CEREBRA
Working wonders for children with brain conditions

Cerebra in Leeds

Preventing neonatal brain injury and childhood disability

Program Grant Update 2022

Cerebra in Leeds 2020-2023: The placental origins of brain injury

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Background

Perinatal brain injury is a common outcome in the fifth of pregnancies that are complicated by pre-eclampsia (PET), fetal growth restriction (FGR) and preterm birth (PTB). Unfortunately, such neurological injury can have irreversible adverse *sequelae* impacting academic performance, social function, cognition and independent living in the long term. Given that many aspects of the aetiology and pathophysiology of these pregnancy complications are increasingly understood to implicate placental dysfunction, understanding the placenta in more detail at an architectural and functional level will open up new avenues of fundamental and translational research to prevent perinatal brain injury. To this end, our integrated research program has centered on developing different strategies for the 'deep phenotyping' of placental disease.

Progress with work packages

Our program of work has been organised around four principal workstreams:

(i) Creation of the Leeds Placenta Bank

The purpose of building a placenta tissue bank was to create a unique resource to support the other facets of the program (see sections below). Our collections focus on targeting both current births as well as historical specimens from the histopathology archive. The Covid-19 pandemic unfortunately had a major adverse impact on fresh specimen collection: the NHS imposed an 18 month moratorium on studies involving face-to-face contact with patients, thus precluding the team from for consenting participants prior to their deliveries. Nevertheless, we have still managed to collect close to 300 of our target of 500 fresh placentae with full obstetric clinical annotations from both normal

pregnancies and those complicated by the obstetric complications most linked to subsequent poor neurodevelopmental outcomes: PET, FGR, and preterm birth. As such, we remain on track. These cases have been complemented by compensatory sourcing from the histopathology diagnostic archive (see below).

(ii) Characterising placental morphometric aberrations

As per our original plan, the principal focus of our program was on establishing placental histoarchitectural correlates with adverse pregnancy outcomes using light sheet microscopy, a cutting-edge technique which we have successfully optimised in-house for fresh specimens. Given the pandemic-related difficulties in fresh placenta collections from caesarean sections and vaginal births, our adaptive strategy was to turn to the histopathology diagnostic archive. Chief amongst the obstacles in making effective use of this surplus diagnostic material was the need to develop laboratory protocols that would enable the application of techniques such as light sheet microscopy to formalin-fixed paraffin embedded tissue – a hurdle that has now been successfully overcome.

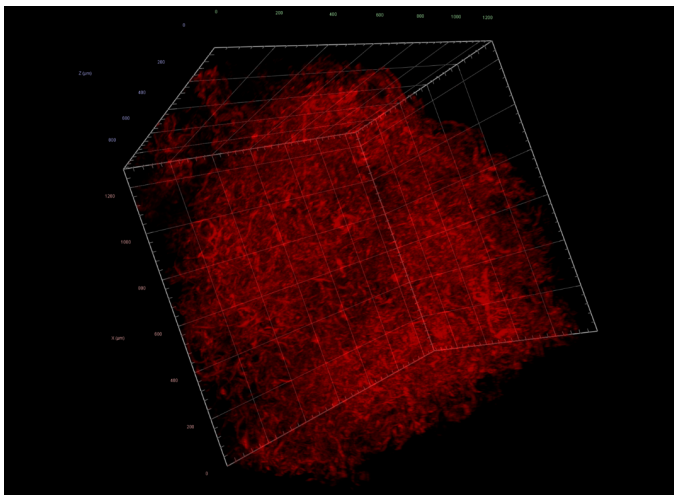


Figure 1: A three-dimensional reconstruction of a placental vascular bed. New imaging modalities enable the user to extract quantitative information about the specimen.

The purchase of high-specification specialist imaging software has been critical to capitalising on these major advances. Specifically, whilst this affords the opportunity to extract meaningful quantitative information from placental specimens (e.g. villous

volumes, villous/vascular density/branching), this can also be achieved with a degree of case throughput not previously possible using alternative techniques, and these were two key objectives of our program. Examples of the high-resolution three-dimensional placental vessel structures that we can handle are given in Figures 1-3.

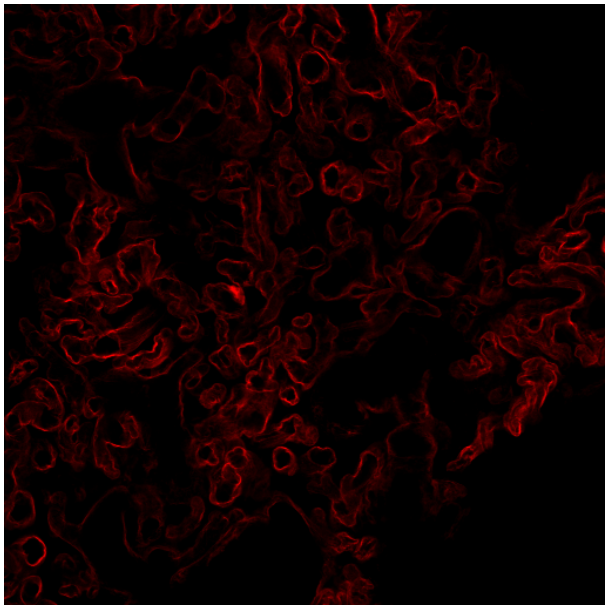
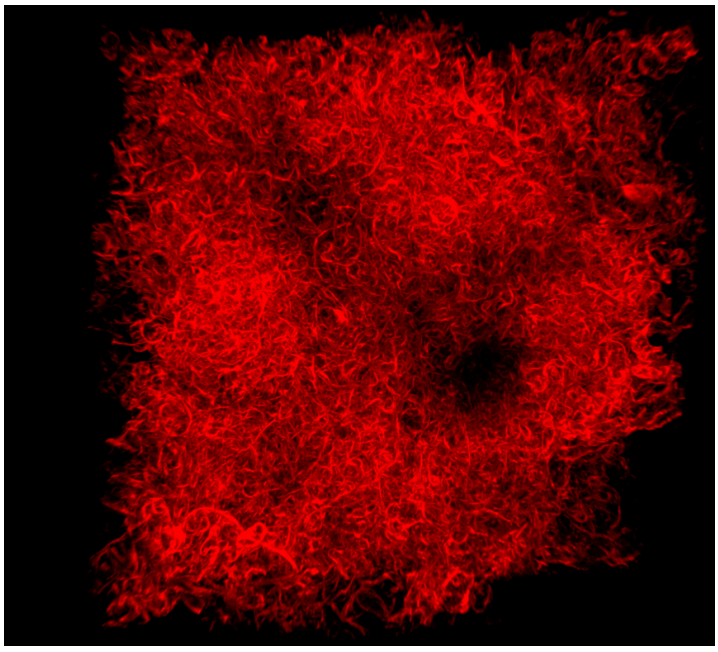


Figure 2: High-resolution view of lectin-labelled placental vessels: this degree of detail is maintained across the entirety of the specimen (see Figure 3). Virtual navigation through such specimens is also supported by our imaging software.

Figure 3: Three-dimensional reconstruction of a placental vascular bed, capturing a level of architectural complexity not afforded by methods other than light sheet microscopy as used herein.



(iii) Biomarker investigations in placental dysfunction in pregnancy complications

The third aspect of the program focuses on identifying phenotypic markers of placental dysregulation. One of the areas that we have been concentrating on these last two years has been amyloid deposition. These deposits represent pathologic accumulations of misfolded proteins that are related to the severity of disorders such as PET. In normal pregnancy, upregulation of pregnancy zone protein (PZP) maintains extracellular proteostasis and prevents accumulation of these pathological deposits. In complicated pregnancies, we surmise that an impairment of this function leads to cellular toxicity and placental dysfunction, although techniques for quantifying these deposits in a meaningful way remains wanting. To this end, we have teamed up with a colleague at the University of Salford, Dr Tiehan Shen, a physicist with world-class expertise in polarisation microscopy. In partnership with him, we are developing a totally unique quantitative method for evaluating amyloid deposition by measuring so-called Stokes parameters, which vary according to changes in the phase of light traversing through a substance (this is particularly prominent in the case of amyloid; see Figures 4 and 5).

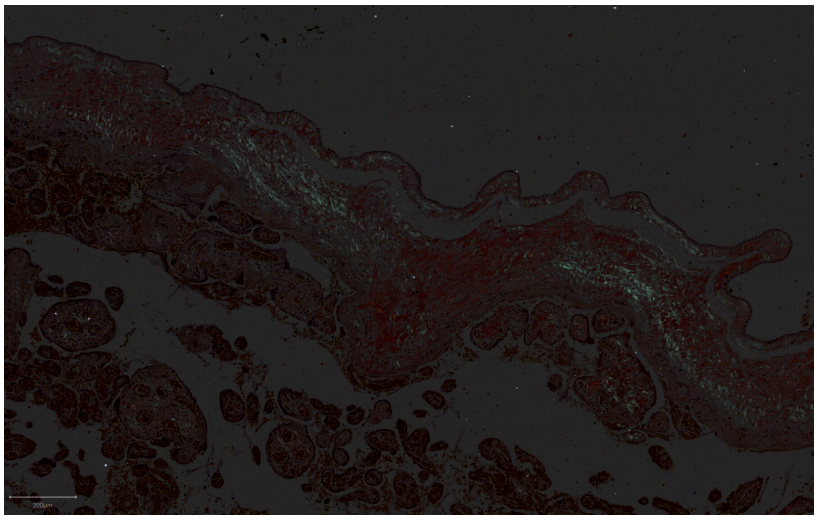


Figure 4: Dark field polarisation image of a placental surface. Note the birefringent signal visible as faint pale lines matching to putative amyloid deposits.

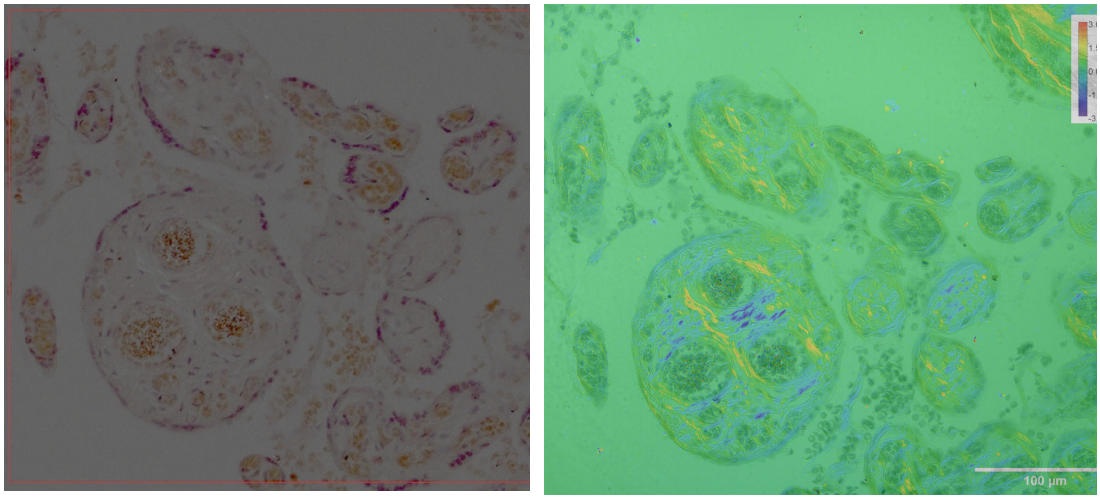


Figure 5: Focal perivascular birefringent signal of a placental villus using Congo Red (left pane) and the same specimen using quantitative polarisation microscopy (right pane); the image is an eAngle contrast colour-scale unit in degrees (x64). Note that the signal can be both visually enhanced and quantified.

These studies will involve confirmatory analyses using immunohistochemistry and other orthogonal measures which will be extended to all obstetric disorders under investigation. Other future areas of development will, as we had initially planned, focus on complementary studies based on developing broader analytical methodologies identifying immunomodulatory elements at the feto-maternal interface as well as capitalizing on our established 2,500 pregnancy *Thousand Women Study* tissue bank where we aim to make more targeted use of currently-used circulatory biomarkers (e.g. placental growth factor (PlGF), soluble fms-like tyrosine kinase (sFlt)-1, pregnancy-associated plasma protein (PAPP)-A) and miRNA 1972).

(iv) Inspiring the next generation through a Leeds-driven Cerebra research network

The last aspect of the program was to strengthen ties with other Cerebra-funded centres (principally Barcelona and Birmingham) by developing synergistic research themes. In

particular, we had planned to recruit nine MRes Medicine/Molecular Medicine students over three years (both graduate scientists and medical students) and align them with the year's prevailing research theme with a view to inspiring the next generation of researchers in this area. Unfortunately, as a result of the pandemic, all laboratory access to undergraduates, MSc and MRes students was curtailed and University access regulations still prevented supervisors from offering laboratory-based projects this current academic year. Normal access to laboratory space has now resumed with a view to opening up facilities to students as before. To this end, we are currently engaging with course coordinators to establish routes for reopening this opportunity to prospective students.

Future directions

The reopening of the laboratory facilities has enabled us to start with wet laboratory-based experiments in earnest again. Our strategy during the lockdown periods has been to minimise staffing levels and avoid rehiring for the Grade 5 technical post as much as possible in order to conserve funding, with existing staff focusing on maintaining ongoing projects. Again, the reopening of our facilities in full this year has meant that we can now readvertise for a technical support post (interviews expected in late May-early June 2022). We have also taken advantage of some of the opportunities offered by the pandemic, such as the regrouping of research work within the University. In this regard, we have strengthened our existing ties with other like-minded research teams through the inception of cross-city program, the principal amongst these being the *Futures Institutes First 1,000 Days of Life* initiative, in which we are key players. The grouping brings together a unique blend of international leaders in reproductive and obstetric biology and pathology, whose mission is closely aligned with that of the present program: determining the impact of events *in utero* on postnatal health. We have also taken part in reinvigorating areas of research neglected during the pandemic, such as improving our

understanding of cervical integrity and dynamics during pregnancy – we are just about to restart a series of studies on the topic in partnership with the Department of Pathology.

Conclusion

In summary, the team at Leeds has been adversely affected by the Covid-19 pandemic, both in terms of clinical tissue availability and physical access to research facilities. The team has adapted by frugally delaying technical hires and refocusing activities by accessing archival rather than fresh material. We are now making good progress, back up to working our potential and will be completing the hire of an outstanding technical post as a matter of priority.