Placental and Fetal Brain Interfaces: How Effective Are They in Protecting Against Drugs and Toxins?
Royal Veterinary College, London, UK | 15 – 16 April 2025

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C01

Prenatal dexamethasone exposure impacts gliovascular interface development more in female offspring than in males

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Fetal exposure to elevated levels of glucocorticoids (GCs) during pregnancy, whether due to maternal stress or the use of antenatal corticosteroid therapy (often prescribed to women at risk of preterm birth), can disrupt normal brain development and increase susceptibility to psychiatric disorders later in life. In male offspring, this has been associated with behaviors relevant to schizophrenia, while in females, it is linked to depression-like symptoms. Previous studies have shown that prenatal exposure to synthetic GCs, such as dexamethasone (DEX), impairs neurogenesis and dendritic spine development, however the impact of prenatal stress on the gliovascular interface remains poorly understood. This interface, the interaction between astrocytes and blood vessels, is critical for brain development and function. Our research aims to explore how prenatal GC exposure impacts gliovascular maturation and potential sex differences across brain regions.

To investigate the effects of prenatal stress on this interface, we administered either saline (control, n=5) or 50 µg/kg of DEX (n=5) subcutaneously to pregnant mice from gestational days 16 to 18. At postnatal day 14 (P14), we collected brains from male and female offspring and label astrocyte endfoot processes using aquaporin-4 (AQP4), a water channel protein that plays a crucial role in maintaining brain homeostasis by regulating water and ion balance. Brain microvessels were labelled with lectin (LEC). Brain slices were then imaged using a slide scanner, confocal and high-resolution Stimulated Emission Depletion (STED) microscopes. Protein expression and vessel tracing were analyzed with Fiji software. A two-way ANOVA ($p<0.05$) tested the effects of treatment (CTR vs DEX) and sex (female vs male).

Physiologically, we observed sex-specific differences in regional brain volume, with males displaying a wider somatosensory cortex and cerebellum at P14. However, there were no significant differences between sexes in AQP4 expression or vascular endfoot coverage. When examining the vasculature, the only notable difference was in the cerebellum, where males had shorter vessel segments compared to females. Our findings also highlighted a more pronounced impact of prenatal stress on female offspring. In the hippocampus of DEX-treated females, we detected an increased expression of AQP4 within astrocytic endfeet, suggesting alterations in astrocyte function due to prenatal stress. In the prefrontal cortex of DEX-treated females, AQP4 showed both increased expression and greater co-localization with blood vessels compared to controls, indicating a shift in localization. Interestingly, this shift occurred without changes in vascular density or segment length. Furthermore, we observed an increased frequency of vessel tortuosity in nearly all brain regions of female offspring, a phenomenon that was not seen in males.

In conclusion, this study underscores the importance of understanding how prenatal GC exposure, a known consequence of maternal stress and medical treatments, disrupts the gliovascular interface, with particularly pronounced effects on female offspring. These alterations could have lasting implications for brain function and may contribute to sex-specific vulnerabilities to psychiatric disorders.

Animal procedures were approved by the Animal Welfare Committee (iCBR, ORBEA 03/2021) and performed by FELASA-licensed users, following European (2010/63/EU) and Portuguese law (Decreto-lei nº 113/2013).

C02

Impact of pesticides exposition in the development on the gliovascular unit

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The European population is daily exposed to pesticide residues found in the food and in the drinking water. This contamination is a major public health issue as it has been link to neurodegenerative diseases (Parkinson's and Alzheimer's) as well as neurodevelopmental diseases (Autism spectrum disorder). To protect food consumers from potential adverse effects, European Union has set an internationally accepted maximum residue limit dose in tap water (0.1 µg/L, EU directive 2020/2184). However, it has been shown in a previous study that chronic exposure to a cocktail of fungicides at this regulatory dose altered neurogenesis in neonates following gestational exposure[1]. Fungicides residues may access the brain via the blood circulation and could then affect the gliovascular unit, a specific interface formed by the vascular compartment and astrocytes, where important brain functions are set such as neurovascular coupling, integrity of the blood-brain barrier, the immune homeostasis and drainage[2]. Thus, the chronic exposure to fungicides results in brain damage which points out the need to evaluate the impact of these residues on brain development. To do so C57Bl/6 female mice were exposed to a cocktail of fungicides (cyprodinil, mepanipyrim and pyrimethanil) at 0.1 µg/L each during gestation and breeding. To perform immunohistochemistry analysis we collected both male and female littermates brains from 5 days post-natal to adulthood. Before tissue collection, mice were injected intra-peritoneally with a mix of ketamine/xylaxine (150 and 15 mg/kg respectively). Experiments were performed in accordance with the French ethical laws (Decree 87–848; Ministère de l'Agriculture et de la Forêt), European guidelines (Directive 2010/63/UE) and ethical committee recommendation (APAFIS #37351). All experiments were performed following the ARRIVE guidelines (www.nc3rs.org.uk), including randomization as well as blinded analysis. Data are represented as mean ± S.D. Data and statistical processing were performed using Microsoft Excel and GraphPad Prism Software. For each data set, normality was tested using Shapiro-Wilk test. Statistical significance across groups defined by one factor was performed using a Kruskall-Wallis test followed by a Dunn's for multiple comparisons. For the pericyte and astrocyte coverage around blood vessel a Chi^2 test was used. A minimum of three mice per condition and four photomicrographies per mice were analyzed. The level of statistical significance was set at $P < 0.05$. Our preliminary results indicate that 1) microglial reactivity occurs in a sex-specific manner, 2) pericyte coverage is reduced as well as 3) perivascular astrocyte coverage but without any changes in astrocytes distribution[3]. This project aims to uncover early pathophysiological modifications caused by chronic exposure to fungicides, as it is essential to comprehend the impact of pesticides on brain development and warn about their usage.

C03

Specific expression of placental MATE1 in rats causes species difference in metformin transfer to the fetus

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[Objectives] The fetal-to-maternal concentration ratio (F/M ratio) of metformin, a substrate of organic cation transporters (OCT) and multidrug and toxin extrusion proteins (MATE), has been reported to be higher in pregnant humans than in pregnant rats. The objective of the present study was to identify transporters that cause species differences in metformin transfer to the fetus.

[Methods] Uncomplicated term human placental tissues were obtained with written informed consent and with the approval of the Institutional Ethics Committee of Keio University Faculty of Pharmacy (150421–2) and Keio University School of Medicine (20110250). The absolute protein expression levels of OCT3 and MATE1 in human, rat, and mouse placentas were quantified using LC-MS/MS. The mRNA expression levels were determined from RNA-seq data. Pregnant rats and mice were continuously infused with [^{14}C]metformin via the jugular vein, and the F/M ratio was measured. Pyrimethamine was used as a potent inhibitor of MATE1. Statistical analyses were performed using an unpaired, two-tailed Student's t-test for comparisons between the two groups.

[Results] OCT3 protein expression was detected in human, rat, and mouse placenta (n=3-4). However, MATE1 protein expression was selectively detected in rat placenta, but not in human and mouse placenta. These protein expression levels exhibited consistency with the mRNA expression levels: while no significant species differences were observed in OCT3 mRNA expression levels, MATE1 mRNA expression levels in the rat placental labyrinth were found to be considerably higher than those in human syncytiotrophoblasts and mouse labyrinth. The F/M ratio of [^{14}C]metformin was significantly lower in rats (n=5) than in mice (n=7). The pre-administration of pyrimethamine at 2 μmol/kg was found to reach a plasma concentration capable of specific inhibition of Mate1, as indicated by the measured IC50 of pyrimethamine to rat Mate1 (0.015 μM) and Oct3 (71.5 μM), and resulted in a significant increase in the F/M ratio of [^{14}C]metformin in rats (n=5) but not in mice (n=7).

[Conclusions] The findings of this study suggest that the fetal transfer of metformin in rats is constrained by placental MATE1 expression. These findings indicate that nonclinical developmental and reproductive toxicity studies of MATE1 substrate drugs in rats may underestimate fetal exposure when extrapolated to humans. This information will be helpful in estimating fetal transfer and toxicity of drugs in human fetuses from rat data.

C04

Determining placental uptake of pollutant nanoparticles using in vitro trophoblast cell models.

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Introduction: Exposure to air pollutants such as black carbon (BC) during pregnancy is associated with adverse outcomes including low birth weight and preterm birth. BC nanoparticles have a mean diameter ranging between 10-100 nm which allows them to cross the alveolar epithelial barrier and enter the systemic circulation where they have been identified in a variety of different tissues including the placenta. Currently, the transport mechanisms underlying uptake of pollutant particles in the placenta are not fully understood; this study aims to elucidate the mechanisms responsible.

Methods: Syncytialised BeWo and trophoblast stem cell (TSC)-derived syncytiotrophoblasts were treated with 0.1-10 µg/ml polystyrene nanoparticles (50 nm) and carbon quantum dots (5 nm) to model BC. Using fluorescence microscopy, cellular uptake of particles was visualised and then quantified (n=5). Uptake of different dose treatments were compared with Wilcoxon test with Bonferroni correction. Samples were also stained with endocytic antibodies to track the trafficking of particles through different intracellular vesicles.

Results: Polystyrene nanoparticles were visualised in syncytialised BeWo trophoblast cell line cultures with uptake occurring in a dose-dependent manner. Particles were rapidly taken up in the first 16 hours of culture where they progressively co-localised with early endosomes, late endosomes and lysosomes. The rate of nanoparticles internalisation slowed after 24 hours. Preliminary data from TSC derived syncytiotrophoblasts treated with carbon quantum dots suggests particles are internalised quicker, visualised intracellularly in the first 2 hours of culture with particles colocalising with endocytic vesicles and lysosomes.

Conclusion: Uptake of pollutant nanoparticles by placental syncytiotrophoblasts is dependent on dose and particle diameter with smaller 5 nm particles internalised quicker than 50 nm polystyrene particles. However, the different cell models used may also influence uptake kinetics. The results from this study will further our understanding of pollutant uptake by the placenta and provide an insight into what extent the trophoblast layer acts as a barrier against carbon-based nanoparticles.

C05

Maternal gut microbiota-derived tryptophan metabolites may alter placental development and function through G-Protein Coupled Receptors.

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Introduction: The microbiome changes during pregnancy and is implicated in pregnancy complications which contribute to nearly 5 million maternal, and neonatal deaths yearly. Impaired placental development primarily accounts for the majority of pregnancy complications although the mechanisms involved are poorly understood. Optimal placental (trophoblast) development depends on the fine balance between the renewal of the cytotrophoblast stem cells and their differentiation into the invasive extravillus trophoblast and the multinucleated syncytiotrophoblast (ST) epithelial cells. While pregnancy is punctuated by finely timed fluctuations in various hormones, that regulate trophoblast differentiation, the reasons for the concomitant changes in the microbiome with advancing gestation are unclear. As the microbiome does not reach the systemic circulation, but its metabolites do, it is possible that the metabolites may mediate microbial-host interactions reflected in microbial changes observed in pregnancy. We show that several microbial-derived tryptophan metabolites dose-dependently decrease energy metabolism and trophoblast differentiation via G-protein coupled receptor mediated cAMP production.

Methods and results: By treating term human primary trophoblast cells and measuring oxygen consumption using Mito stress test, we demonstrate that tryptophan metabolites (tryptamine, Indole-3-carbaldehyde, indole-3-acetic-acid (IAA) mediate differential effects on both energy metabolism and trophoblast differentiation. Tryptamine dose dependently reduced βhCG production (marker of trophoblast differentiation) at 12.6 μM ($p \leq 0.0001$, $n = 5$) and 50μM ($p \leq 0.0001$, $n = 5$). At similar doses (IAA) had no effect on either metabolism or trophoblast differentiation. Tryptamine reduced cyclic adenosine monophosphate (cAMP) production by 20% ($p \leq 0.05$, $N=3$) and shifted the EC50 of forskolin induced cAMP production dose response from 2.6 μM to 4.0 μM.

Discussion: These results demonstrate how microbial metabolites are likely to influence pregnancy outcomes by interacting with signalling pathways important to trophoblast differentiation. As GPCR signalling is an important pathway for various developmental processes including stem cell pluripotency, differentiation, and cell polarity in embryogenesis, these results emphasise the importance of understanding the role of microbial derived metabolites in pregnancy in health and disease.

C06

Prenatal exposure to air pollution is associated with altered neurodevelopmental outcomes in early childhood

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Introduction

Air pollution represents a significant public health challenge, with an estimated 95% of the world's population living with unhealthy air. Prenatal air pollution is associated with altered volumetric brain development (Bos et al 2023), developmental delay (Clifford et al 2016) and increased risk of cerebral palsy (Zhang et al 2024). The aim of this study was to assess the impact of maternal air pollution exposure across each trimester of pregnancy on offspring cognitive, language and motor abilities. We also considered the moderating effect of gestational age at birth (GA), in a representative population cohort from Greater London, UK.

Methods

502 toddlers from the Developing Human Connectome Project (Edwards et al 2022) were included [GA median (range) 39.57 (23.71-43.57), 264 male]. Research Ethics Committee approval was granted (14/LO/1169) and written informed parental consent was obtained. Maternal air pollution exposure was modelled based on residential postcode using the London Air Pollution Toolkit (Beevers et al 2013). Average exposure to NO2, and particulate matter (PM2.5 and PM10) from estimated conception date to date of birth was calculated, as well as average exposure for each trimester. Index of multiple deprivation was calculated from maternal postcode as a measure of socioeconomic status. Cognitive, language and motor abilities were assessed at median (range) 18.43 (17.26-34.49) months corrected age using the Bayley Scales of Infant and Toddler Development - 3rd Edition.

Linear regressions with 5000 permutations were used to assess the relationship between cognitive, language, and motor composite scores and pollutant exposure across pregnancy and within each trimester. The impact of average pollutant exposure across gestation and within Trimesters 1 and 2 were assessed across the whole cohort. Exposure in trimester 3 was assessed excluding infants born <28.00 weeks (n=20). Sex, index of multiple deprivation, GA, and if a parent spoke English as an additional language (N=224, for language analyses) were included as covariates. We also assessed the interaction between GA and pollutant exposure. Benjamini-Hochberg false discovery rate (FDR) correction was used to adjust for multiple comparisons.

Results

Higher maternal exposure to all pollutants in trimester one was associated with lower language composite scores at 18 months (Table 1, Figure 1A-C). Higher exposure to NO2 in trimester one was also associated with lower cognitive composite scores (Figure 1D). GA at birth significantly moderated the relationship between average maternal exposure to all pollutants across gestation and motor composite

scores (Table 2, Figure 2). Posthoc analysis revealed pollutants were significantly associated with motor composite scores in infants born <37.00 weeks GA (all pollutants $p<0.001$) but not infants born ≥37.00 weeks (NO_2 $p=0.728$, $PM_{2.5}$ $p=0.569$, PM_{10} $p=0.424$).

Discussion

Higher maternal exposure to air pollutants during the first trimester is associated with poorer language abilities in toddlers. In infant born prematurely (<37.00 weeks GA), higher air pollutant exposure across gestation was also associated with poorer motor abilities. Our results add to the growing body of evidence that prenatal exposure to air pollution affects neurodevelopment in offspring. Reducing air pollutant exposure should be a public health priority.

Table 1. Associations between maternal pollution exposure and neurodevelopmental outcomes at 18 months

	B (95% CI)	t value	p (p_{FDR})
Cognition, median (range) 100 (55-130)			
NO_2			
Whole pregnancy	-0.135 (-0.302- 0.032)	-1.589	0.112 (0.531)
Trimester 1	-0.273 (-0.455- -0.090)	-2.938	0.004 (0.036)*
Trimester 2	0.167 (0.000- 0.334)	1.968	0.047 (0.210)
Trimester 3	0.069 (-0.100- 0.238)	0.800	0.418 (0.537)
$PM_{2.5}$			
Whole pregnancy	-0.363 (-0.868-0.143)	-1.408	0.160 (0.664)
Trimester 1	-0.565 (-0.995- -0.134)	-2.579	0.010 (0.059)
Trimester 2	0.190 (-0.169- 0.550)	1.041	0.302 (0.473)
Trimester 3	0.197 (-0.202- 0.596)	0.970	0.320 (0.481)
PM_{10}			
Whole pregnancy	-0.166 (-0.558- 0.227)	-0.829	0.398 (0.531)
Trimester 1	-0.427 (-0.777- -0.077)	-2.397	0.017 (0.085)
Trimester 2	0.097 9-0.203- 0.397)	0.635	0.515 (0.625)
Trimester 3	0.283 (-0.021- 0.588)	1.827	0.068 (0.251)
Language, median (range) 97 (47-153)			
NO_2			
Whole pregnancy	-0.202 (-0.430- 0.027)	-1.733	0.086 (0.504)
Trimester 1	-0.559 (-0.809- -0.309)	-4.393	<0.001 (<0.001)*
Trimester 2	0.302 (0.074- 0.529)	2.605	0.008 (0.055)
Trimester 3	0.147 (-0.087-0.381)	1.233	0.220 (0.452)
$PM_{2.5}$			
Whole pregnancy	-0.569 (-1.263-0.125)	-1.612	0.103 (0.625)
Trimester 1	-0.956 (-1.548- 0.364)	-3.174	0.001 (0.025)*
Trimester 2	0.091 (-0.405- 0.586)	0.360	0.718 (0.760)
Trimester 3	)0.311 (-0.244- 0.865	1.102	0.259 (0.452)
PM_{10}			
Whole pregnancy	-0.298 (-0.835- 0.239)	-1.090	0.264 (0.936)
Trimester 1	-0.724 (-1.205- -0.242)	-2.951	0.003 (0.036)*
Trimester 2	0.082 (-0.330- 0.494)	0.389	0.698 (0.760)
Trimester 3	0.398 (-0.026- 0.822)	1.843	0.070 (0.251)
Motor, median (range) 102 (67-130)			
NO_2			
Whole pregnancy	-0.086 (-0.233- 0.060)	-1.155	0.251 (0.662)
Trimester 1	-0.135 (-0.296- 0.026)	-1.646	0.101 (0.284)
Trimester 2	0.085 (-0.062- 0.232)	1.135	0.255 (0.452)
Trimester 3	0.068 (-0.083- 0.219)	0.882	0.370 (0.512)
$PM_{2.5}$			
Whole pregnancy	-0.213 (-0.658- 0.231)	-0.944	0.350 (0.504)
Trimester 1	-0.107 (-0.488- 0.273)	-0.555	0.570 (0.662)
Trimester 2	0.013 (-0.304- 0.331)	0.083	0.936 (0.936)
Trimester 3	0.102 (-0.257- 0.460)	0.558	0.590 (0.664)
PM_{10}			
Whole pregnancy	-0.112 (-0.456- 0.233)	-0.637	0.521 (0.625)
Trimester 1	0.199 (-0.073- 0.472)	1.436	0.154 (0.361)
Trimester 2	0.015 (-0.250- 0.279)	0.108	0.913 (0.936)
Trimester 3	-0.171 (-0.479- 0.138)	-1.087	0.282 (0.461)

*significant p_{FDR} <0.05; adjusting for socioeconomic status, sex, GA and if a parent spoke English as an additional language (for language analyses)

Table 2. Moderating effect of GA on relationship between maternal pollution exposure and neurodevelopmental outcomes at 18 months

	B (95% CI)	t value	p (p_{FDR})
Cognition			
NO_2			
Whole pregnancy	0.031 (-0.004- 0.067)	1.741	0.086 (0.282)
Trimester 1	-0.003 (-0.042- 0.037)	-0.144	0.888 (0.916)
Trimester 2	0.014 (-0.019- 0.047)	0.819	0.416 (0.622)
Trimester 3	0.009 (-0.029- 0.047)	0.458	0.651 (0.837)
$PM_{2.5}$			
Whole pregnancy	0.077 (-0.036- 0.190)	1.339	0.177 (0.374)
Trimester 1	-0.072 (-0.181- 0.037)	-1.291	0.195 (0.390)
Trimester 2	-0.048 (-0.137- 0.041)	-1.066	0.287 (0.511)
Trimester 3	0.020 (-0.082- 0.122)	0.386	0.702 (0.857)
PM_{10}			
Whole pregnancy	0.046 (-0.036- 0.128)	1.109	0.267 (0.505)
Trimester 1	-0.066 (-0.150- 0.018)	-1.546	0.122 (0.313)
Trimester 2	-0.027 (-0.096- 0.043)	-0.760	0.443 (0.622)
Trimester 3	0.030 (-0.048- 0.107)	0.752	0.450 (0.622)
Language			
NO_2			
Whole pregnancy	0.043 (-0.005- 0.092)	1.773	0.078 (0.281)
Trimester 1	0.028 (-0.025- 0.082)	1.038	0.298 (0.511)
Trimester 2	0.043 (-0.002- 0.089)	1.861	0.056 (0.226)
Trimester 3	-0.001 (-0.054- 0.052)	-0.040	0.973 (0.973)
$PM_{2.5}$			
Whole pregnancy	0.178 (0.024- 0.332)	2.270	0.024 (0.128)
Trimester 1	0.028 (-0.121- 0.177)	0.367	0.714 (0.857)
Trimester 2	0.100 (-0.022- 0.223)	1.613	0.103 (0.308)
Trimester 3	-0.015 (-0.158- 0.127)	-0.213	0.837 (0.913)
PM_{10}			
Whole pregnancy	0.130 (0.019- 0.241)	2.294	0.025 (0.128)
Trimester 1	0.019 (-0.095- 0.134)	0.329	0.741 (0.860)
Trimester 2	0.094 (0.000- 0.189)	1.955	0.051 (0.226)
Trimester 3	0.008 (-0.100- 0.115)	0.139	0.891 (0.916)
Motor			
NO_2			
Whole pregnancy	0.061 (0.030- 0.091)	3.884	<0.001 (<0.001)*
Trimester 1	0.041 (0.006- 0.075)	2.321	0.018 (0.128)
Trimester 2	0.038 (0.009- 0.067)	2.553	0.012 (0.106)
Trimester 3	0.026 (-0.007- 0.060)	1.540	0.117 (0.313)
$PM_{2.5}$			
Whole pregnancy	0.202 (0.105- 0.300)	4.063	<0.001 (0.002)*
Trimester 1	0.069 (-0.027- 0.166)	1.413	0.154 (0.370)
Trimester 2	0.021 (-0.057- 0.100)	0.536	0.599 (0.799)
Trimester 3	0.012 (-0.079- 0.103)	0.266	0.785 (0.884)
PM_{10}			
Whole pregnancy	0.148 (0.077- 0.219)	4.116	<0.001 (<0.001)*
Trimester 1	0.052 (-0.022- 0.126)	1.387	0.169 (0.374)
Trimester 2	0.027 (-0.034- 0.088)	0.883	0.380 (0.622)
Trimester 3	0.027 (-0.042- 0.096)	0.776	0.430 (0.622)

*significant p_{FDR} <0.05; adjusting for socioeconomic status, sex and if a parent spoke English as an additional language (for language analyses)

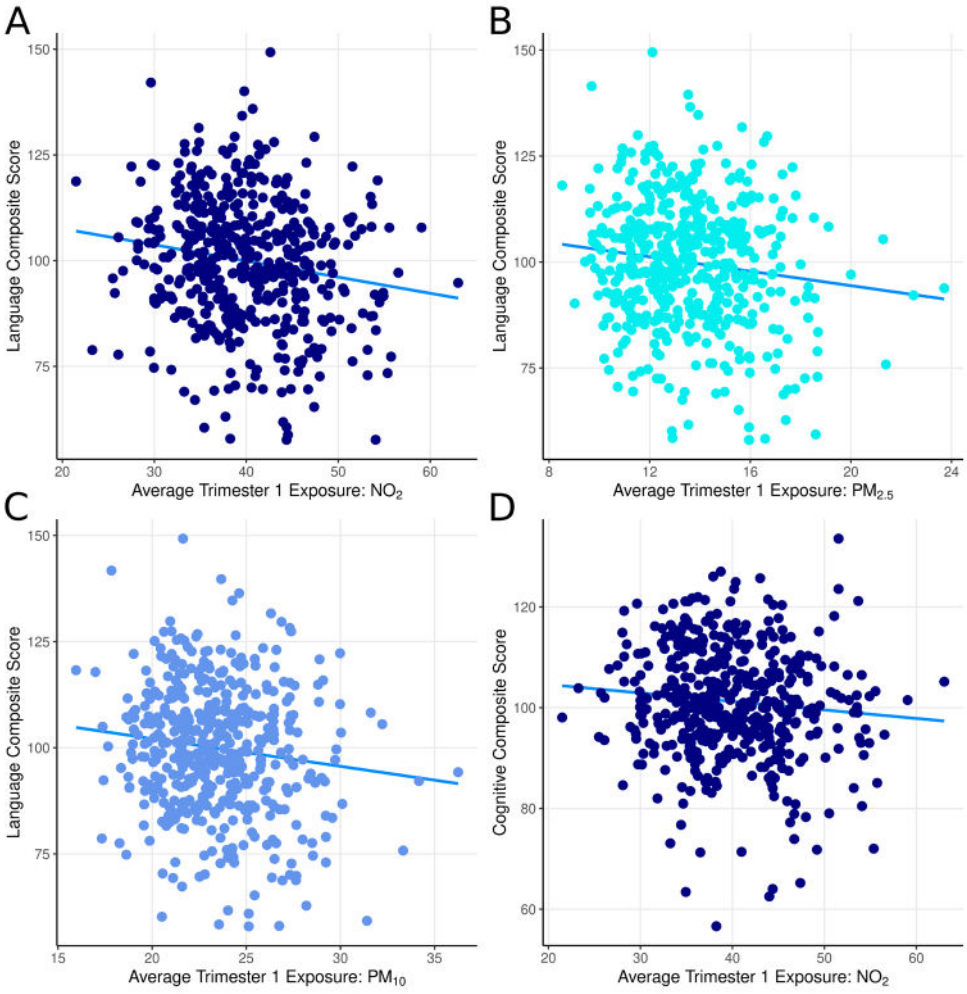

Figure 1. Associations between trimester 1 pollutant exposure and Language (A-C) and Cognitive (D) composite scores adjustingfor sex, GA, socioeconomic status and whether a parent spoke English as an additional language (language analyses)

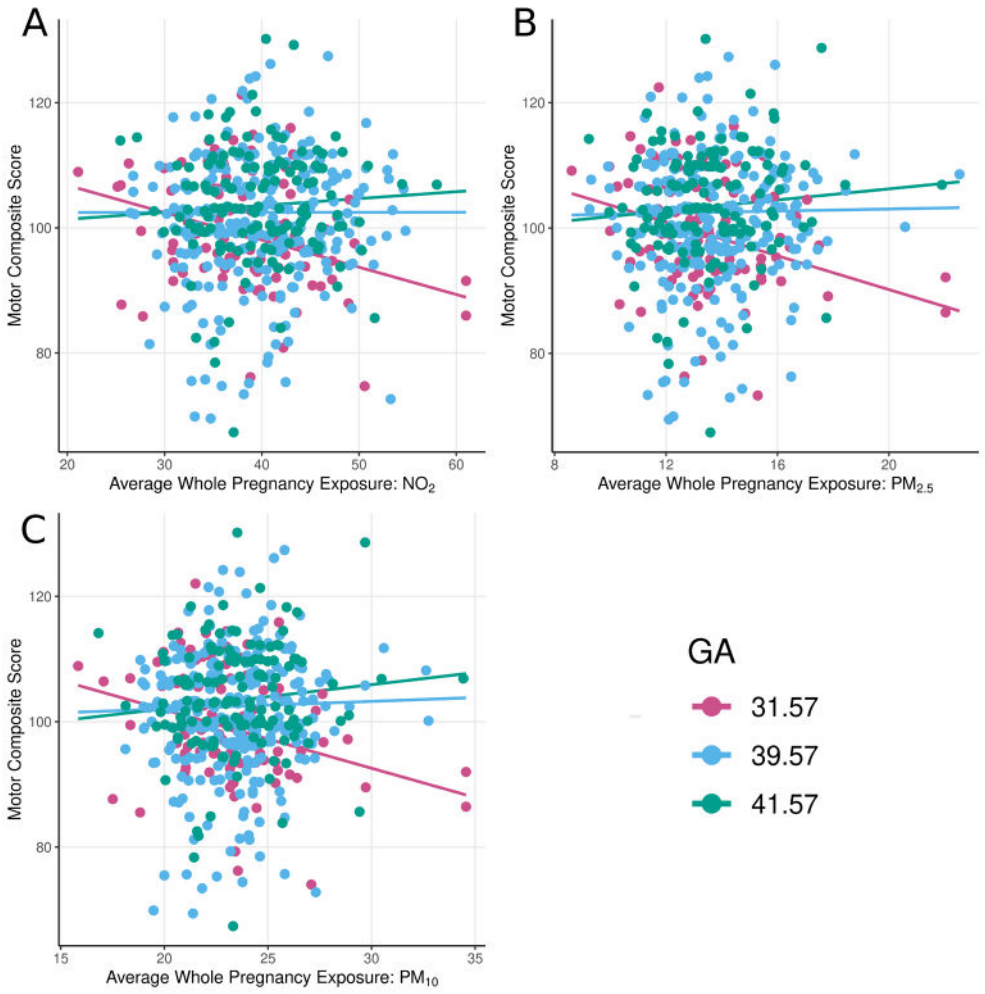

Figure 2. Moderating effect of GA on relationship between motor composite score and whole pregnancy NO2 (A), PM2.5 (B), PM10 (C) adjusting for sex and socioeconomic status

C07

Applications of physiologically based pharmacokinetic models to quantify the placental permeation of xenobiotics

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Introduction: The recent advance in using in silico tools, specially physiologically based pharmacokinetic model (PBPK), has improved our understanding on xenobiotics permeability through biological membrane. Pregnancy PBPK models that integrate longitudinal changes in maternal, placental, and fetal physiology during pregnancy in virtual population together with drugs properties can be applied to quantify transplacental passage and drugs exposure in different maternal and fetal organs [1].

Aim: To demonstrate the ability of pregnancy PBPK models to predict drug exposure in maternal, and umbilical plasma, the placental tissue, and fetal organs.

Method: The Simcyp Pregnancy Simulator was used to predict maternal, placenta, umbilical and fetal organs concentrations for cefuroxime, cefazolin, nifedipine, tenofovir, nelfinavir, and efavirenz [2-4]. Predictions were compared with observations, where available. Permeability through placenta was parametrized using experimental data.

Results: Observed concentrations were within the predicted 5th-95th intervals by the model. The derived PK parameters were also with 2-fold error criteria. Limited observed data on fetal organs exposure (2 compounds) fall in the model prediction space.

Conclusion: Pregnancy PBPK models provide valuable insights into xenobiotics disposition in special individuals that are otherwise difficult to study. These models are “live models” reflecting current knowledge, and their performance can be enhanced by utilizing data derived from advanced experimental tools, such as tissue on chips, ex vivo, and biopsy, to assess drug permeability through maternal, placenta and fetal biological barriers.

C08

Targeting transferrin receptor to enable drug delivery to the neonatal brain

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Intro

Delivery of therapeutics to the brain during early development presents a unique opportunity to treat disease prior to the onset of irreversible brain changes, and at a time with increased tolerance to new proteins. However, the blood-placenta-barrier and fetal blood-brain-barrier have posed substantial challenges to perinatal brain drug delivery.

Objectives

Here we explore the potential of a transferrin receptor (TfR) binding therapeutic platform that we call the “transport vehicle” (TV) to enable delivery of drugs, specifically oligonucleotides, in the neonatal brain.

Methods

To assess this, neonatal mice were treated with subcutaneous injections of a malat1 targeted, oligonucleotide-TV conjugates (OTV) at postnatal days 1, 3, 7, 10 and 13 (n=4-8/group). Brain tissue was collected at postnatal days 3, 7 and 14.

Results

We find that the OTV platform enables delivery of a malat1 targeted ASO, to the brain at early developmental stages. Furthermore, we observe that binding to TfR alters the biodistribution of antibodies and oligonucleotides to the brain.

Conclusions

These data support the TV platform and OTVs as a potential therapeutic platform for systemic delivery of drugs to the perinatal brain.

SA01

Barrier mechanisms limiting drug entry into the developing brain: Entry of antiseizure medications valproate and lamotrigine

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Numerous life-threatening diseases, such as epilepsy, necessitate long-term treatment of patients even during pregnancy and lactation, since discontinuation can cause serious harm to the mother, and subsequently her offspring. However, exposing the highly sensitive developing brain to some drugs, including antiseizure medications valproate and lamotrigine, may result in permanent congenital defects or neurobehavioral issues in later life. The developing brain is protected by its own brain barrier mechanisms (i.e. blood-brain and blood-CSF barriers) but also by the placental interface between maternal and fetal circulations. Factors determining transfer across these barriers include cellular drug transfer mechanisms such as transporters and drug metabolising enzymes but also the extent of drug binding to circulating plasma protein.

GAERS (Genetic Absence Epilepsy Rat from Strasbourg) at embryonic day (E) 19, postnatal day (P) 0, 4 and 21, and young adults were administered valproate (30 or 100 mg/kg) or lamotrigine (6 mg/kg) either alone or in combination with respective [^{3}H]-tracers. In chronic experiments, females commenced on a valproate-containing diet 2 weeks prior to mating and throughout pregnancy. The offspring were studied. Thirty minutes following drug injection, blood, CSF and brain samples were collected from terminally anaesthetised animals (urethane, 2g/kg). Radioactivity was measured and transfer expressed as ratios of radioactivity (Mena±SD%) in fetal over maternal plasma (placental barrier) or in CSF or cortex over fetal plasma (fetal brain barriers). Drug binding to plasma protein was established by ultrafiltration. Transcriptomic analysis of brain cortex and choroid plexus was used to determine expression of cellular drug transfer mechanisms at E19, P5 and adults

Brain entry of valproate was higher in fetal than postnatal animals (76±12%, n=6 E19 and 21±3%, n=4 adults), which may be partially attributed to reduced valproate binding to plasma protein as its free fraction decreased from 85±2% in E19 to 65±10% in adults. Brain entry of lamotrigine was not age-dependent. In acute experiments, combination therapy had no effects on brain and CSF entry of valproate but enhanced entry of lamotrigine into the adult brain (60±9% to 124±20%). Following long-term valproate exposure using a formulated diet, its entry into adult brain decreased. However, maternal exposure to valproate during pregnancy led to increased brain and CSF entry in E19 fetuses and postnatal pups. Placental transfer of valproate at from maternal into fetal circulation decreased substantially from 64±21% to 36±15% (n=8) when co-administered with lamotrigine. Developmental differences in brain entry of valproate may be correlated with expression of some transporters, including Slc22a8, and drug metabolising enzymes.

Present results suggest that combination therapy may mitigate risk of toxicity as placental transfer of valproate decreased when co-administered with lamotrigine. However, in fetuses chronically exposed to valproate throughout gestation, lamotrigine co-administration resulted in increased brain entry of valproate but decreased lamotrigine entry. Overall, findings from this study demonstrated the need to understand mechanisms that influence drug entry into the brain and across the placenta in order to provide an evidence base for devising modulatory strategies to achieve desired clinical outcomes such as reducing collateral exposure of developing brain to maternally administered drugs.

SA02

Placental Transfer Mechanisms

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Understanding transfer systems across the placental barrier is key to understanding the fetal exposure to xenobiotics, including maternal medications.

Although other species, such as the rat and rabbit, are used in developmental and reproductive toxicology (DART) testing of non-biological therapeutics, structural, hemodynamic and cellular physiology differences set them apart from humans in considering biopharmaceutical pharmacokinetics (PK). These are important factors to assist in appropriate dose level selection for further fetal toxicology studies, and for dose margin setting for bio-pharmaceutical risk assessments on human fetal exposure. Furthermore, in the advent of ICH S5 (R3) regulation, there are recommendations for the development, standardisation and use of new alternative methods in DART testing, as drug discovery screens for evaluating adverse effects on embryo and fetal development, to replace the rabbit.

Knowledge on the human syncytiotrophoblast barrier, including transcellular transporters, the paracellular route and endocytosis is still in a juvenile phase; whilst the placental endothelium has been exceptionally understudied. Hence, an appreciation of transfer processes in the PK of the human placental barrier is limited in comparison knowledge of the intestine and the blood-brain barriers. However, state of the art imaging is beginning to reveal the true porous nature of the placental syncytium in the human, which may be in common with other species. Advances in bioengineering and applied mathematics are bringing an added wealth of understanding to placental hemodynamics and reshaping how physiologists think about architectural influences on compound specific barrier transfer rates of nutrients, waste products and xenobiotics.

SA03

Preeclampsia EVs and the placenta-brain axis

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Preeclampsia (PE) is a pregnancy-specific condition characterized by high blood pressure and signs of organ damage. While it primarily affects the cardiovascular and renal systems, PE is also associated with neurological complications often attributed to hypertension-induced alterations in cerebral blood vessels. Small extracellular vesicles (sEVs) have recently emerged as potential drivers of blood-brain barrier (BBB) disruption by impacting its cellular components. We investigated whether circulating sEVs in plasma, including those derived from the placenta, contribute to cerebrovascular alterations in PE. The impact of sEVs on the function of brain endothelial cells (BECs), microglia, and astrocytes was studied, focusing on potential effects related to BBB disruption. Circulating sEVs were isolated from plasma samples of healthy and PE pregnancies and tested in human-based mono-, bi-, or tri-culture models using transwell and microfluidic (BBB-on-a-chip) systems. Our results revealed that PE-sEVs disrupted endothelial barrier integrity evidenced by increased permeability and decreased expression of tight-junction proteins. Furthermore, PE-sEV crossed the BEC layer in a bi-culture transwell setup and reached microglia. There, PE-sEVs were phagocytosed and transferred their miRNA cargo, inducing upregulation of Iba1 expression and amoeboid morphology, suggesting microglial activation. Similarly, using BBB-on-a-chip and direct stimulation models, PE-sEVs triggered a reactive astrocyte phenotype, characterized by increased GFAP expression, elevated secretion of the pro-inflammatory cytokine IL-6, and enhanced migratory capacity. The activation of astrocytes and microglia may amplify neuroinflammation in PE, potentially contributing to exacerbated BBB dysfunction. Our findings suggest that sEVs could play a crucial role in the placenta-brain axis in PE, leading to maternal brain injury by transmitting signals of placental damage. Understanding this mechanism could offer valuable insights for both diagnostic and therapeutic strategies in managing the neurological complications of PE.

SA04

Engineering and characterising large molecule delivery to the brain: receptor-mediated transcytosis at the blood-brain barrier in early development and throughout the lifespan

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Effective delivery of protein therapeutics to the central nervous system (CNS) has been greatly restricted by the blood-brain barrier (BBB), a key border separating the general blood circulation from the brain parenchyma (Badaut et al. Fluids & Barriers of the CNS, 2024). We have developed novel BBB transport vehicles comprising engineered Fc fragments that exploit transcytotic pathways across brain endothelial cells for the CNS delivery of biotherapeutics. These transport vehicles have been engineered using directed evolution to bind either the apical domain of the human transferrin receptor (TfR; Kariolis et al. Science Translational Medicine, 2020) or CD98 heavy chain (Chew et al. Nature Communications, 2023), two highly expressed brain endothelial cell targets. My talk will present results demonstrating how the transport vehicle platform may be paired with a variety of different payloads (e.g. antibodies, enzymes, and antisense oligonucleotides) for increased brain exposures and therapeutic effects in animal models and in human beings. I will also discuss new data demonstrating that healthy neonatal mice exhibit higher vascular TfR expression and TfR-targeted transport vehicle brain exposure than observed in adult mice, whereas BBB transport capacity remains stable across adulthood. Elevated TfR-mediated brain delivery observed in early mouse development suggests the potential of added efficacy in utilizing TfR platforms for the treatment of early childhood diseases, e.g. brain-penetrant enzyme replacement therapies currently being evaluated for certain lysosomal storage disorders (Ullman et al. Science Translational Medicine, 2020; Arguello et al. Journal of Experimental Medicine, 2022). Overall, our work suggests this modular platform approach has great potential for the CNS delivery of multiple protein therapeutics covering a range of neurological disorders including Alzheimer's disease, brain cancers, and neuronopathic mucopolysaccharidoses.

SA05

Methods to investigate BBB function/dysfunction in the developing animal

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Introduction and aims: Investigating blood-brain barrier function in the developing animal can be challenging given the small size of animals and sometimes an in utero setting of experimentation. It necessitates for careful considerations of experimental methodologies in order not to endanger the physiological well-being of the foetus or the newborn animal. These experiments can also be technically difficult requiring different routes of administration into the developing animal. Our aim is to provide methodologies that can be used to successfully complete such experiments.

Methods: To help we have tested and validated a range of molecular weight tracers that can easily be visualised in tissues (down to electron microscopic level) and used to assess BBB integrity. Together with quantifiable markers, experiments can detect both global (across whole brain or brain region) and local changes in BBB function at high resolution. In addition, it might be necessary to administer markers into the developing animal in different ways, depending on species and age of animals, such as injections into the embryo (in utero), foetal membrane vessels or the retroorbital plexus.

Results and Conclusions: Different techniques are often optimal in different settings of experimentation in the developing animal. Optimising the experimental methods will allow for better validity of results and improved animal ethics. By using these techniques, we have over the years revealed changes in BBB function, both spatially and temporally, under various pathophysiological states in the developing animal such as hypoxia-ischemia, neonatal infection/inflammation or following intraventricular haemorrhage.

SA06

Exploring the CRISPR Toolbox: In Vitro and in Vivo

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CRISPR has revolutionized both gene editing and biomedical research since its discovery just over a decade ago. The CRISPR-Cas9 system is comparatively easy to use, cost-effective, and applicable to a wide range of model systems and organisms. In this talk, I will introduce the fundamental principles of CRISPR-Cas9, its applications, and various design and delivery strategies for both in vitro and in vivo studies. Additionally, I will discuss the expanded CRISPR toolbox, including the use of inactivated Cas9 (deadCas9) for gene activation and repression (CRISPRa/i). Finally, I will briefly explore how CRISPR, in combination with human induced pluripotent stem cells (iPSCs), can be leveraged to create in vitro human model systems for developmental biology research and disease modeling.

SA07

Imaging the developing human brain following in utero exposure to drugs

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Exposure to drugs (both therapeutic and to substances of abuse such as opioids) during pregnancy are known to strongly associate with later adverse neurodevelopmental outcomes. However, little is known about how these exposures specifically affect the developing human brain and thus mechanistically how they result in later difficulties. Given emerging challenges such as the recent US opioid crisis, a clear need has emerged to better understand what underlies these difficulties so that difficulties can be identified early and to help develop possible treatments. In recent years, advances in neuroimaging methods and in particular Magnetic Resonance Imaging (MRI) have made it possible to gain images of the developing human brain during the critically important period shortly after birth and even in utero from the fetus. This can not only enable detailed visualisation of the brain's evolving anatomy and help to identify possible injuries, but can also provide quantitative data about volumetric brain growth, tissue composition, and neurometabolite levels. Importantly, it can also provide entirely novel insight about how the brain's framework of white matter pathways (structural connectivity) and coordinated activity (functional connectivity) emerge in early life. Importantly, disruptions to the establishment of this framework have also been found to significantly predict later adverse neurodevelopmental outcome. In this talk I will introduce these imaging methods, highlight the potential insight that they can provide into brain development and pathological processes, and review the existing literature in this area.

SA08

Perinatal specificities of the choroid plexus-CSF system: relevance to neuroprotection against toxic chemicals.

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While the cerebral vascularization develops gradually in two pre- and postnatal waves, the choroid plexuses develop and mature precociously. This confers to the choroid plexus-CSF system a special role in regulating blood-brain exchanges during pre- and early postnatal development. The choroid plexuses fulfill neuroprotective functions that result from the tightness of the choroidal epithelium forming the blood-CSF barrier, from efflux transport systems, and from specific detoxification activities associated to the epithelial cells. The developmental profiles of several of the antioxidant and conjugation enzymes involved in detoxification display a peak of activity centered on the perinatal or early postnatal period, suggesting an adaptation of the choroidal functions to this particularly sensitive period of life. We also provide evidence that the detoxifying function of the blood-CSF barrier can be pharmacologically enhanced through the Nrf2 signaling pathway to better protect the neural fluid environment from drug and toxic accumulation during the neonatal period.

SA09

Air pollution: Fetal and postnatal development

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Abstract

UNICEF estimates that over 100 million infants and 300 million children are exposed to levels of pollution that exceed WHO recommended limits, and around 95% of the world's population lives in regions with unhealthy levels of air pollution, with low- and middle-income countries experiencing the highest levels of exposure. There is growing concern regarding the effect of exposure during gestation as exposure to PM2.5 is associated with low birth weight, preterm birth, still birth and congenital abnormalities.

There is a growing body of evidence linking exposure to air pollution and adverse neurologic consequences in children highlighting that air pollution should be considered a developmental neurotoxicant [1]. In animal studies, prenatal exposure to pollution is associated with altered microglial development [2] disrupted cortical development [3] reduced myelination [4] impaired memory and learning ability, and increased anxiety and persistent behavioural deficits, particularly in male offspring [5]. Human studies show that prenatal and childhood exposure to air pollutants have adverse effects on intelligence, memory, behaviour and mental health in childhood and adolescence [6-8].

Brain magnetic resonance imaging (MRI) studies have reported that in utero and childhood exposure to air pollution is associated with altered microstructural, morphological and functional brain development in childhood. MRI studies in children (imaged between 6 and 12 years) exposed to high levels of ambient pollution in utero demonstrated reduced brain volume, impaired myelination, diminished cortical gyrification and altered functional connectivity [7, 9, 10]. However, research examining the relationship between prenatal exposure to air pollution and fetal or neonatal brain development is limited. The only study to date to assess the relationship between prenatal exposure to air pollution and neonatal brain morphology investigated brain volumes from 469 healthy infants recruited in London, UK to the developing human connectome project (http://www.developingconnectome.org/). Higher gestational exposure to PM10 and lower exposure to NO2 was associated with larger relative ventricular and cerebellar volume, along with modest associations with smaller relative cortical grey matter, amygdala and hippocampus volumes, and larger relative volumes of brainstem and extracerebral CSF [11].

This presentation will discuss studies assessing the relationship between exposure to air pollution and (i) brain MRI findings and (ii) neurodevelopment.

SA10

Methamphetamine in pregnancy: A health crisis!

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Methamphetamine (MA) use in women of childbearing age has now surpassed the use of cocaine and other stimulants globally. The prevalence of MA use during pregnancy has escalated, with studies indicating that up to 5% of all births in some regions are affected by prenatal exposure. The implications of this trend are profound, as exposure affects both the mother's health and well-being and the child's health and development. Women who continue to use MA in pregnancy have higher rates of pregnancy loss, pre-eclampsia, cesarean delivery, placental abruption, preterm delivery, and a higher risk for cardiovascular disease. Maternal MA use during pregnancy is also associated with other drug use, mental illness, a history of physical or sexual abuse, poverty, and domestic violence. The United States (US) and New Zealand (NZ) Infant Development, Environment, And Lifestyle (IDEAL) prospective, longitudinal studies were designed to determine the health, behaviour, and developmental outcomes of prenatal exposure to MA in the context of the child's environments. Women exposed to MA in NZ were enrolled during pregnancy (n = 107) and matched with women who reported no MA use (n = 110) and their infant had a meconium sample negative for MA. Women were matched for education, ethnicity, and their child's birth weight. Figure 1 shows the longitudinal waves of data collection for both studies. The first 36 months of both studies used the same measures. The NZ study continued to collect data throughout middle childhood and early adolescence. At each wave of data collection measures of the child's environment were obtained, including maternal substance use, mental health, and living conditions from the Maternal Lifestyle Questionnaire. Child measures included growth, behaviour, cognitive and motor development, and health and well-being. This talk will provide an overview of the developmental outcomes in the context of the environments of children enrolled in the NZ IDEAL Study. The gaps in our knowledge and how we can mitigate the effects of MA exposure on both the mother and her child will be discussed.

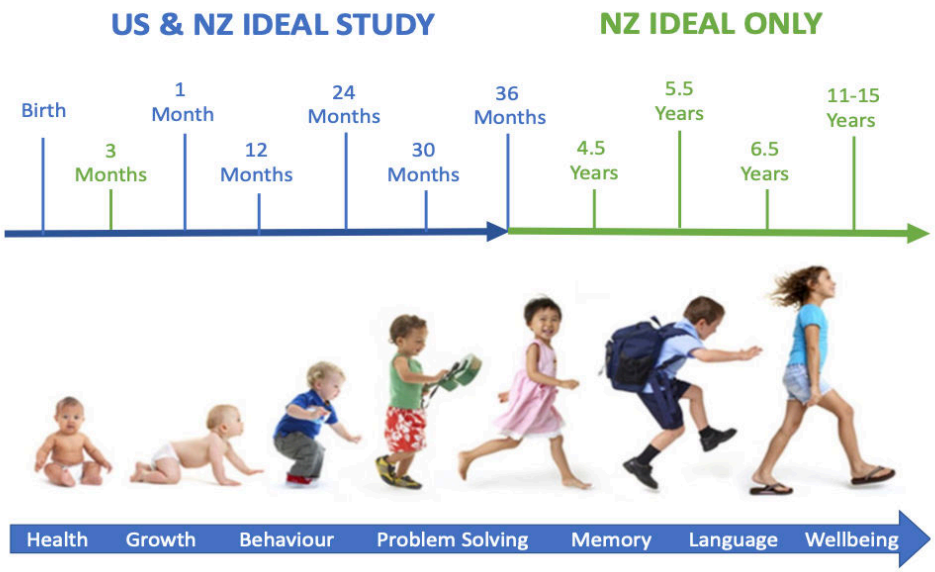

Figure 1. Waves of data collection for the US and NZ IDEAL Studies. The US and NZ IDEAL Studies used the same Lifestyle and child development measures over the first 36 months. The NZ IDEAL Study collected additional data at 3 months and 4.5, 5.5, 6.5, and 11-15 years follow-up.