ 30 th centile). To adjust for slower recruitment than initially planned, the number of participating sites has been increased from 45 to 50 and recruitment has been extended by one year. To date (30th June 2017) there are: 54 participating sites, 1060 (n=1850) women who have consented, from which 230 (n=500) are randomised.

Implications: The Arabin pessary is an inexpensive, readily available device. If STOPPIT 2 shows it to be effective, it has the potential to reduce pre-term birth; with significant cost savings and wider benefits for women and babies.

Reference: Liem SM, van Pampus MG, Mol BW, Bekedam DJ. Cervical pessaries for the prevention of preterm birth: a systematic review. *Obstet Gynecol Int.* 2013;2013:576723.

Funding: This study is being funded by the National Institute for Health Research (NIHR), Health Technology Assessment (HTA) Programme (**Project: 13/04/22**). The views and opinions are those of the authors and do not necessarily reflect those of the HTA programme, NIHR or the Department of Health.

NUMBER 34

Author(s): Juan S. Gnecco^{a,b}, Tianbing Ding^a, Caroline Smith^a, Kaylon L. Bruner-Tran^a, and Kevin G. Osteen^{a,b,c*}

Affiliation(s):

^aWomen's Reproductive Health Research Center, Vanderbilt University Medical Center, Nashville TN, USA.

^bDept. of Pathology, Immunology and Microbiology, Vanderbilt University Medical Center, Nashville TN, USA.

^cVeteran Affairs Tennessee Valley Healthcare System, Nashville TN, USA

Contact author(s): Juan Gnecco (juan.s.gnecco@vanderbilt.edu) or Kevin Osteen, PhD (Kevin.Osteen@vanderbilt.edu)

Title: Shear-Stress Induced Prostanoids Promote Decidualization of Perivascular Stroma in the Human Endometrium.

Abstract: Decidualization is the differentiation process of the endometrial stromal fibroblasts under the influence of progesterone that is required for the establishment of pregnancy. In the human endometrium, decidualization originates at the stroma surrounding the terminal vascular arterioles during the late secretory phase of the menstrual cycle. However, the mechanisms that drive why perivascular stromal cells are the first to undergo decidualization have not been identified. Herein, we utilized a novel microfluidic Organ-on-Chip model of the endometrial vascular microenvironment to examine the crosstalk between primary human uterine microvascular endothelial cells and stromal fibroblasts under hormonal and physiological signals. We morphologically and biochemically measured the decidualization process in the model after 14 days of progestin treatment in static and perfused conditions. We observed an enhanced decidualization response when stromal cells were co-cultured with vascular cells under hemodynamic forces, such as shear stress, from laminar microfluidic perfusion (1 μ L/min). To identify the cellular communication mechanism, we examined the secreted paracrine factors between the cell types and identified that endothelial-derived prostanoids, specifically prostacyclin (PGI₂) and prostaglandin (PGE₂) significantly enhanced decidualization of the stromal cells under progestin signaling via the cAMP pathway. We confirmed these findings and demonstrated that the hemodynamic forces acting on the vascular endothelium actively induce COX-2 expression to promote prostaglandin secretion. Altogether, these findings show that the endometrial vascular endothelium plays a key role during the molecular initiation of decidualization of the perivascular stroma in the human endometrium via paracrine prostanoids. Clinical implications of these findings suggest a novel cellular target for therapeutic design for disorders such as infertility, preeclampsia and endometriosis.

Decidualization with flow (1 μ l/min)

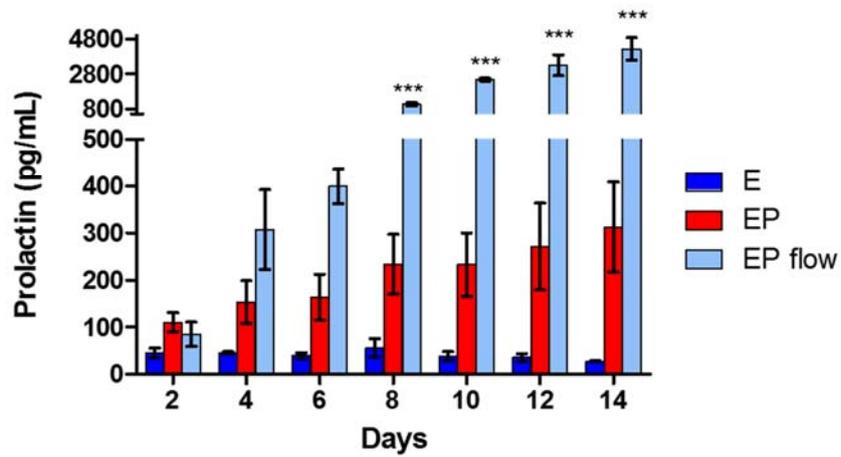


Figure 1. Decidualization is enhanced by the perfusion of the vascular endothelium in a microfluidic co-culture model of the perivascular stroma. (N=4) E, EP and flow represents oestrogen only, oestrogen with progesterin and flow at 1 μ l/min, respectively.

Session Preference:

Preterm labour/miscarriage/implantation

Poster (or oral)

Author is an "In-training presenter"

NUMBER 35

A Novel Role for sST2: Promotion of First Trimester Trophoblast Invasion.

Zahraa Alyahyaei¹, Alison Wallace², Judith Cartwright², Ian Sargent¹, Ingrid Granne¹, Jennifer Southcombe¹

¹ Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, U.K.

² Molecular and Clinical Sciences Research Institute, St. George's, University of London, London, U.K.

Regulation of the growth and differentiation of trophoblast cells is critical for successful placentation. Cytokines are key players in modulating the maternal immune response to prevent rejection of the conceptus. We have investigated the expression of the Interleukin 1 family member cytokine IL-33, its receptor ST2L and the soluble sST2 (decoy factor), in first trimester placenta with the aim to better understand their role in early pregnancy.

IL-33 is a dual function cytokine; it is a nuclear protein which may repress transcription and also acts as an 'alarmin' signalling damage to the immune system. IL-33 binds to the receptor ST2L, which in combination with the IL-1 Receptor Accessory Protein triggers Th2 cytokine responses. In addition sST2 acts as a decoy receptor for extracellular IL-33, however sST2 has an alternate role and can also promote breast cancer cell motility and enhance metastasis.

IL-33 and ST2 mRNA and protein were both expressed in first trimester placentas from 6-12 weeks of gestation, with no correlation between expression versus gestational length. sST2, rather than ST2L, was the predominant isoform in the placenta, ten-times more sST2 mRNA or protein was expressed than ST2L. IL-33 was localized to cells in the villous stroma, whereas ST2 was present in the syncytiotrophoblast and villous cytotrophoblast with high expression on the invasive extravillous cytotrophoblast of the cell columns. Secretion of sST2, but not IL-33, by first trimester placenta explants was found. sST2 binds to the first trimester trophoblast cell line SGHPL5, and functions *in vitro* to inhibit proliferation (MTT assay) and stimulates trophoblast invasion (spheroid outgrowth experiment). Our study has shown that sST2 may play an important role in placentation through controlling trophoblast invasion.

NUMBER 36

The impact of hypoxia inducible factor on the inflammatory response at menstruation

Jacqueline A Maybin, †¹, Alison Murray¹, Philippa Saunders², Peter Carmeliet³ and Hilary OD Critchley¹. ¹MRC CRH & ²MRC CIR, University of Edinburgh, United Kingdom and ³VIB, KU, Leuven, Belgium.

Introduction: The inflammatory process of menstruation is followed by coordinated repair of the denuded endometrial surface. Suboptimal endometrial repair is associated with heavy menstrual bleeding (HMB), a common and debilitating complaint. Progesterone-withdrawal in the late secretory phase causes vasoconstriction of endometrial spiral arterioles, but the presence and role of hypoxia in the endometrium remains undefined. Hypoxia inducible factor (HIF)-1 α is stable in hypoxic conditions and co-ordinates the cellular response to hypoxia.

Hypotheses: (1) hypoxia is present in the menstrual endometrium (2) HIF-1 α is necessary for efficient endometrial repair at menstruation (2) the hypoxic response alters endometrial innate immune cells at menses.

Methods: Endometrial biopsies were collected from healthy women and menstrual blood loss objectively measured (modified alkaline-haematin method: HMB>80ml). HIF-1 α was detected by Western blot and downstream targets by PCR (n \geq 4 per group/cycle stage). Ovariectomy, administration of estradiol/progesterone and decidualization induction enabled modelling of endometrial shedding/repair in mice, allowing manipulation HIF-1 *in vivo* (n \geq 6/group). Histological grading quantified endometrial repair.

Results: HIF-1 α was present in nuclear extracts from human endometrium exclusively during the perimenstrual phase. Women with HMB had decreased menstrual endometrial HIF-1 α and its downstream targets (*CXCL4/VEGF*) vs those with normal loss (P<0.05, P<0.001). Women with HMB bled for 2 days longer than those with normal loss (P<0.01). Mouse studies revealed a transient hypoxic episode in the endometrium during menses, detected by pimonidazole staining (marker of O₂<10mmHg). Pharmacological inhibition of HIF-1 during simulated menses (i.p. echinomycin vs vehicle) significantly delayed endometrial repair (P<0.05). Echinomycin treatment resulted in reduced endometrial neutrophil numbers at the time of repair. Endometrial macrophage numbers were not significantly reduced. However, markers of a Th2 response (*Arg-1/Il10*) were significantly decreased in uterine tissue from mice treated with echinomycin, suggestive of an altered macrophage phenotype.

Conclusions: Hypoxia is present in the mouse endometrium during active bleeding and HIF-1 is necessary for normal endometrial repair at menses. The delayed repair observed with inhibition of HIF-1 may be due, at least in part, to an altered innate immune response at menses.