BMFMS Bursary Interim Report

Microbiome sequencing in term fetal membranes in labour versus not in labour and fresh frozen versus formaldehyde fixed paraffin embedded (FFPE) membranes

Inge Christiaens, Andrew Nelson, Judith Bulmer, and Stephen Robson

Background

More than 1 in 10 infants are born prematurely worldwide, making preterm birth the leading cause of neonatal mortality and morbidity [1, 2]. The high incidence of preterm labour (PTL) and the severity of its consequences for societal health, dictates the need for our continued search for novel therapeutic strategies to treat this condition.

The onset of labour, both term and preterm, is characterised by placental and amniotic inflammation. Recent studies have suggested that the placenta harbours microbial communities and that this 'placental microbiome' may be of importance in the etiology of preterm birth [3]. The use of 16S rDNA PCR, to facilitate microbiome analysis of samples, in combination with standard microbiological culture assessments has indicated a much greater microbial colonisation of amniotic fluid and placental tissue than previously envisaged [4, 5]. ~50% of spontaneous PTL (sPTL) cases with preterm prelabour rupture of membranes (PPROM), and 15% of sPTL cases with intact membranes, had microbial invasion in amniotic fluid using this approach with the detection of microbial DNA showing an inverse prevalent correlation with gestational age. 16S rDNA PCR has also been used to analyse bacterial species in the placenta and amniochorionic membranes of term labour and (very) preterm labour; bacteria were more prevalent in sPTL <32 weeks gestation whether with intact membranes or with PPROM compared to term samples [6]. Furthermore, there was increased diversity of bacterial species detected in the preterm group. Doyle *et al.* recently confirmed that bacterial DNA is present in the majority of placental membranes from both term and preterm deliveries, irrespective of mode of delivery and there are consistently identifiable bacterial species in preterm labour [7].

Rationale

Rigorous and extensive sPTL phenotyping studies examining molecular, cellular and clinical changes that are occurring in uteroplacental tissues during labour, preterm and at term are required. These include histological changes and identification of bacteria in the placental membranes. In order to set up these integrated studies and make optimal use of the data accrued, it is first necessary to establish best practise methodologies.

To use term placentas as 'controls' in future studies examining the microbiome, it is imperative to know whether mode of delivery and the presence of labour are associated with different microbial community structures and if so, are we able to easily identify these differences. Previous research showed a marked difference between the relative levels of Lactobacillus in the vaginal deliveries compared to caesarean sections, which was easily identifiable [7].

At the RVI, placentas from preterm deliveries (<34 weeks) are all sent to Pathology for histological examination. As a standard, placental tissues are processed and formaldehyde fixed paraffin embedded (FFPE). As FFPE tissue blocks do not need to be frozen at -80 degrees Celsius, this is a cheaper and more convenient way for long-term storage for subsequent research. Previous studies of the placental microbiome all used fresh or fresh frozen samples for

DNA extraction and sequencing. Earlier pilot data from our laboratory showed that it is possible to extract DNA from FFPE placental tissues, however, whether the process of FFPE alters the microbiome community structures is unknown.

Objectives

The aim of this study was to determine if mode of delivery and the presence of labour are associated with different microbial community structures.

More specifically, our objectives were:

1. To compare bacterial transcriptome-wide 16S rRNA diversity profiles of placental membranes from low risk term spontaneous vaginal deliveries (i.e. term in labour) and term elective caesarean sections (i.e. term not in labour)
2. To examine whether 16s DNA microbiome sequencing gives similar results from FFPE membranes compared with fresh frozen placental membranes

Methods

Term (37-42 weeks gestation) placentas were collected following written informed consent, within one hour either after a spontaneous vaginal delivery (VD) from the Newcastle Birthing Centre or elective caesarean section (ELCS) from low risk women with a singleton pregnancy. We defined 'low risk' as the absence of known maternal and/or fetal complications. Exclusion criteria included, but were not limited to, fetal congenital abnormalities, clinical signs of infection/chorioamnionitis during labour or at the time of delivery, prolonged ruptured membranes (>24 hours), and antibiotics use in labour.

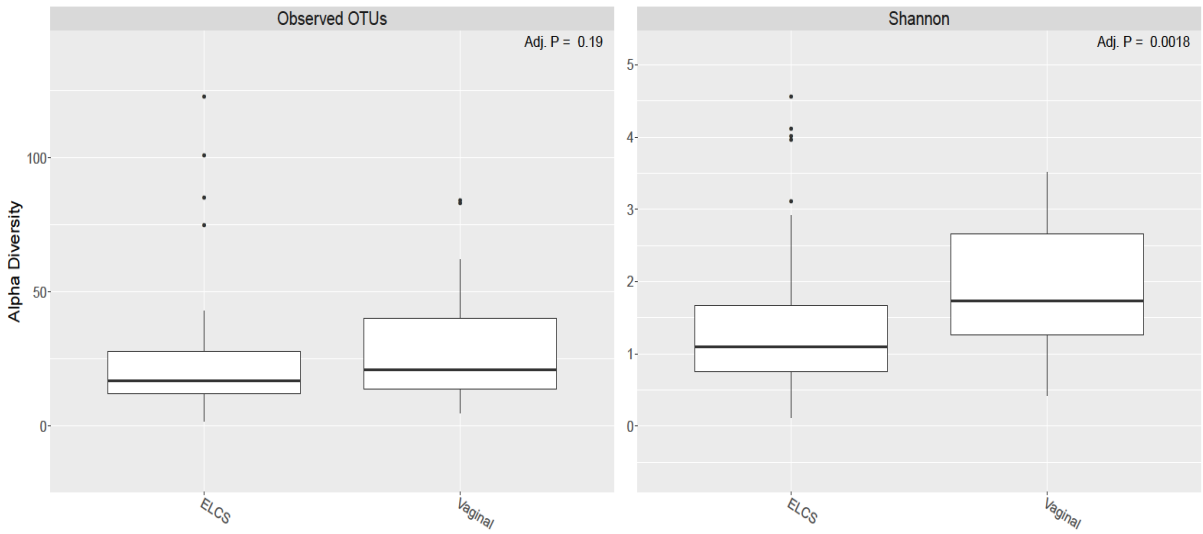
Amniochorionic membranes were carefully dissected free from other material, ensuring that both amnion and chorion laeve are included. For each placenta, two fresh frozen membrane rolls were prepared: one from the reflective amnion (P1) and one from near the placental bed (P2). Correspondingly, two FFPE membranes were prepared: P1 and P2. For each sample, an H&E section was examined histologically to assess inflammation and signs of chorioamnionitis.

DNA was extracted from all fresh frozen samples without histological chorioamnionitis in the corresponding FFPE tissue block and (n=43 versus n=69 for VD and ELCS, respectively) bacterial profiling analysis by 16S rRNA gene sequencing.

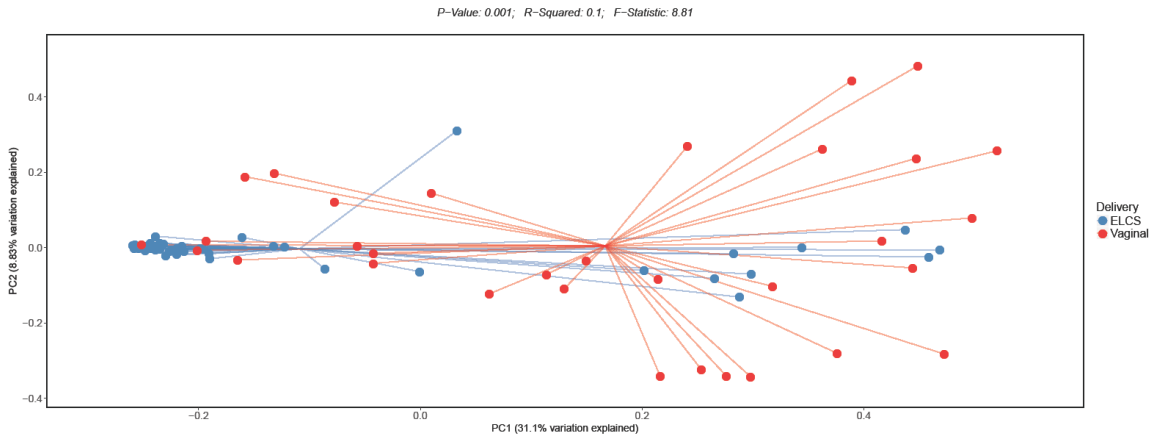
As ongoing research in our laboratory revealed that DNA extracted from FFPE membranes was of too poor quantity and quality (assessed by spectrophotometry and gel electrophoresis), the decision was made to only analyse DNA from fresh frozen samples by 16S rRNA PCR for this project.

Results

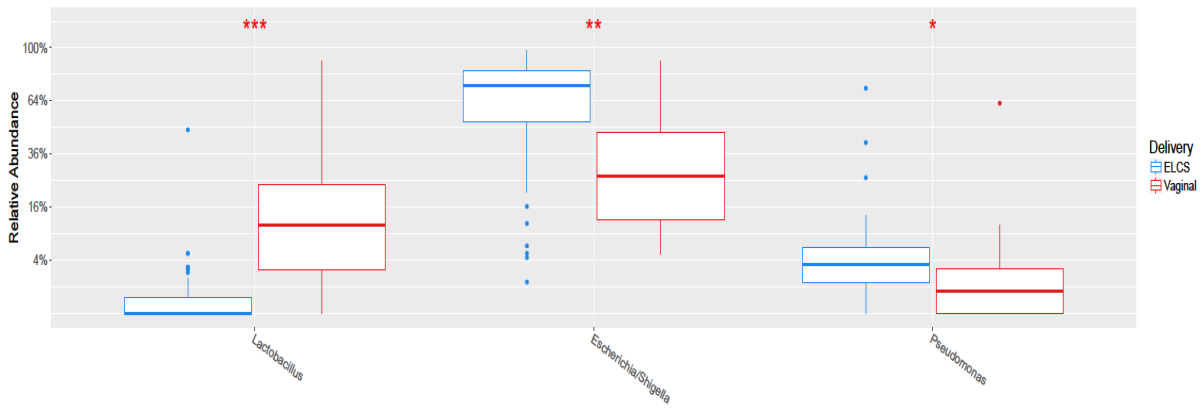
The number of putatitive bacterial species (alpha diversity) were similar between VD and ELCS samples (p=0.19) but Shannon diversity index, which takes into account species richness and proportion of each species, showed a difference (p=0.0018).



Beta diversity identified differences in bacterial community structure and membership between VD compared to ELCS (p = 0.001).



More specifically, the relative abundance of Lactobacilli was higher in membranes from VD versus ELCS samples. This was not unexpected as this confirmed what Doyle *et al.* had shown in previous research.



In conclusion, amniochorionic membranes collected after VD and ELCS in low risk term pregnancies showed different bacterial diversity profiles, which likely reflects Lactobacilli acquired from the vagina during delivery.

Further research experiments to be completed

To validate and to quantify the above results, we have to perform quantitative PCR. The appropriate probes and primers have been ordered and the PCR experiments are expected to be completed within the next few months.

Dissemination of results

An abstract has been submitted for the BMFMS Conference in April 2018. We are also in the process of drafting a manuscript to be submitted later this year.

References

1. March of Dimes, PMNCH, Save the Children, WHO. Born Too Soon: The Global Action Report on Preterm Birth. Geneva: World Health Organization 2012.

2. Goldenberg RL, Rouse DJ. Prevention of premature birth. The New England journal of medicine. 1998;339(5):313-20. doi:10.1056/NEJM199807303390506.

3. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Science translational medicine. 2014;6(237):237ra65. doi:10.1126/scitranslmed.3008599.

4. DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, Gotsch F et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PloS one. 2008;3(8):e3056. doi:10.1371/journal.pone.0003056.

5. DiGiulio DB, Romero R, Kusanovic JP, Gomez R, Kim CJ, Seok KS et al. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. American journal of reproductive immunology. 2010;64(1):38-57. doi:10.1111/j.1600-0897.2010.00830.x.

6. Jones HE, Harris KA, Azizia M, Bank L, Carpenter B, Hartley JC et al. Differing prevalence and diversity of bacterial species in fetal membranes from very preterm and term labor. PloS one. 2009;4(12):e8205. doi:10.1371/journal.pone.0008205.

7. Doyle RM, Alber DG, Jones HE, Harris K, Fitzgerald F, Peebles D et al. Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery. Placenta. 2014;35(12):1099-101. doi:10.1016/j.placenta.2014.10.007.