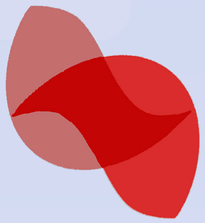
ONLINE SYMPOSIUM

05-06 May 2021

Novel perspectives in maternal and fetal health

SYMPOSIUM PROCEEDINGS

in collaboration with



ANZPRA

Australian and New Zealand Placental Research Association

Edited by the iPLACENTA symposium committee

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FOREWORD

This booklet collects orals and posters presented at iPLACENTA's "Novel perspectives in maternal and fetal health" symposium which took place online on 5-6 May 2021.

iPLACENTA ("Innovation in modelling Placenta for Maternal and Fetal Health") is a Marie Skłodowska-Curie Innovative Training Network funded by the European Union and coordinated by Colin Murdoch at the University of Dundee. The multi-disciplinary, multi-sectoral network encompasses fifteen early-stage researchers and their supervisors at eleven institutions in eight countries.

The aim of the symposium was to bring together early-career and more senior researchers within and outside the network to share current research and develop new connections in placental research.

The abstracts and posters are categorised in four sections:

- Early-career researcher oral abstracts
- Poster session 1: Imaging/Modelling and new technologies in Maternal and Fetal Health
- Poster session 2: Pathology and Maternal Health
- Poster session 3: Proteo-genomics of the placenta

Prize-winning contributions are indicated by a .

We would like to thank Jo James in particular for ensuring the fruitful collaboration with the Australian and New Zealand Placental Research Association (ANZPRA).

Thanks also go to everyone who gave a talk and/or showcased their poster, and especially to the early-stage researchers who acted as moderators during the symposium.

Members of iPLACENTA:

Wednesday, May 5th 2021, 9:00 – 10:00 am BST (British Summer Time)

Parallel poster sessions:

- Session 1: Imaging/modelling and new technologies
- Session 2: Pathology and Maternal Health
- Session 3: Proteo-genomics of the placenta

Thursday May 6th 2021, 8:00 am – 1:00 pm BST (British Summer Time)

8:00 am BST	**Welcome** **Section 1: Next generation models and diagnostics of PE and IUGR**	
8:10 - 8:30	Carlos Salomon, U Queensland	Placental extracellular vesicles across gestation and their potential as early biomarkers of preeclampsia
8:30 - 8:40	Natalia Gebara, U Turin	Characterization and function of amniotic fluid derived extracellular vesicles in pre-eclampsia
8:40 - 9:00	Jo James, U Auckland	Knowledge is power: Linking anatomical and functional changes to improve the prediction and detection of fetal growth restriction
9:00 - 9:15	*Q&A*	
9:15 - 9:25	Samprikta Manna, UC Cork	Investigation of premature cellular senescence in Pre-eclampsia and Intrauterine Growth restriction
9:25 - 9.35	Jessica O'Callaghan, Queensland U of T	Sex-specific methylation and expression differences in the placenta of infants born small for gestational age
9:35 - 9:45	Gwenaëlle Rabussier, Mimetas	Development of a 3D microphysiological placenta in-vitro model as a tool for drug transport studies
9:45 - 10:05	Patricia Maguire, UC Dublin	AI_PREMie: a novel risk stratification tool for preeclamptic toxaemia
10:05 - 10:20	*Q&A*	
10:20 - 10:50	**Panel discussion: Challenges in pregnancy research**	

10:50 am BST	**Section 2: Adverse pregnancies, a stress test for further maternal complications**	
10:50 - 11:10	Susan Ozanne, U Cambridge	Exploring the consequences of obesity during pregnancy for mother and child: a window of opportunity
11:10 - 11.30	Asma Khalil, St George's Hospital, U of London	Impact of Covid-19 on pregnancy
11:30 - 11:45	*Q&A*	
11:45 - 11:55	Antonia Hufnagel, U Cambridge	Diet-induced maternal obesity leads to reduced uterine artery compliance and fetal growth-restriction in a murine model
11:55 - 12:05	Rachael Crew, U of Western Australia	Characterising the structural, haemodynamic and molecular phenotype of the feto-placental vascular network in a rat model of fetal growth restriction
12:05 - 12:25	Peter Barrett, UC Cork	Hypertensive disorders of pregnancy and the risk of chronic kidney disease: A Swedish registry-based cohort study
12:25 - 12:40	*Q&A*	
12:40 - 13:00	**Prizes** **Prizewinning posters: Elevator pitches** **Close**	

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Poster Session 3: Proteo-genomics of the placenta

OA01 - Investigation of premature cellular senescence in Pre-eclampsia and Intrauterine Growth restriction

Samprikta Manna, University College Cork, Ireland

Introduction: Placental ageing is a normal physiological response in progressing pregnancy with the organ exhibiting extreme morphological and physiological senescence at term. Pre-eclampsia and intra-uterine growth restriction can cause premature placental ageing, resulting in maternal and foetal morbidity and mortality worldwide. Aim: To investigate senescence associated secretory phenotype (SASP) in maternal blood and markers of cellular senescence in the placenta in pre-eclampsia and intra-uterine growth restriction.

Methods: Maternal plasma samples were taken at term gestation from nulliparous women with pre-eclampsia (n=9), intra-uterine growth restriction (n=10) and age-matched control uncomplicated pregnancies (n=15). SASP panel of cytokines were evaluated using a multiplex Mesoscale ELISA assay in all groups. Statistical analysis was performed using GraphPad Prism 8®. Placental samples were collected after caesarean sections from pre-eclampsia (n=6), intra-uterine growth restriction (n=11) and control uncomplicated pregnancies (n=16) for absolute telomere length analysis by RTqPCR. Multiple regression and statistical analysis was performed using IBM SPSS v26.

Result: SASP consists of both inflammatory cytokines and mediators including, Interferon-γ (INF-γ), Interleukins (IL-13, IL-6, IL-8), Monocyte chemoattractant protein-1 (MCP-1) and Macrophage Inflammatory Protein 1 alpha (MIP-1α) and Matrix Metalloproteinase-3 (MMP-3). There was a significant increase in IL-6 in pre-eclampsia when compared to healthy controls (0.54 pg/ml ± 0.271 v 0.3 pg/ml ± 0.102; P=0.017). In addition, there was a non-significant increase in MMP3 in both pre-eclampsia (15.049 ng/ml ± 4.98 v 8.005 ng/ml ± 1.078; P=0.095) and intra-uterine growth restriction (16.12 ng/ml ± 5.40 v 8.005 ng/ml ± 1.078; P=0.088) when compared to controls. No significant differences were seen between study groups for other cytokines in the SASP panel. The calculated absolute telomere length of the placenta showed no significant difference between controls (4.87 ± 3.74 kbp) compared to pre-eclampsia (7.45 ± 3.17 kbp) and IUGR (4.87 ± 4.07 kbp). When adjusted for maternal age, BMI, gestational age and individualised customised centile of the foetus, there were no significant differences, however there was difference between IUGR (Coefficient 0.0195 with 95% CI) and pre-eclampsia (Coefficient 2.5755 with 95%CI).

Conclusion: Our data shows increased inflammatory markers such as IL-6 in maternal plasma in pre-eclampsia only suggesting altered inflammatory signalling, which may modulate senescence. Absolute telomere length of the placenta at term may be dependent on other variable factors such as BMI and gestational age.

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Manna, Samprikta (1,3), McCarthy, Fergus P (1,3), McElwain, Colm (2), Maher, Gillian (3), Martín, Marta Giralt (2), McCarthy, Cathal (2)

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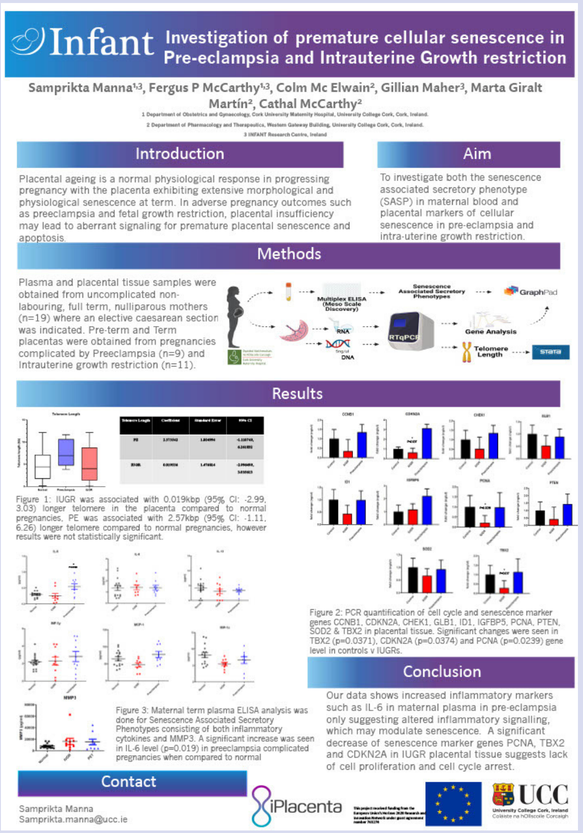

OA02 - Diet-induced maternal obesity leads to reduced uterine artery compliance and fetal growth-restriction in a murine model

Antonia Hufnagel, Institute of Metabolic Science, University of Cambridge, United Kingdom

Best Oral, Runner-up

Author(s):

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Background/Aims: Worldwide around 50% of babies are born to obese mothers. This is concerning as it leads to complications such as preeclampsia, stillbirth, and gestational diabetes which has long-term health consequences for mother and child. The aim of this study is therefore to elucidate the direct effects of an obese pregnancy on the mother and in turn the fetoplacental unit.

Methods: C57Bl6/J mice were fed with chow or obesogenic diet from 10 weeks prior to mating and during pregnancy. Throughout pregnancy body weight of the dams was monitored and ipGTTs were performed at day E17.5. Uterine artery and fetal blood flow were assessed via ultrasound at the end of pregnancy (E18.5), at which timepoint the dams, fetuses and placentas were dissected.

Results: Maternal obesity led to impaired glucose tolerance and hyperinsulinemia. Additionally, the uterine artery pulsatility index was increased significantly in the obese dams (0.49 +/- 0.02 in control dams vs. 0.59 +/- 0.01 in obese dams, p=0.0009). SFlt levels were higher in the maternal serum from obese compared to control dams (26.2 +/- 3.0 ng/mL in control vs. 37.3 +/- 3.3 ng/mL in obese dams, p=0.02). Together with observed placental structural impairments these changes are likely contributing to the reduced fetal weight observed in the obese group (1.16 +/- 0.01 in control fetuses vs. 0.93 +/- 0.02 in obese fetuses, p<0.0001).

Conclusions: We conclude that our model provides a clinically relevant platform for further mechanistic research as human obese pregnancies are known to be at an increased risk for preeclampsia. The results highlight the detrimental effects of maternal obesity on pregnancy complications and the health and development of the placenta and the fetus.

OA03 - Characterising the structural, haemodynamic and molecular phenotype of the feto-placental vascular network in a rat model of fetal growth restriction

Rachael Crew, The University of Western Australia

Author(s):

Crew, Rachael C. (1), Tongpob, Yutthapong (1,2), Bappoo, Nikhilesh (3), Khinsoe, Georgia (3), Doyle, Barry (3) & Wyrwoll, Caitlin S. (1)

(1) School of Human Sciences, The University of Western Australia, Australia, (2) Faculty of Medical Science, Naresuan University, Thailand, (3) Harry Perkins Institute of Medical Research, Australia

Best Oral

Objective: Since the developing fetal heart beats directly against placental vascular bed resistance, an optimal placental vascular network likely drives healthy fetal cardiac development. Fetal growth restriction (FGR) is strongly associated with diminished placental vasculature, however the exact mechanisms involved and the implications for fetal cardiovascular development are unclear. Here, we use a rat model of glucocorticoid excess to examine the feto-placental vasculature in FGR, using a novel, integrative approach encompassing structural and molecular analysis techniques.

Methods: Wistar rats were administered dexamethasone acetate (DEX) from embryonic day (E)13 until E21 (term is E22). Three-dimensional feto-placental vascular casts were obtained via perfusion of radiopaque contrast medium and assessed with micro-CT scans. Expression of key angiogenic genes was measured in the placental labyrinth zone (LZ) and fetal heart by qPCR.

Results: DEX induced fetal (20%; $P<0.001$) and placental (34%; $P<0.01$) growth restriction by E21. Three-dimensional casting revealed a 50% decrease in the DEX placental arterial network volume ($P<0.005$); attributed to reduced arteriole length (45%; $P<0.01$) and branching (58%; $P<0.05$), with a corresponding reduction in LZ Nos3 expression (40%; $P<0.05$). DEX increased fetal heart volume relative to body weight (50%; $P<0.01$) and elevated cardiac Vegfa expression (68%; $P<0.05$).

Conclusions: While the placental vascular structure was severely compromised by DEX, cardiac sparing mediated via Vegfa may have occurred in the fetus, and nitric oxide sensitivity may contribute to placental vascular disturbances in this model. Ultrasound analysis and computational modelling of placental blood flow are currently underway to define the placental haemodynamic environment. This may drive the development of more accurate diagnostic criteria for at-risk pregnancies, to prevent adverse fetal cardiovascular development and corresponding health complications in the offspring of compromised pregnancies.

IM04 - Investigating the role of S-glutathionylation as a redox switch in preeclampsia using iPSC-derived models

Agathe Lermant, University of Dundee, United Kingdom

Objectives: Preeclampsia (PE) is a severe pregnancy complication associated with hypertension, increased oxidative stress and high circulating levels of anti-angiogenic factors produced by the placenta. Yet antioxidant therapy has failed, in some cases worsening pregnancy outcomes. S-glutathionylation is a common reversible oxidative post-translational modification (oxPTM) modulating protein activity. Although this oxPTM is emerging as an important “redox-switch” in angiogenesis and cardiovascular diseases, its role has not been investigated in pregnancy-induced vascular complications. We aimed to investigate the molecular basis for how thiol modification removal may promote PE phenotype by disrupting angiogenic signalling at the maternofoetal interface.

Methods: We combined physiological in vivo assessment with bioinformatics proteomic analysis and exon-level microarray analyses to identify potential redox-sensitive targets involved in the development of PE phenotype. In vitro studies investigated the role of oxPTM in angiogenic signalling in individual placenta cell types. Primary endothelial cells and iPSC-derived trophoblasts were implemented in 3D models to replicate key placental functions and assess the consequences of oxPTM removal in early pregnancy.

Results: Transgenic mice (TG) overexpressing glutaredoxin (Grx) were generated to promote S-glutathionylation removal during timed pregnancy. TG mice developed gestational hypertension and elevated plasma sFlt-1 compared to their littermate controls. Grx overexpression disrupted endothelial cell sprouting in a 3D organoplate®, inhibited trophoblast migration and syncytialisation. Grx disrupted angiogenic signalling in a cell-type specific manner by increasing sFlt1:PlGF ratio in endothelial cells and syncytiotrophoblasts while reverting it in extra-villous trophoblasts, and preventing endoglin expression in all cell types. An exon-level profiling of TG vs WT mice placenta revealed a genome-wide alteration of alternative splicing and bioinformatic analysis identified relevant redox-sensitive targets. Of significance, S-glutathionylation removal from the spliceosome machinery affected Flt-1 splicing.

Conclusion: Grx-dependent removal of ox-PTMs regulates angiogenic pathways in the placenta via the modulation of redox-sensitive targets in the spliceosome machinery, which may promote the PE phenotype.

Author(s):

Lermant, Agathe (1), Rabussier, Gwenaëlle (2), Sneddon, Claire (1), Kerr, Jennifer (1), Lanz, Henriëtte (2), Murdoch, Colin E. (1)

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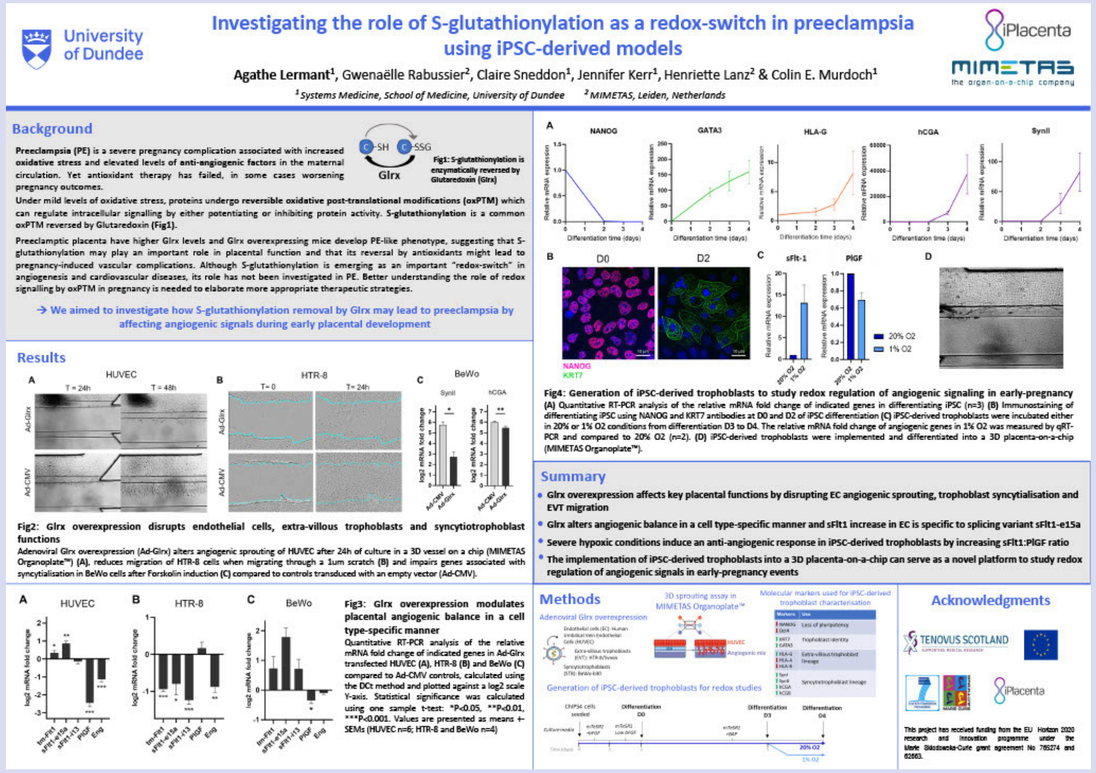

IM05 - Assay development to quantify reactive oxygen species in the OrganoPlate® to investigate oxidative stress in healthy and diseased placenta models

Camilla Soragni, MIMETAS BV, Leiden, The Netherlands

Oxidative stress (OS) is an imbalance between reactive oxygen species (ROS) and antioxidant response. OS is an important parameter in healthy and diseased pregnancies and the placenta plays a crucial role, as it generates ROS. Therefore quantification of ROS is an important feature to analyse in in vitro and in vivo models mimicking the placenta. In this study we developed an image-based assay to quantify ROS accumulation and depletion in the OrganoPlate® 3- lane 40 for high-throughput (HT) application. Mono-culture of HUVEC was used for the development as it is a wellestablished OrganoPlate® model and HUVECs are classically used in placenta co-culture models together with cell types such as BeWo, which are trophoblasts. HUVECs were cultured as micro-vessels against extra-cellular matrix in the OrganoPlate 3-lane 40 for at least 2 days before performing the ROS assay. The assay is image-based, using DHR to stain ROS multiplexed with Hoechst to stain nucleic acids for cell counting. In terms of controls for ROS accumulation we selected TBHP and standard culture media as positive and negative control respectively. In order for the assay to be feasible, and HT compatible, only images from the top and bottom of the tubule were acquired instead of the entire tubules. The assay was successfully applied on the placenta co-culture HUVEC-BeWo with/out synciatilization media to induce trophoblast fusion. Future application will be diseased placenta model characterization and studies to investigate effect of antioxidant drugs to treat these conditions.

Author(s):

Soragni, C. (1,2), Rabussier, G. (1,2), Lanz, H.L. (1), Bircsak, K. (1), De Windt, L.J. (2), Trietsch, S.J. (1), Ng, C.P. (1)

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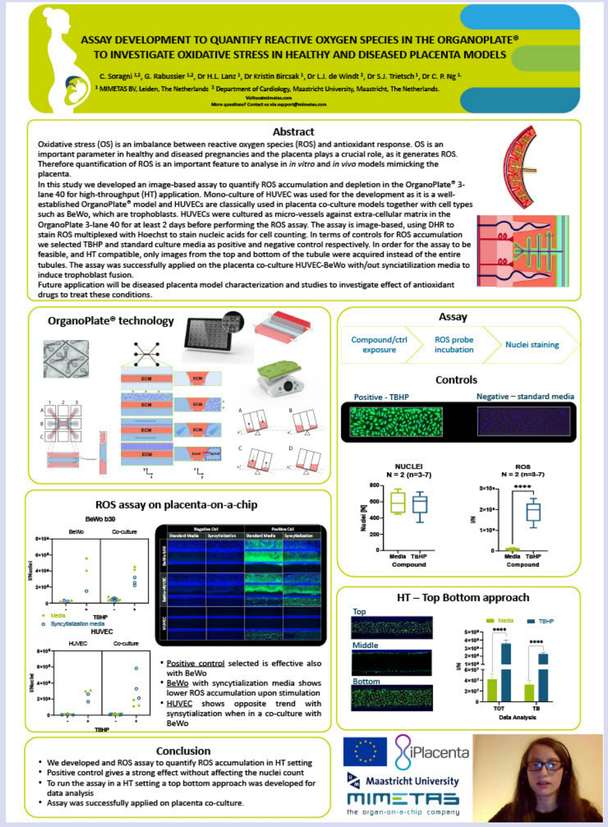


IM06 - Advanced Magnetic Resonance Imaging of the sheep placenta with and without carunclectomy

Dimitra Floury, King's College London, United Kingdom

Author(s):

Flouri, Dimitra (1,2), Darby, Jack R. T. (3), Holman, Stacey L. (3), Perumal, Sunthara R. (4), David, Anna L. (2, 5), Morrison, Janna L. (3), Melbourne, Andrew (1,2)

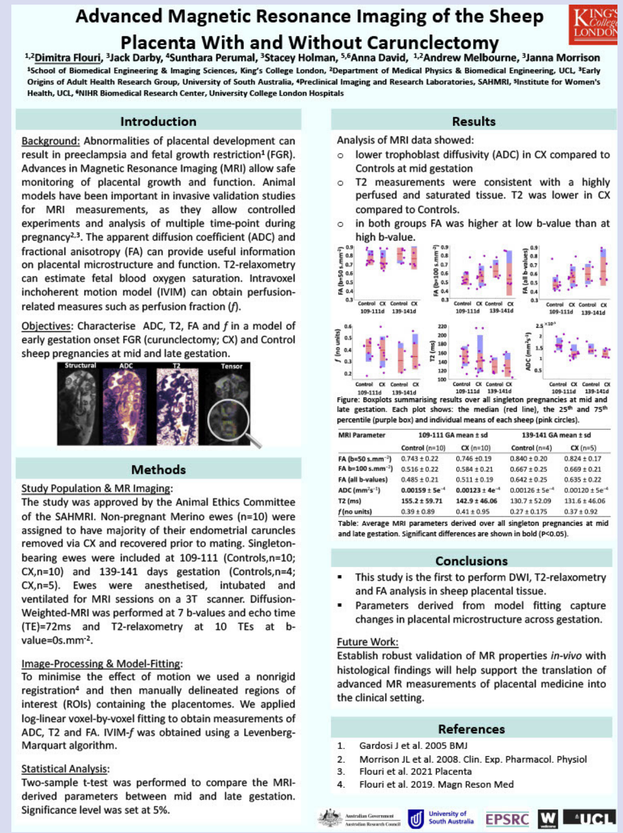
(1) King's College London, (2) University College London, (3) , University of South Australia, (4) South Australian Health & Medical Research Institute, (5) University College London Hospitals

Objectives: Animal models have been important in invasive validation studies for Magnetic Resonance Imaging (MRI) measurements, as they allow controlled experiments and analysis of multiple time-points during pregnancy. Here we characterise multiple placental MRI markers. Apparent diffusion coefficient (ADC) reflects changes in cellular diffusivity, T2 measurements are related to oxygenation levels, fractional anisotropy (FA) measures direction of diffusion and perfusion fraction (f) can be measured from intravoxel incoherent motion (IVIM) analysis.

Methods: The study was approved by the Animal Ethics Committee of the South Australian Health and Medical Research Institute. Non-pregnant Merino ewes (n=10) were assigned to have majority of their endometrial caruncles removed via carunclectomy (CX) and recovered prior to mating. Singleton-bearing ewes were included at 105-111 (Controls,n=10; CX,n=10) and 139-141 days gestation (Controls,n=4; CX,n=5). Ewes were anesthetised (induction:diazepam (0.3mg/kg), ketamine (7mg/kg); maintenance:2.5% isoflurane), intubated and ventilated with 1:5L oxygen to air gas mixture for MRI sessions on a 3T Siemens Skyra Scanner. Diffusion-Weighted MRI was performed at 7 b-values and echo time (TE)=72ms and T2-relaxometry at 10 TEs at b-value=0s.mm-2. Regions of interest containing placentomes were manually delineated. We applied log-linear voxelwise fitting to obtain measurements of ADC, T2 and FA. IVIM-f was obtained using a Levenberg-Marquart algorithm.

Results: Analysis of the MRI data showed lower trophoblast diffusivity in CX compared to Controls (ADCCX=0.00129mm2s-1, ADCcontrol=0.00162mm2s-1, P=0.007) at mid gestation. T2 measurements were consistent with a highly perfused and saturated tissue in CX and Controls (T2CX=142.9ms, T2control=155.2ms, P=0.02). In both groups, FA was significantly higher at low b-value (FAcontrol=0.74 FACX=0.75) than at high b-value (FAcontrol=0.52, FACX=0.58), suggesting that it is related to the radial blood flow component of the sheep placentome. No difference between groups was observed for f-VIM.

Conclusions: Establishing robust validation of MR properties in-vivo will support the translation of advanced placental MRI measurements into the clinic.



Advanced Magnetic Resonance Imaging of the Sheep Placenta With and Without Carunclectomy

[1,2]Dimitra Flouri, [3]Jack Darby, [4]Sunthara Perumal, [3]Stacey Holman, [5,6]Anna David, [1,2]Andrew Melbourne, [3]Janna Morrison
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Introduction

Background: Abnormalities of placental development can result in preeclampsia and fetal growth restriction[1] (FGR). Advances in Magnetic Resonance Imaging (MRI) allow safe monitoring of placental growth and function. Animal models have been important in invasive validation studies for MRI measurements, as they allow controlled experiments and analysis of multiple time-point during pregnancy[2,3]. The apparent diffusion coefficient (ADC) and fractional anisotropy (FA) can provide useful information on placental microstructure and function. T2-relaxometry can estimate fetal blood oxygen saturation. Intravoxel inchoherent motion model (IVIM) can obtain perfusion-related measures such as perfusion fraction (*f*).

Objectives: Characterise ADC, T2, FA and *f* in a model of early gestation onset FGR (curunclectomy; CX) and Control sheep pregnancies at mid and late gestation.

Methods

Study Population & MR Imaging:
The study was approved by the Animal Ethics Committee of the SAHMRI. Non-pregnant Merino ewes (n=10) were assigned to have majority of their endometrial caruncles removed via CX and recovered prior to mating. Singleton-bearing ewes were included at 109-111 (Controls,n=10; CX,n=10) and 139-141 days gestation (Controls,n=4; CX,n=5). Ewes were anesthetised, intubated and ventilated for MRI sessions on a 3T scanner. Diffusion-Weighted-MRI was performed at 7 b-values and echo time (TE)=72ms and T2-relaxometry at 10 TEs at b-value=0s.mm^{-2}.

Image-Processing & Model-Fitting:
To minimise the effect of motion we used a nonrigid registration[4] and then manually delineated regions of interest (ROIs) containing the placentomes. We applied log-linear voxel-by-voxel fitting to obtain measurements of ADC, T2 and FA. IVIM-*f* was obtained using a Levenberg-Marquart algorithm.

Statistical Analysis:
Two-sample t-test was performed to compare the MRI-derived parameters between mid and late gestation. Significance level was set at 5%.

Results

Analysis of MRI data showed:
- lower trophoblast diffusivity (ADC) in CX compared to Controls at mid gestation
- T2 measurements were consistent with a highly perfused and saturated tissue. T2 was lower in CX compared to Controls.
- in both groups FA was higher at low b-value than at high b-value.

Figure: Boxplots summarising results over all singleton pregnancies at mid and late gestation. Each plot shows: the median (red line), the 25th and 75th percentile (purple box) and individual means of each sheep (pink circles).

MRI Parameter	109-111 GA mean ± sd		139-141 GA mean ± sd	
	Control (n=10)	CX (n=10)	Control (n=4)	CX (n=5)
FA (b=50 s.mm^{-2})	0.743 ± 0.22	0.746 ±0.19	0.840 ± 0.20	0.824 ± 0.17
FA (b=100 s.mm^{-2})	0.516 ± 0.22	0.584 ± 0.21	0.667 ± 0.25	0.669 ± 0.21
FA (all b-values)	0.485 ± 0.21	0.511 ± 0.19	0.642 ± 0.25	0.635 ± 0.22
ADC (mm^{2}s^{-1})	**0.00159 ± 5e^{-4}**	**0.00123 ± 4e^{-4}**	0.00126 ± 5e^{-4}	0.00120 ± 5e^{-4}
T2 (ms)	**155.2 ± 59.71**	**142.9 ± 46.06**	130.7 ± 52.09	131.6 ± 46.06
f (no units)	0.39 ± 0.89	0.41 ± 0.95	0.27 ± 0.175	0.37 ± 0.92

Table: Average MRI parameters derived over all singleton pregnancies at mid and late gestation. Significant differences are shown in bold (P<0.05).

Conclusions

- This study is the first to perform DWI, T2-relaxometry and FA analysis in sheep placental tissue.
- Parameters derived from model fitting capture changes in placental microstructure across gestation.

Future Work:
Establish robust validation of MR properties *in-vivo* with histological findings will help support the translation of advanced MR measurements of placental medicine into the clinical setting.

References

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3. Flouri et al. 2021 Placenta
4. Flouri et al. 2019. Magn Reson Med

IM07 - In vivo Magnetic Resonance Imaging Measurement of Fetal Blood Oxygen Saturation in Normal Pregnant Sheep

Dimitra Flouri, King's College London, United Kingdom

Objectives: The lack of possibilities to safely measure fetal oxygen saturation (FO2) is still one of the major problems in obstetrics. Recent studies suggest that FO2 can be measured non-invasively using Magnetic Resonance Imaging (MRI). The aim of this study was to assess the feasibility of non-invasively determine FO2 with MRI using a sheep multi-compartment model and compare the MRI measurements against reference oxygen saturation by catheterisation.

Methods: The study was approved by the Animal Ethics Committee of South Australian Health and Medical Research Council and abided by the Australian code of practice for the care and use of animals for scientific purposes. Singleton-bearing ewes were included at 105-111 (n=10) and 139-141 (n=4) days gestation. Ewes were anesthetised (induction: diazepam (0.3mg/kg), ketamine (7mg/kg); maintenance:2.5% isoflurane), intubated and ventilated with 1:5L oxygen to air gas mixture for MRI session on 3T Siemens Skyra Scanner. Diffusion-Weighted MRI was performed at 7 b-values and echo time (TE)=72ms. Diffusion-Weighted MRI was performed at 7 b-values (b = [0, 10, 20, 30, 50, 70, 100, 200, 300, 500, 600] s.mm-2) with echo time (TE)=72ms and T2-relaxometry at 10 TE = [81, 90, 96, 120, 150, 180, 210, 240, 270, 300]ms at b-value=0 s.mm-2. The dependence of blood oxygenation on the effective T2-relaxation time is characterised by the Luz-Meiboom model.

Results: The linear relationship between MRI FO2 estimation with Luz-Meiboom and reference measurements was significant (y=1.44x-72.5, R2 = 0.88, P=0.0004). Bland-Altman analysis showed a mean difference of 28.7% where the limits of agreement ranged from 21.7% to 35.7%.

Conclusion: We presented in-vivo validation of a multi-compartment model of placental MRI to estimate FO2 in uncomplicated sheep pregnancy at mid- and late-gestation. Future work will estimate FO2 in sheep model of fetal growth restriction.

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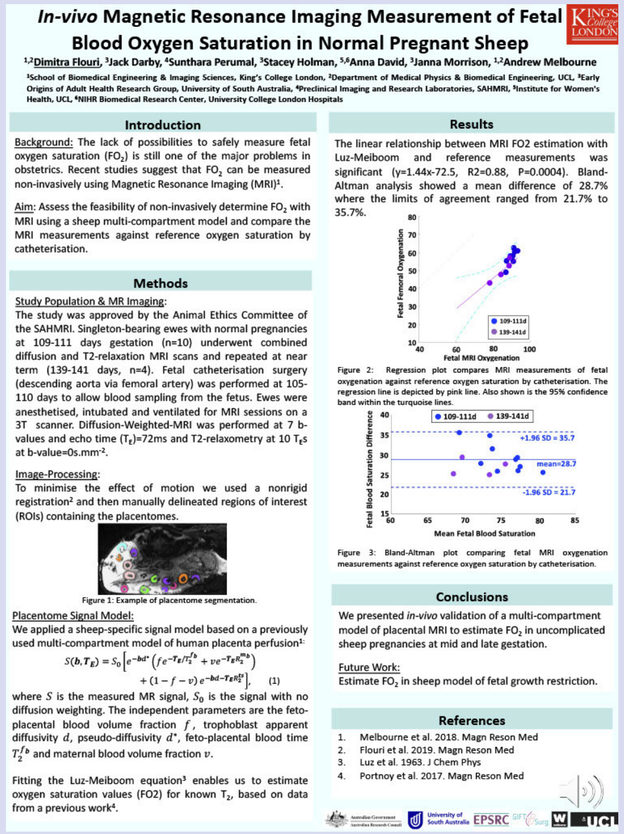

***In-vivo* Magnetic Resonance Imaging Measurement of Fetal Blood Oxygen Saturation in Normal Pregnant Sheep**

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Introduction

Background: The lack of possibilities to safely measure fetal oxygen saturation (FO_2) is still one of the major problems in obstetrics. Recent studies suggest that FO_2 can be measured non-invasively using Magnetic Resonance Imaging (MRI)[1].

Aim: Assess the feasibility of non-invasively determine FO_2 with MRI using a sheep multi-compartment model and compare the MRI measurements against reference oxygen saturation by catheterisation.

Methods

Study Population & MR Imaging:
The study was approved by the Animal Ethics Committee of the SAHMRI. Singleton-bearing ewes with normal pregnancies at 109-111 days gestation (n=10) underwent combined diffusion and T2-relaxation MRI scans and repeated at near term (139-141 days, n=4). Fetal catheterisation surgery (descending aorta via femoral artery) was performed at 105-110 days to allow blood sampling from the fetus. Ewes were anesthetised, intubated and ventilated for MRI sessions on a 3T scanner. Diffusion-Weighted-MRI was performed at 7 b-values and echo time (T_E)=72ms and T2-relaxometry at 10 T_Es at b-value=0s.mm^{-2}.

Image-Processing:
To minimise the effect of motion we used a nonrigid registration[2] and then manually delineated regions of interest (ROIs) containing the placentomes.

Figure 1: Example of placentome segmentation.

Placentome Signal Model:
We applied a sheep-specific signal model based on a previously used multi-compartment model of human placenta perfusion[1]:

$$S(\boldsymbol{b},T_E) = S_0\left[e^{-bd^*}\left(fe^{-T_E/T_2^{fb}} + ve^{-T_E R_2^{eff}}\right) + (1-f-v)\,e^{-bd-T_E R_2^{pl}}\right], \quad (1)$$

where S is the measured MR signal, S_0 is the signal with no diffusion weighting. The independent parameters are the feto-placental blood volume fraction f, trophoblast apparent diffusivity d, pseudo-diffusivity d^*, feto-placental blood time T_2^{fb} and maternal blood volume fraction v.

Fitting the Luz-Meiboom equation[3] enables us to estimate oxygen saturation values (FO2) for known T_2, based on data from a previous work[4].

Results

The linear relationship between MRI FO2 estimation with Luz-Meiboom and reference measurements was significant (y=1.44x-72.5, R2=0.88, P=0.0004). Bland-Altman analysis showed a mean difference of 28.7% where the limits of agreement ranged from 21.7% to 35.7%.

Figure 2: Regression plot compares MRI measurements of fetal oxygenation against reference oxygen saturation by catheterisation. The regression line is depicted by pink line. Also shown is the 95% confidence band within the turquoise lines.

Figure 3: Bland-Altman plot comparing fetal MRI oxygenation measurements against reference oxygen saturation by catheterisation.

Conclusions

We presented *in-vivo* validation of a multi-compartment model of placental MRI to estimate FO_2 in uncomplicated sheep pregnancies at mid and late gestation.

Future Work:
Estimate FO_2 in sheep model of fetal growth restriction.

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4. Portnoy et al. 2017. Magn Reson Med

IM08 - Endothelial assessment and endoscopic blood flow visualisation in a murine model of pregnancy

Lukas Markwalder, University of Dundee, United Kingdom

Author(s):

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Endothelial dysfunction is important in the pathophysiology of preeclampsia often preceding the onset of the clinical disease, suggesting a major contributor to placenta dysfunction. Animal models offer unique opportunities to study both placental insufficiency and maternal endothelial function during pregnancy, though there is a need new tools for validation. We developed an endoscopic probe for real-time perfusion imaging of the placenta and miniaturisation of iontophoresis hardware to assess maternal skin microvascular function in mouse models.

Methods: A minimally invasive probe featuring an endoscopic Laser Speckle Flow Imaging (LSFI) system to visualise real-time perfusion in organs was developed. LSFI is a real-time wide-field scanning technique, used clinically for assessment of skin microvascular function.
For endothelial assessment the transdermal drug delivery method iontophoresis is an established technique. With the help of 3d- printing, the use of new materials, a flexible drug delivery chamber has been developed and validated in a vasculature model of C57Bl/6 mice.

Results: Our LSFI-device combines a miniature camera with a fibre-coupled near-infrared laser in the tip of an endoscopic probe. A 1mm diameter camera successfully visualized blood flow activity of phantoms and of human finger.
Ion-mini is a small, flexible chamber attached to the skin which allows for fast measurements, induces low strain on the tissue and allows for cost-effective application.

Conclusion: The hardware for LSFI has been successfully miniaturized and tested. The technique could provide wider clinical impact to visualize blood vessels and organ perfusion in real-time during surgery without the need for different imaging modalities or injection of dyes.
New iontophoresis hardware has been developed for the use in rodents. This technology will provide a platform to assess animal models of pregnancy and compare against clinical parameters to validate model characteristics.

MH09 - Phenotypic analysis of human choriodecidua in relation with parturition

Léa Chicoisne, Institut Cochin, U1016, INSERM, France

Both preterm and post-term birth are associated with an increased risk of adverse pregnancy events. Chorion, the outermost leaf of fetal membranes, is fused with decidua, providing a large surface of maternal-fetal interaction. Choriodecidua presents the largest gene expression variability in the context of labor. To examine cell state changes, single-nucleus RNA-sequencing (snRNA-seq) approach was performed as it achieves comparable gene detection to single-cell RNA-seq, with reduction of cell dissociation bias and stress response. Our aim is to gain knowledge in the cell populations present in the choriodecidua at term pregnancy, before and after the onset of labor. Choriodecidua samples were obtained at term pregnancy from 7 non-laboring women delivered by caesarean section and from 7 women who delivered spontaneously after labor. Nuclei are isolated from frozen tissues using dounce homogenization in lysis buffer. Further encapsulation and snRNA-seq were performed using the 10X Genomics technology. Data computational analyses were processed using 10X Cell Ranger software and Seurat v.4 pipelines. From 62,752 transcriptomic profiles, we identified the expected cell populations, ranked from the most to the less abundant: trophoblasts, stromal cells, immune cells -myeloid and lymphoid subtypes-, endothelial cells -lymphatic and vascular- and epithelial endometrial cells. For the first time, we can define five distinct subsets of trophoblasts in chorion. Mesenchymal cells comprise decidual stromal cells and a new population of fibroblasts, poorly characterized. The largest number of deregulated genes with labor was observed in these fibroblasts, followed in a less extent by trophoblasts. As expected, an upregulation of inflammatory genes was associated with labor in all the different cell populations. Our preliminary results suggest a broader degree of cellular heterogeneity in trophoblasts and stromal cells, not reported before. Our unbiased snRNA-seq approach holds the promise of a detailed molecular characterization of the distinct cell types and their changes with labor.

Author(s):

Chicoisne, Léa (1), Karunanithy, Vaarany (1), Bertholle, Céline (1), Izac, Brigitte (1), Letourneur, Franck (1), Vaiman, Daniel (1), Miralles, Francisco (1), Andrieu, Muriel (1), Méhats, Céline (1)

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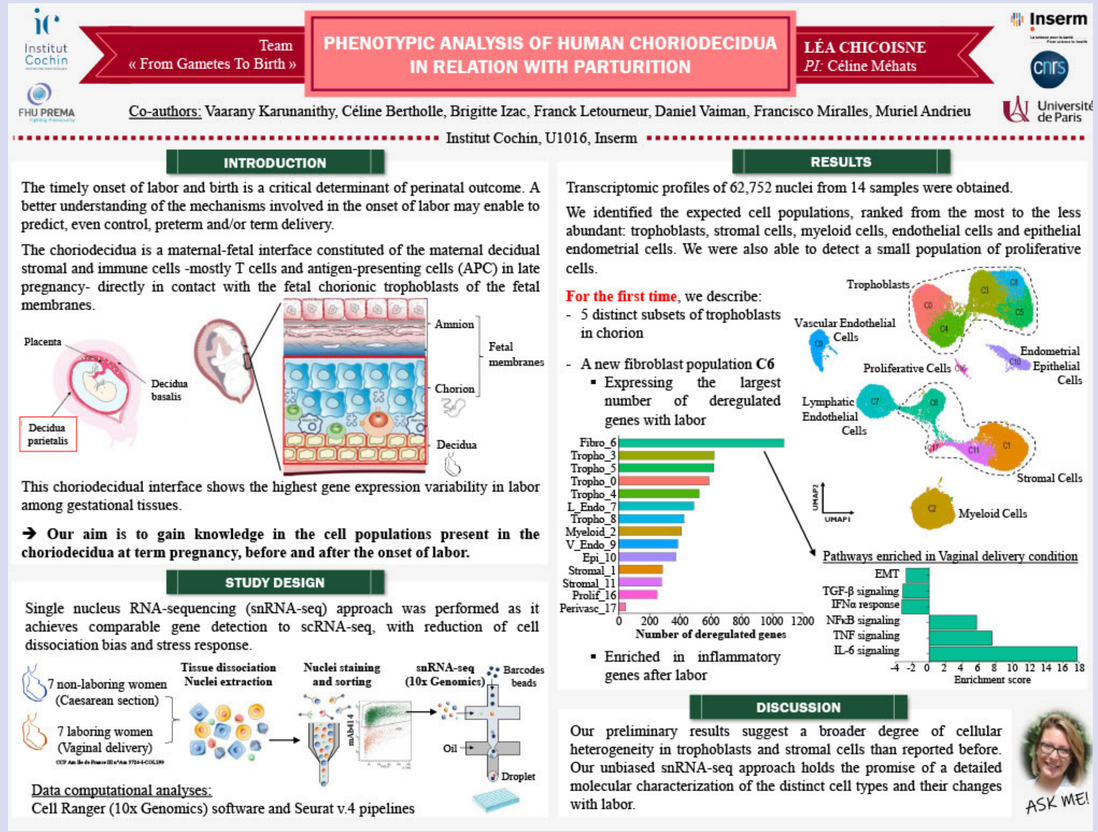
Best Poster



MH10 - Perinatal Outcomes in Small for Gestational Age in Twin Pregnancies: Twins vs. Singleton Charts

Carolina Di Fabrizio, St George's University Hospital, United Kingdom

Twin pregnancies are commonly assessed using singleton growth and birth weight reference charts. This practice has led to a significant number of twins labelled as small for gestational age (SGA), causing unnecessary interventions and increased risk of iatrogenic preterm birth. However, the use of twin-specific charts remains controversial. This study aims to assess whether twin-specific estimated fetal weight (EFW) and birth weight (BW) charts are more predictive of adverse outcomes compared to singleton charts. Centiles of EFW and BW were calculated using previously published singleton and twin charts. Categorical data were compared using Chi-square or McNemar tests.

The study included 1740 twin pregnancies, with perinatal adverse outcomes recorded: perinatal death, preterm birth <34 weeks, hypertensive disorders of pregnancy (HDP) and admissions to the neonatal unit (NNU). Twin-specific charts identified prenatally and postnatally a smaller proportion of infants as SGA compared to singleton charts. However, twin charts showed a higher percentage of adverse neonatal outcomes in SGA infants than singleton charts. There was no significant association between HDP and SGA using the singleton charts. In SGA infants, according to the twin charts, the incidence of abnormal umbilical artery Doppler was significantly more common than in SGA using the singleton chart. In conclusion, singleton charts misclassify a large number of twins as at risk of fetal growth restriction. The evidence suggests that the following twin-specific charts could reduce unnecessary medical interventions prenatally and postnatally.

Author(s):

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MH12 - Primary human trophoblast expression of renin-angiotensin system components in response to hyperglycaemia

Alyssa Jade Lochrin, University of Newcastle, Australia

Gestational diabetes mellitus (GDM) affects approximately 10% of pregnancies worldwide, and is associated with immediate and long-term health complications for mothers and their offspring. The placenta is important in GDM as placental factors induce maternal insulin resistance and hyperglycaemia, which characterise GDM pathology.

The placental renin-angiotensin system (RAS) plays an important role in placental development, however it is yet to be characterised in GDM. The RAS is upregulated in non-pregnant type II diabetes, and is involved in hyperglycaemia-induced tissue injury e.g. diabetic retinopathy and nephropathy. We propose that the placental RAS is dysregulated in women with GDM in response to hyperglycaemia, and that this alters placental structure and function.

Primary human trophoblast cells were isolated from the placentae of women with uncomplicated pregnancies delivering by caesarean section ≥37weeks gestation (n=5). Trophoblast cells were cultured in normoglycaemic media [5mM glucose] for the initial 24h and subsequently cultured in normoglycaemic or hyperglycaemic media [25mM glucose] for an additional 48h (n=3 replicates per group per placenta). The mRNA expression of RAS components was measured in cell lysates by qPCR.

Culture in hyperglycaemic conditions significantly reduced the mRNA expression of 2 RAS genes, angiotensin converting enzyme (ACE; p=0.008) and angiotensin II type 1 receptor (p=0.035), and possibly reduced the expression of ACE2 (p=0.055). Work is currently underway to quantify placental RAS components at the protein level and determine if hyperglycaemia alters their abundance and/or activity.

These data suggest that placental RAS expression in primary trophoblast cells is sensitive to circulating glucose levels. Further research is now required to determine whether these changes in placental RAS expression alter placental nutrient transport and/or contribute to the inflammation and fibrosis that has been reported in the placentae of GDM pregnancies.

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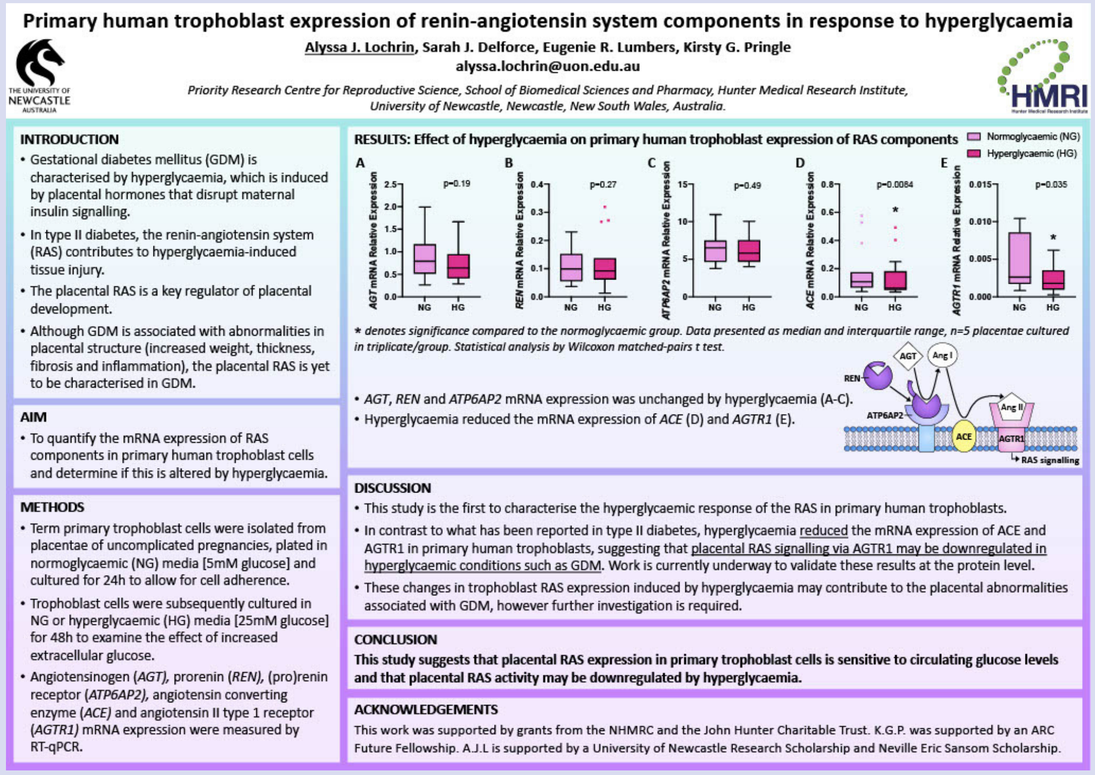

PG13 - Expression Quantitative Trait Loci identified in Human Placenta

Clara Apicella, Institut Cochin, INSERM U1016, France

The key role of placental dysfunction in the aetiology of numerous pregnancy pathologies, such as preeclampsia (PE) and intra-uterine growth restriction (IUGR) is now well established. Nonetheless, molecular mechanisms that orchestrate placental function and that are behind disease development are still incompletely understood. In this study, we seek to identify expression Quantitative Trait Loci (eQTLs) that describe the correlation between a change in sequence of a specific locus on the genome (eSNP) and levels of expression of a target gene (eGene). We perform this analysis in healthy and pathological placentas (17 control, 6 PE, 3 PE+IUGR and 10 IUGR) by combining transcriptome (microarray ClariomD, Affymetrix) and genotype (array Infinium OmniExpress, Illumina) for each sample. 10559 transcripts and 438590 SNPs (MAF> 0.15) were analysed with the Matrix eQTL software to perform multivariate linear regression for each gene-SNP pair. The multivariate models included 14 covariates (placental sex, gestational age, disease group, experimental batch and genotype principal components). Preliminary results identified 177 cis-QTLs, with a False Discovery Rate < 0.05, corresponding to 43 unique eGenes; 62.8% of these had been previously identified as associated to placental eQTLs, including the well described ERAP1 and ERAP2 genes, corroborating the robustness of our study design. Additionally, we describe 16 novel eGenes. Among these, TP53BP1 and ASCC3 are involved in DNA repair, and the latter in enhancing the NF-kappa-B-mediated inflammatory response, NT5E is necessary for correct lymphocyte differentiation, ACSS3 in the synthesis of Acetyl-CoA. Combining gene expression alterations with genetic variants opens the possibility to gain insights into key molecular hierarchies governing complex tissues. Our work allowed to validate previous findings and to identify novel eQTLs that will contribute to clarify the functional landscape of the human placenta.

Author(s):

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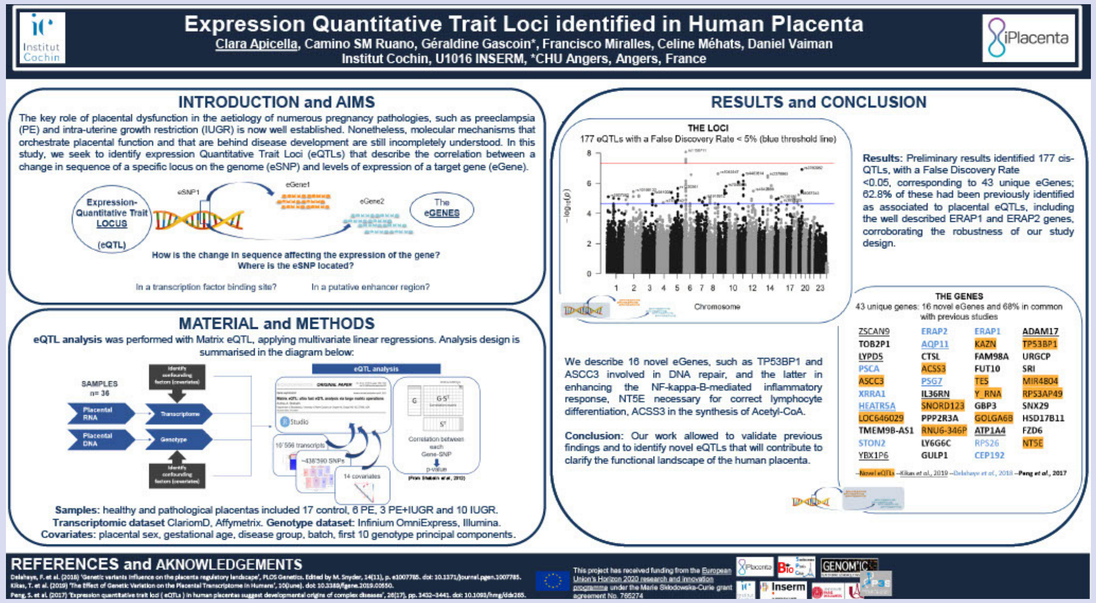

PG14 - DNA methylation in Placental diseases

Camino Ruano San Martin, Institut Cochin (160 INSERM), France

Objective: Preeclampsia (PE) and Intrauterine Growth Restriction (IUGR) are the two most common placental pathologies. DNA Methylation differences have already been reported to be altered in diseased placentas. However, biological and experimental factors can influence the methylation level, leading to an increased risk of false positive findings not directly related with the disease. Herein, we aimed to identify CpGs, linked with the disease status with minimal influence of other clinical (such as gestational age, baby's sex, maternal age) and experimental covariates.

Materials and Methods: DNA Methylation pattern of 31 placentas was obtained using EPIC Illumina methylation Array. These placentas come from two different sample sets; Sample set 1 formed by 6 Early Onset of PE (EOPE), 3 PE+IUGR and 6 Ctrl and Sample set 2 composed by 6 Late Onset of PE (LOPE), 2 IUGR and 8 Ctrl. Gestational age, ethnicity, cell composition, and infant sex were predicted using the Planet R package. Minfi and Champ R package were used for quality check and normalization. The M-values of 741,184 CpGs were linearly regressed towards all individual covariates. These analyses were performed in each individual set and both datasets together. Gene Ontology analysis was performed using GOnet online resource.

Results: The removal of all the CpGs influenced by covariates leads to a remarkable reduction of the number of CpGs correlated only with disease: 6731 in Sample Set 1, 2050 in Sample Set 2 and 5697 in the combination of the two sets. No CpG was found to be in common between the different subsets. However, when considering the gene location of these CpGs, we observed that 137 genes are shared. The most enriched biological processes are Neurogenesis, Nervous system development and multicellular organism processes, suggesting a role in membrane projection.

Conclusion: These preliminary analyses led to identifying genes associated with different DNA methylation patterns in diseased placentas, despite the cohort's limited size. Differentially methylation regions will be further studied.

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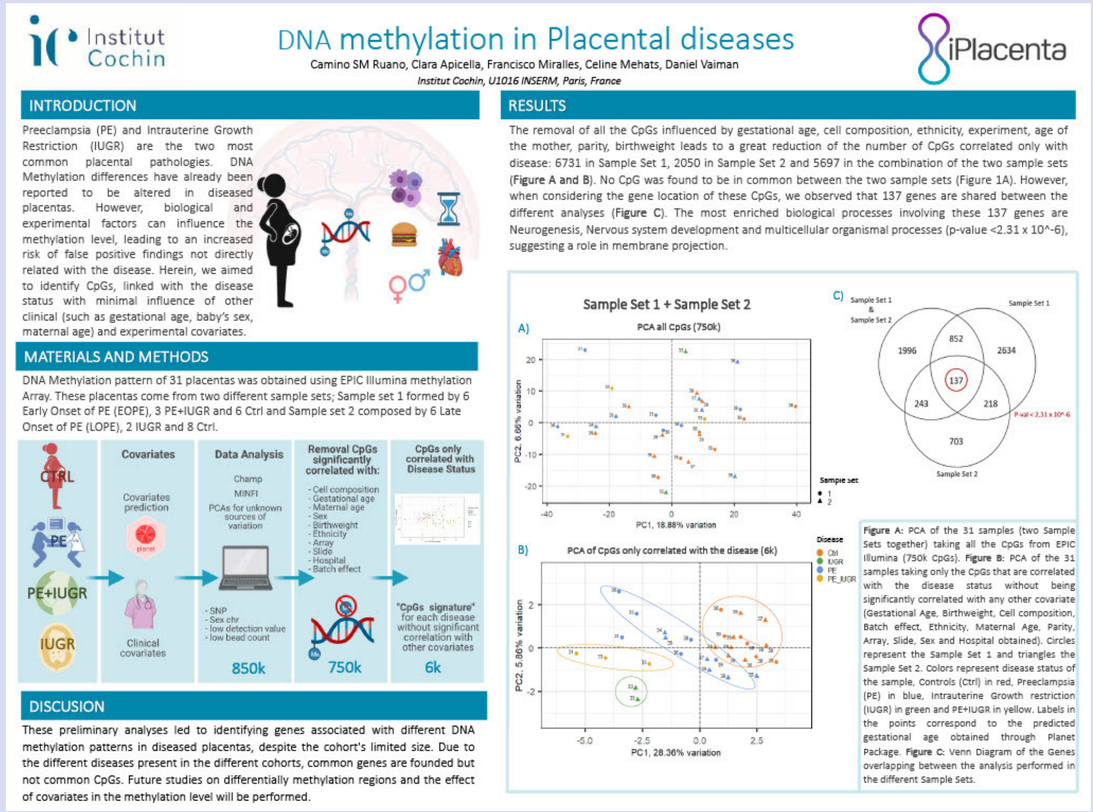

PG15 - Developmental changes of human cardiomyocyte differentiation at single cell resolution

Jana-Ch. Hegenbarth, Maastricht University, The Netherlands

Background: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have emerged as a promising experimental tool for translational cardiac research and drug development. However, uncertainty surrounding their maturation status raise questions about their value as bona fide human adult cardiomyocytes. Accordingly, our goal is to identify all developmental changes in the transcriptome that hiPSC-CMs undergo from pluripotency to 3D-engineered heart muscle at single cell resolution.

Methods: We cultured two distinct Caucasian hiPSC clones, differentiated them to beating CMs, casted them into 3D-engineered heart muscle (EHM) and dissociated cells at decisive developmental time points (day 0, 2D hiPSCs; day 3, 2D mesodermal precursors; day 10, 2D early hiPSC-CMs; day 25, 2D hiPSC-CMs and 3D EHM) and subjected samples to single-cell RNA-seq and bulk RNA-seq technologies during hiPSC cardiac differentiation.

Results: A total of 62.398 cells were sequenced across all 5 developmental stages. Our results highlight the cellular heterogeneity of cell types within their developmental stages indicative of diverse developmental origins. Furthermore, we assessed the differences between the male and female clone. These results suggest no direct impact of differentiation susceptibility, but differences in cellular composition at each stage. Even though hiPSC-derived CMs are a powerful tool to study cardiac cells, certain criteria including their maturity still remain a limitation. In our results, we were able to show the transcriptional changes during CM differentiation on a longitudinal study including the more mature EHMs on a single cell basis. Furthermore, we intend to use an machine learning algorithm to fully grasp their maturity level when comparing it to the developmental stages of the human heart.

Author(s):

Hegenbarth, Jana-Ch. (1), De Majo, Federica (1), Shahvar, Dena Esfandyari (2), Engelhardt, Stefan (2), Tiburcy, Malte (3), Zimmermann, Wolfram H. (3), Stoll, Monika (4), De Windt, Leon J. (1)
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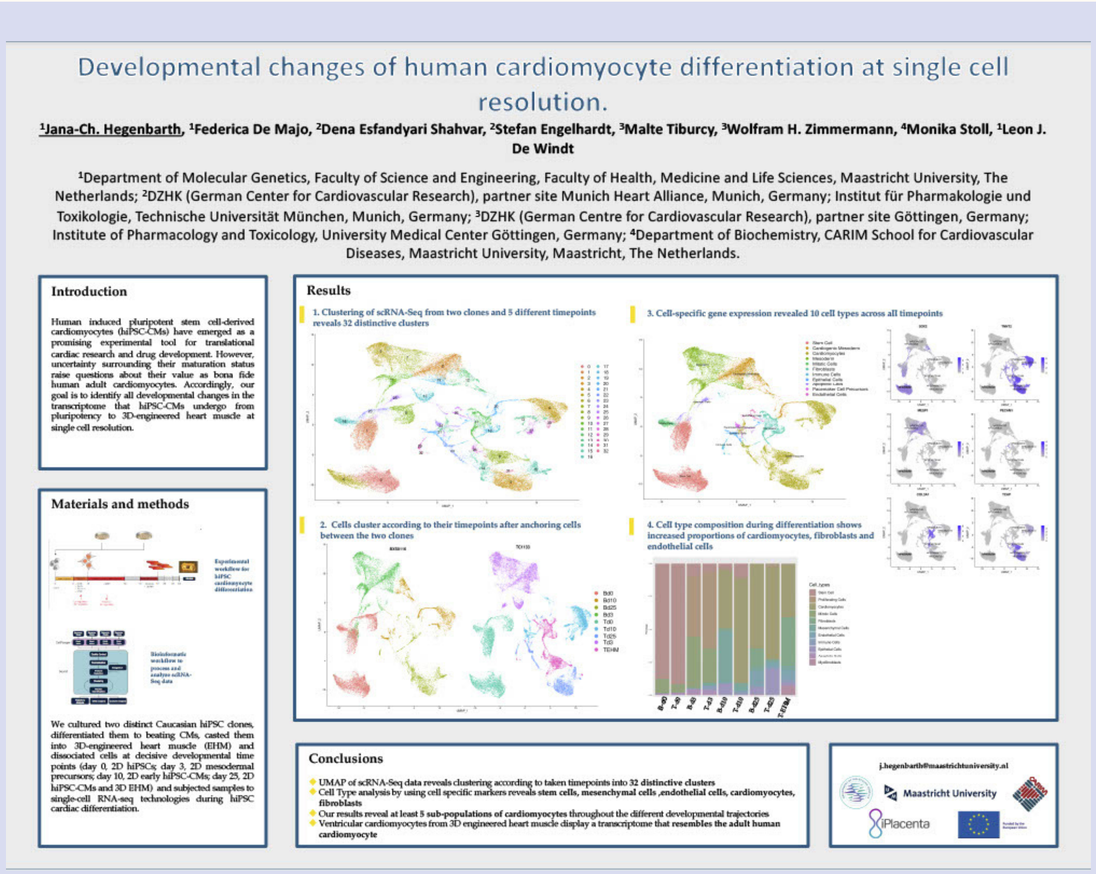

PG16 - MicroRNA-132 is overexpressed in fetuses with late-onset fetal growth restriction

Gabriela Loscalzo, Hospital Universitario y Politécnico La Fe, Spain

Objective: to evaluate the expression of micro RNA 132 (miR-132) in normal fetuses and in fetuses with late-onset growth restriction (FGR). Material and methods: In a prospective study, 48 fetuses (24 with late-onset FGR and 24 with normal growth) were scanned with Doppler ultrasound after 34 weeks and followed until birth. Subsequently, blood samples from the umbilical cord were collected to evaluate the expression of miR-132 by means of Real-time qPCR, determining the existence of normality cut-offs and studying the diagnostic ability for the diagnosis of FGR and adverse perinatal outcome (APO).

Result: In comparison with normal fetuses, late-onset FGR fetuses expressed up-regulation of miR-132 (2-ddCt = 33.94 ± 45.04 versus 2.88 ± 9.32, P<0.001). This overexpression showed an AUC of 0.82 (P<0.001) for the diagnosis of FGR and improved the prediction accuracy (AUC) of CPR MoM for APO (from 0.73, P<0.05 up to 0.80, P<0.01) and FGR (from 0.89 up to 0.99, both P<0.0001).
The majority of miR-132 overexpression and APO cases were concentrated within the lowest BW centiles. The use of 5 as cut-off value for miR-132 expression showed a sensitivity and specificity of 50% and 96% for the diagnosis of FGR, while the sensitivity and specificity for the diagnosis of APO was 46% and 80%.

Conclusions: fetuses with late-onset FGR up-regulate miR-132. Future studies are needed to investigate the role of miR-132 expression in the diagnosis and management of late-onset FGR.

Author(s):

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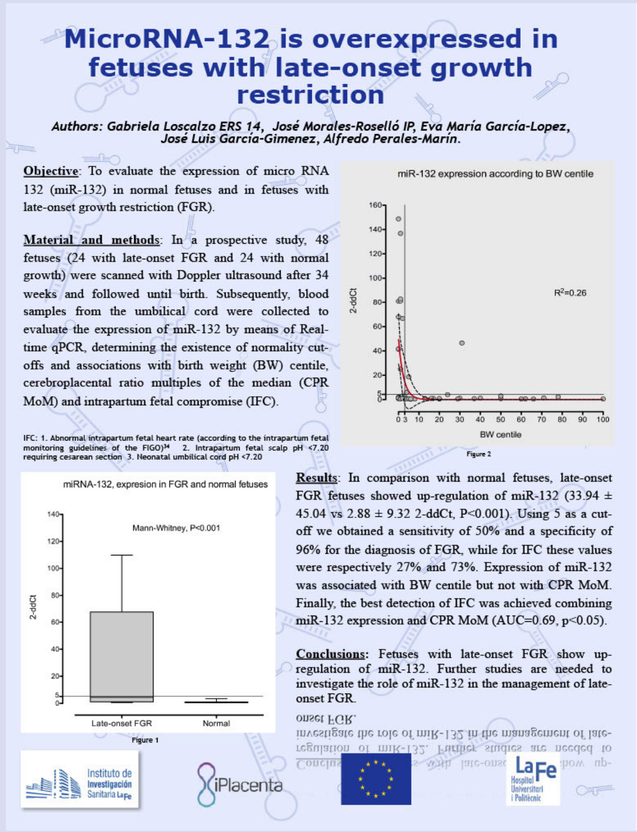

PG17 - Towards a “Disease Map” for Placental Dysfunctions

Julia Scheel, Universität Rostock, Germany

Objective: The placenta is a highly vascularized and complex organ. Placental defects are believed to be the leading cause of major pregnancy-related complications, such as preeclampsia, intrauterine growth restriction, and stillbirth. Nevertheless, it is one of the least studied human organs. Computational approaches have been used for various aspects of research and development and are particularly useful in complex disease research. Data produced by computational approaches remain largely poorly integrated and inaccessible to the public. Here, we identify gaps, in which computational approaches have not been exploited, in the context of placenta research. We further make recommendations for an integrative workflow for heterogeneous data, in the disease map NaviCenta.

Methods: We identified important processes of placental development based on primary literature. The identified processes were then integrated into the placenta specific disease map, which consists of three layers (i) disease context, (ii) process layer, and (iii) molecular interaction map. Processes were translated into machine and human readable molecular interaction networks in CellDesigner,integrated into the process layer, and uploaded for further (public) use on Minerva (www.sbi.uni-rostock.de/minerva/index.xhtml?id=NaviCenta).

Results: There are a plethora of placenta associated models. Given it’s complex nature, most models are specific to certain processes and data types, and thus difficult to combine. The disease map approach, on the other hand, has been applied specifically for heterogeneous data integration. The NaviCenta, the disease map specific to placental dysfunction currently contains 10 process sub maps and is being further developed.

Conclusion: Computational models are useful tools to study specific aspects of the placenta and placenta related diseases. The impact of isolated models can be increased by combined approaches. Systems biology approaches have been used on some datasets, however, the disease map approach has not been exploited yet in this context.

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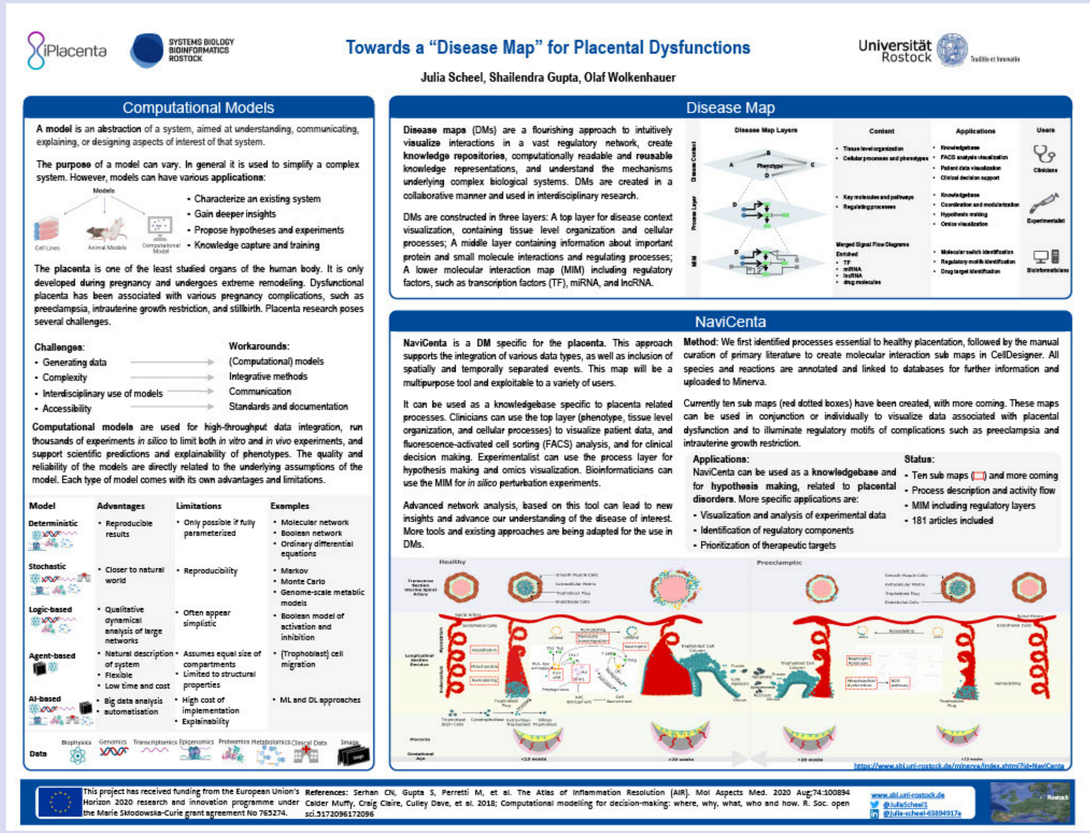
Best Poster, Runner-up



PG18 - Maternal serum extracellular vesicle microRNAs alter human placental proteome and function in pregnancies complicated by gestational diabetes mellitus and fetal overgrowth

Rachel C. Quilang, University of Leeds, United Kingdom

Author(s):

Quilang, Rachel C. (1), Cartland, Sarah J. (1), Godhinho, Emmanuelle (1), Timms, Kate (1), Scott, Eleanor M. (1), Forbes, Karen (1)

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Background: Large-for-gestational-age (LGA) infants have increased risk of developing cardiometabolic complications. LGA is elevated in pregnancies affected by gestational diabetes (GDM). The mechanisms responsible are unclear, but are associated with altered placental development/function. We have recently reported that 9 specific miRNAs contained within extracellular vesicles (EVs) are altered in maternal circulation prior to the onset of LGA in GDM. Maternal EVs can be internalised into the placenta. We propose that maternal EV-miRNAs contribute to LGA by influencing placental development/function.

Methods: Term placental tissue was collected from GDM pregnancies with appropriate-for-gestational-age (AGA; n=14), or LGA infants (n=10). miRNAs altered in maternal serum EVs in GDM-LGA, and their primary transcripts, were measured by QPCR. For miRNA overexpression, normal term human placentas were collected, and villous explants cultured for 4 days prior to exposure to miRNA mimics or non-targeting (NT) control (100nM) for 72 hours. QPCR confirmed overexpression (n=4) and TMT mass spectrometry assessed placental proteome (n=4). Proteins were considered differentially expressed (DEP) when $P<0.05$; $-0.41 \geq \log2$ fold-change ≥ 0.58. Data was validated by Western blotting (n=4). Functional enrichment analysis (FEA) of DEP was performed using DAVID, STRING and Ingenuity Pathway Analysis.

Results: All 9 EV miRNAs were detected in placenta. miR-375 (4.74-fold; $p<0.01$) and miR-200c-3p (2.78-fold; $p<0.05$) were increased in GDM-LGA vs GDM-AGA. Mature miR-375 and miR-200c-3p were present in placental tissue, not primary transcript, suggesting that these mature miRNAs are being delivered exogenously, likely via maternal EVs. miR-375 and miR-200c-3p overexpression altered the placental proteome. FEA revealed potential roles for miR-200c in inflammation, mitochondrial function and angiogenesis, and for miR-375 in cellular growth, lipid metabolism and endocrine disorders.

Conclusions: These data demonstrate that miR-200c-3p and miR-375 are elevated in maternal serum EVs and placenta with concurrent GDM and LGA. These EV-miRNAs may potentially be contributing to LGA by influencing placental events.

Best Poster, Runner-up

PG19 - Extracellular vesicle bound myomiRs in circulation are associated with large-for-gestational-age outcomes in pregnancies complicated by gestational diabetes

Margeurite Kennedy, University of Leeds, United Kingdom

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Introduction: Gestational diabetes (GDM) affects 1 in 6 pregnancies globally, increasing babies' risk of pathological fetal growth – particularly being born large-for-gestational-age (LGA). This can cause birth injuries and predisposes offspring to developing cardio-metabolic disease in adulthood. The cause of LGA is unclear, although it is associated with abnormal placental morphology. microRNAs (miRNAs) regulate placental development; they are synthesised within cells but can be released into circulation inside extracellular vesicles (EVs), which can be transported into target cells and tissues to influence cellular processes. We aimed to characterise circulating EVs in pregnancies complicated by GDM-LGA and determine if EV-derived miRNAs have the potential to influence placental development.

Methods: Maternal serum and plasma samples were collected from women with pregnancies complicated by GDM at 24-32 weeks gestation. Placental tissue was collected at delivery, babies were appropriately-grown-for-gestational-age (AGA) or LGA. Serum and plasma EVs were isolated by size exclusion chromatography and characterised by electron microscopy (morphology), nanoparticle tracking analysis (size/concentration; NTA), and Western blotting for EV-enriched proteins. miRNA QPCR arrays were performed on EVs. miRNAs were quantified in placental tissue via QPCR.

Results: EM and Western blotting confirmed isolation of EVs and NTA revealed no significant difference in size/concentration in GDM-LGA pregnancies (n=7) compared to GDM-AGA (n=13; $p>0.05$). Several EV miRNAs were altered in maternal circulation in GDM-LGA compared to GDM-AGA (n=7/group; >2-fold-change; $p<0.05$), including four skeletal muscle-specific 'myomiRs': miR-1-3p, miR-133a-3p, miR-133b, and miR-499a-3p (all increased). All four myomiRs were present in placenta but only miR-1-3p and miR-133a-3p were significantly altered in GDM-LGA (n=15) compared to GDM-AGA (n=12; $p<0.05$).

Conclusions: EV-encompassed myomiRs may have predictive value for LGA offspring in cases of GDM. miR-1-3p and miR-133a-3p regulate vascular development in other systems so they may contribute to aberrant fetal growth by altering placental vascularisation. However, further research is needed to confirm this.

PG20 - Changes induced by high glucose exposure in the proteomic profile of placental cells and derived extracellular vesicles

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Objectives: This study aims to evaluate the effect of a hyperglycemia mimetic condition on the proteomic profile of a model of placental cells and derived extracellular vesicles (EVs).

Methods: BeWo cells were cultured under 5 mM D-glucose (Control) or 25 mM glucose (HG) (n=3). After 48 hours of exposure, cell-conditioned media and lysate were collected. 3 different populations of EVs were isolated based on centrifugation speed (2.ooog, 10.000g and 100.000g). Protein content was quantified in EVs and lysate. Tryptic peptides were obtained from each sample and used in a combination of sequential window acquisition of all theoretical fragment ion spectra mass spectrometry (SWATH) and information-dependent acquisition (IDA) protocol. A comparison analysis was performed between control and high glucose, and between groups.

Results: Analysis of the protein content in BeWo cells showed that high glucose treatment significantly changed the expression of 177 proteins. Changes were also observed in EVs isolated in the 2.000g step. In this population of EVs, 281 proteins were significantly affected by high glucose. In the case of EVs from 10.000g pellet, 238 proteins changed expression because of the treatment. EVs isolated by ultracentrifugation at 100.000g showed differences in the expression of 179 proteins. Analysis of the proteins aforementioned identified 18 proteins which are significantly affected by high glucose but also expressed in cells and the 3 populations of EVs. Conversely, 107 proteins were only identified in BeWo cells, 195 proteins observed only in 2.000g pellet, 160 proteins only in 10.000g pellet and 119 proteins only in EVs from 100.000g pellet.

Conclusion: High D-glucose exposure modifies the proteomic profile of a placental model. The identification of proteins uniquely expressed in a specific set of EVs can potentially provide future biomarkers for pathologies involving alteration in the levels of glucose in maternal circulation.

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