2018-2019

Annual Report

The University of Liverpool

Harris-Wellbeing Preterm Birth Centre

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Liverpool

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V2.0

1.	Objectives of original application

To build an internationally recognised sustainable research centre that brings together synergistic, multidisciplinary research teams focussing on personalised approach to mechanisms, phenotyping and treatments of preterm birth.

i) Genetic and metabolomic approach to preterm birth phenotyping. Our goal is to enable sophisticated biomarker phenotyping leading to personalised risk assessment in early pregnancy and better and safer use of preventative therapies. We will use high-throughput technologies to examine simultaneously millions of genes, transcripts and metabolites in an unbiased "hypothesis generating, multi layering" approach in women who suffered spontaneous preterm birth. We will then validate predictive value of these preterm birth gene/multifactorial/systematic signatures in low risk women.

ii) Developing more effective tocolytic regimens. We will elucidate the contribution of uterine environment and gestation on tocolytic efficacy by using a range of techniques, including imaging, metabolomics and transcriptomics, to determine key pathways and molecular events, allowing us to identify novel tocolytic targets.

iii) Evaluating different preventative strategies by research synthesis – global approach. Liverpool is an acknowledged world leader in research synthesis. By pioneering methods such as network meta-analysis, along with national and international collaborations, including RCOG, NICE and WHO, the best insights into effective preventative strategies will arise. Our Harris-Wellbeing of Women Research Centre will become the international reference centre for research synthesis related to preterm birth.

Our overarching aim is to use pan-omic analyses, systems biology and research synthesis to bridge the gaps between molecular mechanisms, physiology and therapeutics. Our ultimate goal is to use principles of personalised medicine to deliver targeted preventative strategies by sophisticated stratification of pregnant populations.

Our 3 work packages (WP) described below will take place between 1 April 2015 and 31 March 2020. Regular interdisciplinary meetings take place to discuss progress and the potential implications of data emerging from each work package. In essence, we aim to create a highly complementary flow of information, which is designed to maximize the translational potential of each work package. Primary data from targeted clinical studies will feed into computational integration and analysis of complex-omic profiles, thereby providing insight into improved diagnostic markers and potential pathophysiological mechanisms. Established physiological systems will then be used to investigate better methods of regulating tocolytic responses and functional consequences of condition specific molecular signatures. Finally, data from all studies will be integrated and assessed to inform new clinical trials.

2.	Progress so far – WP1

WP1 Genomic and metabolomic approach to spontaneous preterm birth (sPTB) phenotyping

AIM 1 – To investigate preterm birth phenotypes by using multiple omics and systems biology approaches in high risk population

AIM 2 – To validate predictive value of preterm birth gene/multifactorial/systematic signatures in low risk women

Biomarkers of preterm birth project

This project has finished recruitment and is now in the analysis stage. In total, we have 566 participants. Our 'high risk' arm has 296 participants with a previous spontaneous preterm birth (sPTB) or preterm premature rupture of membranes (PPROM) under 34 weeks gestation. Our 'low risk' arm has 270 parous women with all previous births at term. Participants had two study visits in pregnancy: the first at 15-18 weeks gestation and the second at 19-22 weeks gestation, and they gave permission for the study team to review their medical records to obtain pregnancy outcome data. At each study visit participants gave: detailed medical histories; samples of blood, urine and vaginal swabs; and cervical length measurements. Some participants also donated stool samples.

Details about pregnancy complications and birth outcomes have now been carefully obtained. The clinically available data (such as cervical length and quantitative fetal fibronectin) have been used to assess the utility of an algorithm called 'QUiPP'. QUiPP gives a risk prediction for preterm birth and is being adopted in clinical practice but has had little analysis outside of London. Our paper analysing QUiPP's utility in our cohort is currently under review.

The first stage of our systems biology analysis is to determine the utility of clinically available data to predict preterm birth in this cohort. The clinically available data are currently being used to form a model of preterm birth prediction. We will then use this model to inform the analysis of other arms of the biomarker project to assess whether and how each of these 'omics' can improve our preterm birth prediction.

Two clinicians have carefully reviewed the medical notes of all participants who delivered preterm to determine whether there were clinically important 'contributing factors' that led to the preterm birth. This information will be used in the analysis of each 'omic' stream to try to better understand the multiple causes of preterm birth.

Genome – Scientific Update

Results from the Genome-wide association studies (GWAS) of the large patient cohort, using the UK Biobank Axiom array (Thermo Fisher/Affymetrix), have now been analysed using PLINK software. These results have been combined with the raw data from the pilot cohort, increasing patient samples and allowing for comparison of the phenotypic groups: spontaneous preterm birth (SPTB), preterm premature rupture of membranes (PPROM), high-risk patients that delivered at term (HTERM) and "low risk" or healthy parous women with previous births delivered at term (LTERM). When comparing cases to controls, no genome-wide significant differences were identified in frequencies of any single nucleotide polymorphisms (SNPs). Further investigation is underway to analyse SPTB and PPROM groups.

2.	Progress so far – WP1

Transcriptome – Scientific Update

RNA is currently being extracted from maternal blood samples from a further 550 patient samples, using the PreAnalytix RNA extraction kit. The samples are currently undergoing Nanodrop to check for samples quality prior to processing on the same Clariom D (Thermo Fisher) array as the pilot cohort. The aim will be to combine the pilot cohort and the large cohort array datasets for analysis.
In addition to this, work has been carried out with a medical student (supervised by Ana Alfirevic) to analyse the gene expression of two genes, AP2A2 and NUP93, identified as suggestive genes in the GWAS analysis (discussed above) and the previous transcriptomics analysis. Statistical analysis for gene expression data is currently underway.

Metabolomics – Scientific Update

In addition to the previous smaller cohort, serum samples from a further 647 samples have been processed on the NMR (Nuclear Magnetic Resonance) machine at the University of Liverpool's NMR Centre. These further samples consisted of more high-risk patients (who delivered at term and preterm) and a healthy control group. Analysis of the metabolomics results from the 600 MHz (combining the small and large cohort) using statistical analysis (ANOVA and Neural Network), has shown potential for good prediction when comparing high-risk patients to a healthy control. Further investigation is taking place to identify more metabolites from the datasets for a complete list of potential metabolic biomarkers. An abstract of these initial findings has been submitted for a talk to the Royal Statistical Society Conference taking place in Belfast 2019.

Proteome – Scientific Update

We are collaborating with the Department of Clinical and Molecular Pharmacology at the University of Liverpool for proteomics analysis of the plasma samples using sequential window acquisition of all theoretical mass spectra (SWATH-MS).

We are also collaborating with the March of Dimes Prematurity Research Center Ohio Collaborative and the University of Cincinnati (Ohio, USA) for further plasma samples to be processed on Inductively Coupled Plasma Mass Spectrometry (ICP-MS). This work will measure the level of selenium and other metals and non-metals in the participant samples. The samples have been processed and our collaborators are currently in the 'data checking' stage of the analysis.

Microbiome – Scientific Update

The vaginal microbiome analysis is now in its final stages. In addition to the planned 16s rRNA gene sequencing, the samples underwent a second analysis using 'qPCR' of the 16s gene to estimate the bacterial load in each participant. This allows a better understanding of the burden of pathological bacteria in a given woman, to assess whether this contributes to her risk of preterm birth. The quality control process has been completed. We have 216 'low risk' results for the first visit, and 206 for the second visit. For the 'high risk' women we have 132 results available for the first visit, and 135 for the second visit. We are currently in the process of relating the detailed vaginal microbiome results to the birth outcomes.

System Biology Analysis Update

Pathway analysis is underway for the individual omics, with analysis plans implemented for the integration of the overall multiple omics dataset. Statistical methods such as neural networks and random forest have been identified from individual omics analysis as having potential to apply to the full dataset. This work is being completed by Professor Bertram Muller-Myhsok, Max Planck Institute for Psychiatry, Germany) and Juhi Gupta (PhD student).

2.	Progress so far – WP2

WP2 Developing better tocolytics

AIM 3 – Determine which tocolytic in combination with $MgSO_4$ is the most promising for treating threatened preterm birth

In clinical practice $MgSO_4$ is currently given for fetal neuroprotection, whilst a tocolytic is administered to delay delivery. The objective of this aim therefore was to investigate which tocolytic would be most promising in combination with $MgSO_4$.

In years 1-3 we determined the effect of combining 3 different tocolytics, atosiban, indomethacin and nifedipine with MgSO4. Our data show: combining $MgSO_4$ and atosiban was more effective in inhibiting contractions than atosiban alone; the potency of $MgSO_4$ is no greater in the presence of indomethacin than in its absence; and combining $MgSO_4$ with nifedipine does not result in any further inhibition of contractions compared to use of each drug alone.

Overall, the combination of $MgSO_4$ and atosiban inhibited contractions to the greatest extent compared to $MgSO_4$ plus indomethacin or $MgSO_4$ plus nifedipine. Hence we established a rank order of potency for the three tocolytics to be $MgSO_4$+atosiban > $MgSO_4$+nifedipine > $MgSO_4$+indomethacin.

This work formed a large part of the research undertaken by Blessing Osaghae during her PhD (submitted December 2018, viva March 2019). It is currently being prepared for publication in Reproductive Sciences, planned submission July 2019.

AIM 4 – To elucidate the contribution of uterine environment and gestation on tocolytic efficacy

We are pleased to report that this work has been completed and published in Reproductive Sciences – see publication list below.

With a masters student we are investigating a novel signalling pathway involving lactate, to gain preliminary data and to assess whether this may have a role in preterm labour initiation, as it is linked to inflammatory pathways.

With a new PhD student, Tshikaya Kaleta, we have started to investigate the roles of glycogen and glucose in uterine activity. As part of this, we will investigate if these metabolites affect the efficacy of both uterine stimulants, especially oxytocin but also the tocolytics magnesium and atosiban.

2.	Progress so far – Other research activity

Combining tocolytics with hydrogen sulphide (H2S)

Hydrogen sulphide (H_2S), similar to nitric oxide and carbon monoxide, is an endogenous gaseous signalling molecule, shown to play a prominent role in the regulation of contraction of a number of smooth muscles, including myometrium. In mammalian tissues, H_2S is predominantly produced from L-cysteine via the enzymatic activity of cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS) both of which are expressed in the uterus. Hydrogen sulphide and H_2S-releasing compounds are potent inhibitors of myometrial contractions in both the rat and human uterus, with evidence to suggest activities at the K_{ATP} channels, Cl^- channels and on Ca entry. H_2S also exerts anti-inflammatory, anti-oxidant and cytoprotective actions.
ATB-346 is a non-steroidal anti-inflammatory drug (NSAID) derived from the NSAID, naproxen, but it is coupled to a H_2S-releasing moiety, 4-hydroxy-thiobenzamide (TBZ). As other NSAIDs are used as tocolytics, e.g. indomethacin, we aimed to compare the effects of ATB-346 with naproxen and TBZ alone and the H_2S donor, Na_2S, on human myometrial contractility.

Acute application of Na_2S to contracting strips of human myometrium produced a concentration-dependent decrease in activity. The decrease in force amplitude of contraction was significant at 30µM Na_2S and for AUC at 100µM Na_2S ($P<0.01$ and $P<0.001$ respectively). ATB-346 also produced a significant decrease in force amplitude and AUC which was observed at 10µM: 74.5 (±8.4) % and 70.8 (±5.8) % of control activity respectively ($P<0.05$). This effect was further potentiated at 30µM; 41.2 (±14.0) % and 39.0 (±11.3) % of control activity respectively ($P<0.01$). Naproxen and TZB alone had small but non-significant effects on contraction amplitude and AUC at both concentrations ($P>0.05$).

Our data suggest that using H_2S donors in combination with COX inhibition provides greater inhibition of contraction than single therapies alone. We therefore wish to further explore other combination approaches involving H_2S donors as potential tocolytic therapies in myometrium. We also plan to further test the mechanism of action of H_2S in myometrium.

This work has been submitted to The Physiological Society for presentation by Dr Arrowsmith at their Annual meeting in Aberdeen, July 2019.

Oxytocin and vasopressin signalling in the uterus

Our work of establishing the contribution of oxytocin, vasopressin and their receptors to uterine contraction has centred around using novel OTR and AVPR selective ligands and antagonists to delineate their role.

In years 1-3, we have shown that in addition to V1aR, the myometrium responds to selective V1bR and V2R agonists, and we have data suggesting these receptors are expressed in human myometrium, which is a novel finding.

Our data involving OTR and V1aR antagonists suggest that the OTR is the predominant receptor through which oxytocin signals, but there is some activation of the V1aRs at supraphysiological concentrations. For vasopressin, our data suggest that AVP signals predominantly via its own receptors, and there is no involvement of the OTRs in its response.

This paper will be submitted for publication in the British Journal of Pharmacology (planned submission June 2019) with Dr Arrowsmith as first author. The delay in publishing this paper has been due to the primary author (Dr Arrowsmith) being on maternity leave.

Dr Arrowsmith has also been invited to contribute to a review on oxytocin and vasopressin signalling in the uterus for a special issue of *Current opinion in Physiology* focussing on ‘Pregnancy and the myometrium’ (planned submission August 2019).

2.	Progress so far – Other research activity

Nanomedicine techniques

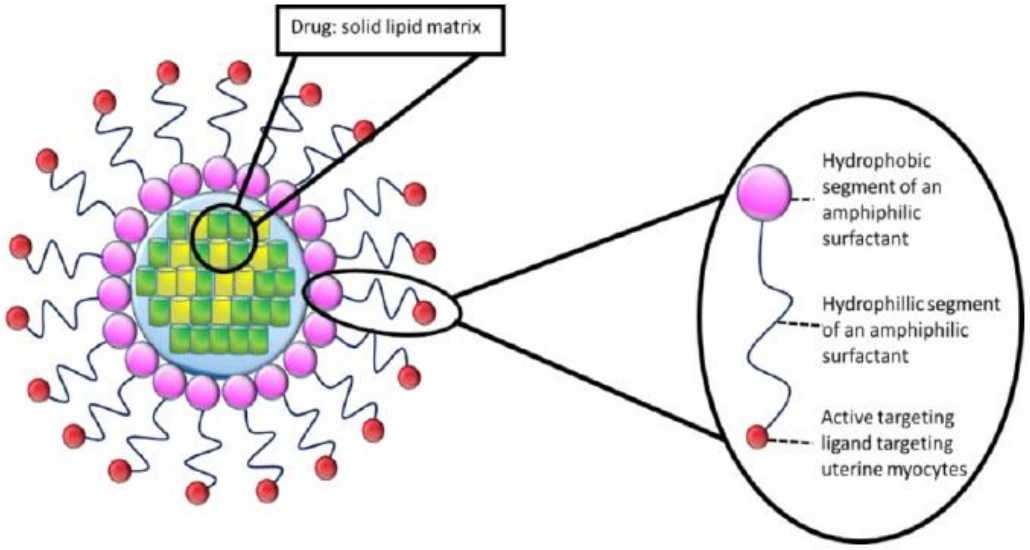
The aim of this ongoing project is to develop a range of nanoformulations with active targeting capabilities to the myometrium tissue, in order to reduce the incidence of preterm birth. We endeavour to develop library of nanoformulations through the optimisation of materials chemistry. These formulations will enable us to incorporate a tocolytic agent that otherwise causes clinical implications.

Indomethacin is a highly hydrophobic chemical entity that falls into the Biophamaceutical Classification System (BCS) as a class II drug, a highly permeable moiety exhibiting poor aqueous solubility. These properties result in high dosage requirements and ultimately result in toxicity risks with a high possibility of maternal-fetal side effects. Briefly, these side effects may include oligohydramnios, ductus arteriosus, kidney implications, hypertension, jaundice and in some cases mortality. The field of nanomedicine has previously showed immense benefit in formulating pre-existing drugs that have poor aqueous solubility and associated toxicity when they exist as small molecule drugs; including the renowned discoveries of Doxil® and Vyxeos®, both as nano-anticancer treatments. The key benefits of nanomedicines are attributed to lower dosage requirements for the same or enhanced therapeutic effects, site specific delivery, reduced toxicity and biodegradability. Additionally, for to the nanoformulation of molecules that have already received approval by the Food and Drug Administration (FDA), the translation from laboratory research to clinic is somewhat quicker in comparison to the approval of new medicines. As a result, the nanomedicine field has received a huge amount of interest for new formulations for both acute and chronic clinical implications; with intense focus on drugs with poor pharmacokinetic parameters. Moreover, this opens a door towards further development within obstetrics, with nanomedicine at the heart of this exciting, novel research. Within the field of obstetrics, there is an enormous requirement for new developments of drug formulations, with our main focus being the prevention of preterm birth. There are several sub-divisions of nanomedicine types which are predominantly determined by the excipients used in each formulation. The main categories are derived from lipid-based formulations, polymeric nanoparticles or inorganic nanoparticles.

Our primary focus is to develop a lipid-based formulation due to the high degree of biodegradability and biocompatibility of the excipients. In particular, solid lipid nanoparticles (SLNs) are of significance due to documented advances including improved storage stability, easy scale up at a low cost and enhanced solubility profiles.

2.	Progress so far – Other research activity

Nanomedicine techniques



Solid lipid nanoparticles consist of an interior solid matrix of a solid lipid, commonly a triglyceride derivative and the drug that solidifies in solution and stabilised by an amphiphilic surfactant. An amphiphilic surfactant has a hydrophobic and a hydrophilic region which provides stability in an aqueous dispersion, therefore protecting the drug from rapid clearance and undesired pharmacological effects. In addition, these surfactants have the ability to be functionalised with decorative endogenous ligands which provides active targeting capabilities to the myometrial tissue.

In turn, this decreases unwanted accumulation in other cell types throughout the body, hence decreasing unwanted side effects. Having particles with a more specific delivery vehicle such as the lipid nanoparticle, causes a decrease in the dosage required and therefore decreasing potential placental transfer and improving maternal-fetal benefits.

Thus far, the project has involved materials chemistry optimisation in order to develop an understanding of the formation of these nanoparticles and how the experimental parameters can change the particles defining characteristics. The characteristics of high importance include size, polydispersity and crystallinity which can be used to predict stable formulations to take forward for further development. In addition, these parameters can also be collated to understand the capability to enhance drug loading in each of the variant formulations developed. To date we have developed an indomethacin loaded formulation containing 0.8 wt% drug that can be lyophilised and stored as a powder. Current optimisation is ongoing to enhance the drug loading through developing a library of different formulations through the usage of different lipids and varying the chemical stabilisers.

Simultaneously, there are other projects underway looking at alternative nanoformulations including solid drug nanoparticles, in order to discover a different lead candidate that may also be used with potential higher drug loading capabilities. The different nanoformulations may be compared through *ex vivo* studies and the most appropriate candidates will be taken forward for further testing.

2.	Progress so far – Other research activity

Multiple pregnancy - Examining the myometrial transcriptome in twin pregnancy

Premature activation of uterine contractions underlies spontaneous preterm delivery. There is an urgent need for a better understanding of why twins are at high risk of preterm delivery and for better modes of treatment and prevention. Key to this is determining the differing pathophysiological mechanisms of preterm birth in singleton and twin pregnancy, including the role of the myometrium and premature uterine contraction. We hypothesised that there would be differences in myometrial gene expression between the two pregnancy groups.

In year 3, Dr Sharp and Dr Arrowsmith secured funding from The British Maternal and Fetal Medicine Society (BMFMS) in association with The Twin and Multiple Pregnancy Association (TAMBA) to undertake a high throughput RNA sequencing study, to investigate the key differences in the human myometrial transcriptome between singleton and twin pregnancies.

In year 4, total RNA was extracted from 24 biopsies of myometrium from women undergoing pre-labour CS with singleton or twin pregnancy at term or preterm (n=6 per group). RNA integrity was assessed using an Agilent Bioanalyser. RNA with an integrity number (RIN) of 7 or above was considered acceptable.

Transcriptomics analysis of myometrium from singleton and twin pregnancies at term or preterm was performed by RNA-sequencing at the University of Liverpool's Centre for Genomic Research facility. Total RNA underwent PolyA-selection, cDNA synthesis, fragmentation and indexing to create 24 RNASeq libraries which were prepared using a protocol for Illumina technology. Paired-end sequencing was performed on the Illumina HiSeq 4000 platform using sequencing by synthesis chemistry to generate 2 x 150bp paired-end reads. Mapping and alignment of reads to the human reference genome (Ensembl GRCh38.dna) was performed using TopHat2 version 2.1.0. Reads were counted according to the gene features which they mapped to using HTSeq-count version 0.6.1p1. Identification of differentially expressed genes (DEGs) was performed using DESeq2 package in R software. Gene Ontology (GO) functional term and Kyoto Encyclopaedia of Genes and Genomes (KEGG) response pathway analysis was performed using the R package 'gage'.

Four contrasts were performed:

1. Singleton Preterm (SP) vs. Twin Preterm (TP)
2. Singleton Term (ST) vs. and Twin Term (TT).
3. Singleton Preterm (SP) vs. Twin Term (TT)
4. Singleton Term (ST) vs. Twin Preterm (TP)

Contrasts 1 and 2 were performed to identify differences between singleton and twin myometrium when matched and controlled for gestational age. Contrast 3 was performed to loosely look for the effects of uterine over distension and contrast 4 was performed to act as control for contrast 3.

Across the 24 sample cDNA libraries, 1786.7 million reads were sequenced, producing an average of 37.2 million paired-end reads per sample. Most of the reads were successfully aligned (91-93%) using TopHat2. Technical and biological variation between samples and sample groups was assessed by pairwise scatterplot of read counts. Between groups, the variation was not strong. Principal component analysis (PCA) of the first two principal components using the log10 count data from all libraries also confirmed that sample groups could not be clearly separated. A sample correlation heat map of the correlation of all libraries also showed that the correlations are similar both within and between groups and hence suggested that the number of differentially expressed genes would be small.

2.	Progress so far – Other research activity

Multiple pregnancy - Examining the myometrial transcriptome in twin pregnancy

When matched for gestation (contrasts 1 and 2), only 3 DEGs were identified between singleton and twin myometrium. These genes correspond to: *ADRA1D* (alpha 1D adrenergic receptor) and *DPY19L2* (dpy-19-like 2), which were up-regulated in preterm twin pregnancy compared to preterm singleton pregnancy, and *HLA-DQA2* (major histocompatibility complex class II, DQ Alpha 2) which was down-regulated in twin pregnancy at term compared to term singleton pregnancy. This suggests that there is little, if any, difference in the transcriptome of singleton and twin pregnancies.
Uterine over-distention is thought to be one mechanism that may contribute to the higher rates of preterm birth in twin pregnancy. To examine the effects of stretch on myometrial gene expression, we compared singleton preterm samples with twin term samples (contrast 3) as this where differences in neonatal birthweight (a surrogate for stretch) would be at its maximum. This revealed 99 differently transcribed elements, including protein-coding transcripts, pseudogenes and lincRNAs. 24 were at a higher level in the twin myometrium and 75 were at a lower level in the twin myometrium. 84 were known protein coding genes (14 were upregulated and 70 were down regulated in twins) and included changes in pro-inflammatory genes and genes associated with regulation of myometrial contraction. Contrast 4 (singleton term vs. twin preterm) identified only one DEG- *CA1* (Carbonic anhydrase) which again suggests little difference exits between the transcriptome of singleton and twin myometrium.

Genes were assessed for biological process Gene ontology (GO) term enrichment and KEGG pathway enrichment. The top GO-term biological processes identified as being altered in twin term myometrium compared to preterm singleton myometrium included immune response, regulation of immune response and inflammatory response. Active KEGG pathways included cytokine-cytokine receptor interaction, chemokine signalling, toll-like receptor signalling and nod-like receptor signalling, which are all important inflammatory pathways.

This work was the first to explore changes in the myometrial transcriptome between singleton and twin pregnancy groups. It was disappointing to find so few changes in myometrial gene expression between them. However, it may reflect the fact that these samples were all obtained pre-labour. We chose to examine samples from these women with the intention of being able to study changes in gene expression between groups independently of changes that may occur with labour onset. Samples collected at the time of labour (preterm or term), irrespective of the initial mechanistic trigger, may be too biologically similar to distinguish changes in expression between sample groups. For example, the activation of similar final common pathways leading to labour such as inflammation, may mask any significant detectable changes between groups. Therefore, further examination of changes in expression with labour onset for both groups may yield more meaningful analysis and point to different mechanisms (if any) responsible.

This work has been written up for submission to PLoS ONE with Dr Arrowsmith as first author and Dr Sharp as final author. Planned submission is May 2019.

2.	Progress so far – WP3

WP3 Evaluating current preventative strategies by research synthesis – global approach

AIM 5 – To establish an ongoing research synthesis of data from clinical trials investigating strategies for preventing labour in women with short cervix

Network meta-analysis (NMA)

Our previous year-end report proposed a Cochrane systematic review and network meta-analysis of interventions for women of high risk of preterm birth, in order to rank the effectiveness of treatments for pregnant women with short cervix, in particular. Our project has expanded to review and compare multiple treatments for two different populations of pregnant women at high risk of preterm birth. The first population are at risk due to factors related to pregnancy history or to biomarkers (ie, when women without symptoms of preterm labour are found to have a short cervix on ultrasound or a positive fetal fibronectin test). The second population of women are at risk due to factors linked to an infection, including women with a history of preterm premature rupture of membranes or chorioamnionitis, or a women with a positive urine culture or vaginal swab. Our network will include randomised clinical trials of interventions to prevent preterm birth in these two populations of women, and we will rank the usefulness of the interventions for each population. The two networks will likely comprise different interventions, but we anticipate including: progesterone, cervical cerclage, cervical pessary, prophylactic antibiotics, prophylactic tocolytics, bed rest, vitamin supplements and any combination of the named interventions. Our study protocol is forthcoming:

Medley N, Donegan S, Tudur-Smith C, Goodfellow L, Caldwell D, Alfirevic, Z. Interventions to prevent preterm birth in high risk women with singleton pregnancy: a systematic review and network meta-analysis (Protocol). Cochrane Database of Syst Rev 2019 Issue XX forthcoming.

We plan for publication by the end of 2019.

Cochrane overview of systematic reviews

Medley N, Vogel JP, Care A, Alfirevic Z. Interventions during pregnancy to prevent spontaneous preterm birth: an overview of Cochrane systematic reviews. Cochrane Database Syst Rev 2018 Issue 11. Art. No.:CD012505. DOI: 10.1002/14651858.CD12505.pub2

Our overview of all Cochrane systematic reviews relevant to prevent preterm birth was published in November, 2018. The publication has succeeded in summarising all preterm birth evidence in an accessible way for a diverse reader audience. A detailed report on the impact of this Cochrane review is available at https://cochrane.altmetric.com/details/51177818. With an Altmetric attention score of 193, the preterm birth Overview is in the top 5% of all outputs ever scored by Altmetric. (Altmetric has tracked 12,789,324 outputs to date.) For the Cochrane context, the Overview is no. 127 of 10,430 outputs and no. 3 of Cochrane outputs of a similar age. The Overview has been shared in 343 tweets from 253 users, with an upper bound of 500,681 followers. The Overview was mentioned in an Evidently Cochrane blog from November 16, 2018, http://www.evidentlycochrane.net/premature-birth and was the subject of 'Students 4 best evidence' blog - https://www.students4bestevidence.net/interventions-for-reterm-birth-a-cochrane-review. The Overview is available in Spanish, Bahasa Malaysian, Portuguese, Russian, Thai and Chinese.

2.	Progress so far – WP3

WP3 Evaluating current preventative strategies by research synthesis – global approach

Progesterone trials individual patient data (IPD) repository

N Medley and Z Alfirevic secured funding to pilot methods to establish a Cochrane-affiliated data repository (NIHR Cochrane Collaboration Programme grant, Dec 2017 – June 2020). The project will build a website and infrastructure to host cleaned, individual-patient datasets (IPD) from clinical trials of progesterone to prevent preterm birth. We will also establish management systems to facilitate onward data-sharing via an approvals panel. Researchers who want to work on progesterone datasets will submit a study protocol and signed legal agreements before sharing data.

The question of progesterone and preterm birth prevention is not yet solved, and our goals for this project were twofold – 1. To avoid research waste and enable onward data-sharing of cleaned progesterone datasets sitting with the EPPIC project (https://www.pcori.org/research-results/2017/evaluating-progestogen-prevention-preterm-birth-international-collaborative), and 2. To enable Cochrane to pilot different methods for hosting and sharing clinical trials data, to improve Cochrane systematic reviews and other evidence synthesis projects. Alongside the data repository built at Liverpool University, we have proposed that Cochrane collaborate with two organisations with experience of hosting and sharing clinical trials data – the UK Data Service, https://www.ukdataservice.ac.uk and Vivli, https://vivli.org. By comparing these three options for Cochrane to facilitate re-use of clinical trials' data, we aim to identify barriers to data sharing and to propose workable solutions.

3.	Impact

Publications in Print

Medley N, Vogel JP, **Care A, Alfirevic Z**
'Interventions during pregnancy to prevent preterm birth: an overview of Cochrane systematic reviews'
Cochrane Database of Systematic Reviews 2018, Issue 11. Art. No.: CD012505
DOI: 10.1002/14651858.CD012505.pub2

Osaghae B E, Arrowsmith S, Wray S
'Gestational and Hormonal Effects on Magnesium Sulfate's Ability to Inhibit Mouse Uterine Contractility'
Reproductive Sciences 1-10
DOI: 10.1177/1933719119828089

Care A, Ingleby L, **Alfirevic Z, Sharp A**
'The influence of the introduction of national guidelines on preterm birth prevention practice: UK experience' (2018)
BJOG https://doi.org/10.1111/1471-0528.15549

Carter J, Tribe RM, Sandall J, Shennan AH; **UK Preterm Clinical Network**
'The Preterm Clinical Network (PCN) Database: a web-based systematic method of collecting data on the care of women at risk of preterm birth'
BMC Pregnancy Childbirth 2018 Aug 17;18(1):335

Ivandic J, **Care A, Goodfellow L**, Poljak B, **Sharp A**, Roberts D, **Alfirevic Z**
'Cervical pessary for short cervix in high risk pregnant women: 5 year experience in a single centre'
The Journal of Maternal-Fetal & Neonatal Medicine 2018 Sep 25:1-7

3.	Impact

Sterpu I, Anfelter P, **Wray S**, Kaihola H, Åkerud H, Wiberg-Itzel E
'The association of second trimester biomarkers in amniotic fluid and fetal outcome'
J Matern Fetal Neonatal Med. 2018 May 6:1-6. doi: 10.1080/14767058.2018.1469127

Almohanna A, **Wray S**
'Hypoxic Conditioning in blood vessels and smooth muscle tissues: effects on function, mechanisms, and unknowns'
Am J Physiol Heart Circ Physiol. 315(4):H756-H770

Publications submitted

Goodfellow L, Care A, Sharp A, Ivandic J, Poljak B, Roberts D, **Alfirevic Z**
'Effect of QUiPP prediction algorithm on treatment decisions in women with a previous preterm birth: a prospective cohort study'
Submitted to BJOG January 2019.

Any Implications for Policy and Practice

There are multiple implications for health policy and clinical practice following from the diverse streams of research described above. For example, if the exploration of QuiPP yields data to support use of the algorithm to prevent preterm birth in hospital clinics, then the tool may be considered for testing in clinical trials and, ultimately, endorsed by NICE guidelines for use across the UK. Likewise, the results of investigations of the effects of combinations of tocolytics on the myometrium has the ultimate aim of identifying new ways to stop uterine contraction in women in preterm labour. If we better understand the pathway through which tocolytics work to stop contractions, we can better-target specific drugs, reduce their side-effects, and ultimately improve the clinical treatment of pregnant women. Finally, the evidence synthesis project described above found several Cochrane systematic reviews to be out of date. Current evidence is needed to guide clinical treatment, and as described below with Omega-3, the results of Cochrane reviews have the potential to change clinical practice recommendations for pregnant women (see section 'Other evidence of Impact' below).

Any other Funding Obtained

January 2019 – Wellcome Trust Public Engagement Grants Scheme
Liverpool Fetal Growth Research Centre (LFGRC): Reaching out to Improve Outcomes.
A Sharp Primary applicant – Successful
£1,955

July 2018 – NIHR RfPB
PLANES: Placental Growth Factor led Management of the Small for Gestational Age Fetus: a Feasibility Study
A Sharp Primary applicant, Z Alfirevic Co-applicant - Successful
£249,998

3.	Impact

Details of User Involvement and Engagement

The Harris-Wellbeing Preterm Birth Centre has been involved in public and patient involvement and engagement activities.

20 November 2018
Faculty of Health and Life Sciences Public Engagement Showcase Event, University of Liverpool
The Harris-Wellbeing Preterm Birth Centre PPI group was shortlisted for a public engagement award. Dr Angharad Care presented an overview of the group and members of our PPI group also attended the showcase.

17 November 2018
World Prematurity Day
The Harris-Wellbeing Preterm Birth Centre supported raising awareness for World Prematurity Day. As part of the awareness day, the Harris-Wellbeing Preterm Birth Centre had a research information stand in the reception area of the Liverpool Women's Hospital and held a staff bake sale. We were supported on the day by members of our Public and Patient Involvement group.

28 September 2018
Public & Patient Involvement Meeting with national patient group "Little Heartbeats" and the Harris-Wellbeing Preterm Birth Centre PPI group to develop the content for the UKOSS PPROM study application (to investigate the role of previable prelabour rupture of the membranes).

17 July 2018
Initial discussion/meeting with national patient group "Little Heartbeats" about the design of the UKOSS PPROM study and application to UKOSS (to investigate the role of previable prelabour rupture of the membranes).

4 July 2018
NHS@70 at the Liverpool Women's Hospital. Dr Laura Goodfellow and Juhi Gupta attended a Public Engagement event, discussing the preterm biomarkers project, the microbiome and different bugs within the body.

4 May 2018
Public & Patient Engagement Meeting at the Bluecoat Chambers, Liverpool. An overview/update of the Harris-Wellbeing Preterm Birth Centre research was presented by Dr Angharad Care. Dr Andrew Sharp also presented and received feedback on two new research projects that he is leading on. Plans to broaden the membership of the PPI group were also discussed.

Social Media

Facebook – 5* rated and 147 likes
https://www.facebook.com/harriswellbeing/

Twitter – 132 followers
@WellbeingHarris
https://twitter.com/WellbeingHarris

Website
http://www.harris-wellbeingptbcentre.co.uk/

3.	Impact

Collaborations

- We are working with the March of Dimes Prematurity Research Center Ohio Collaborative and the University of Cincinnati (Ohio, USA) to perform a section of the proteomics analysis for the biomarkers project.
- Oxford Centre for Genomics, Wellcome Centre for Human Genetics, Oxford University.
- Centre for Genomics Research, University of Liverpool.
- NMR Centre for Structural Biology, University of Liverpool.
- Department of Molecular and Clinical Pharmacology, University of Liverpool.
- The Cincinnati Children's Hospital Medical Centre and the University of Cincinnati (Ohio, USA)
- Vanderbilt University and March of Dimes.
- Dr Arrowsmith and Professor Wray have ongoing collaborations with Ass Professor M Muttenthaler and C Gruber, University of Vienna

Awards

The Academy of Medical Sciences has won the Royal Society Athena Prize for an innovative scheme to increase the number of women experts in the media with the help of Professor Susan Wray - 01/08/2018.

Any Other Evidence of Impact you think relevant

Being the Harris-Wellbeing Preterm Birth Centre has given us the momentum and authority to adopt new research findings quickly. In November 2018 Cochrane published a review into omega 3 supplementation in pregnancy. This recommended omega 3 supplementation for preterm birth prevention, and was the best evidence for a new therapy to prevent preterm birth for over a decade. Using the authority developed through the Harris-Wellbeing Preterm Birth Centre we were able to discuss this evidence with our hospital Trust and we introduced omega 3 supplementation in our preterm birth prevention clinic in January 2019. We are now in discussion with other universities to assess how best to assess the mechanism of this finding, and how this should be incorporated into supplementation in pregnancy throughout the UK.

3.	Impact

Presentations

Scientific meetings attended/presented/poster/chair, etc

3rd Annual Invited Lecture – Harris-Wellbeing Preterm Birth Research Centre (13/12/2018)
Speaker: Mr Nigel Simpson, Leeds Teaching Hospital NHS Trust
Lecture Title: "Cerclage – past present and future"
(40 attendees)

The lecture is available on our website
https://www.harris-wellbeingptbcentre.co.uk/news-events/events/
and on YouTube https://youtu.be/Ruvy6tpNVxo

Zarko Alfirevic

Keynote Lecture - "Core Outcomes for Spontaneous Preterm Birth" **3rd European Spontaneous Preterm Birth Congress** *'One Problem, One Congress, One Session at a Time'*, Edinburgh (16-18/05/2018)

Keynote lecture – "Preterm birth prevention. What the evidence says?" **Strategies to prevent preterm birth, Preis School,** Florence (05 & 06/07/2018)

4th Annual UK Preterm Birth Research Conference, Bristol (13 & 14/09/2018)

Sue Wray

2018 - Plenary Lecturer - 10th Anniversary Celebration **Institute for Translational Research in Biomedicine Kaohsiung, Taiwan**
"Myometrial activity and labour outcomes"

2018 - Invited Faculty Lecturer - **University of Malaga, Spain**
"Update on the myometrium and clinical relevance"

2018 - Invited Faculty Lecturer - **University of Debrecen, Hungary**
"Uuterine environment and consequences for labour"

2018 - Invited Plenary Lecturer - **International Medical Students Quiz, Malaysia**
"How to teach and research myometrial physiology and its relevance to labour"

Angharad Care

Oral Presentation - "Three Arm Randomised Trial of Cervical Cerclage, Arabin Pessary and Vaginal Progesterone to Prevent Spontaneous Preterm Birth in Asymptomatic High Risk Women; a feasibility study (RECAP)" **3rd European Spontaneous Preterm Birth Congress** 'One Problem, One Congress, One Session at a Time', Edinburgh (16-18/05/2018)

Oral Presentation - "A 5-year review of the provision and practise of specialist preterm labour clinics: UK survey of practice" **4th Annual UK Preterm Birth Research Conference**, Bristol (13 & 14/09/2018)

3.	Impact

Scientific meetings attended/presented/poster/chair, etc

Andrew Sharp

Multiple Pregnancy - International Society for Ultrasound in Obstetrics and Gynaecology (ISUOG) meeting, London (February 2019)

Laura Goodfellow

Poster Presentation – **"**Cervical Length and Quantitative Fetal Fibronectin in Parous Women at Low Risk of Preterm Birth: An Evaluation of Normality" Goodfellow L, Care A, Alfirevic Z **3rd European Spontaneous Preterm Birth Congress** 'One Problem, One Congress, One Session at a Time', Edinburgh (16-18/05/2018)

Oral Presentation – "Is the use of the QUiPP app superior to cervical length screening alone for targeting preterm birth prevention treatment in high risk women?" **4th Annual UK Preterm Birth Research Conference**, Bristol (13 & 14/09/2018)

British Society for Microbial Technology, Autumn Microbiology Symposium 2018 "Going Overboard with Microbiology – Women and Children First", Liverpool (19/10/2018)

Oral presentations at North of England Gynaecology Society meeting, April 2019 "Is our management of preterm labour evidence based?" and "Should we be offering more early inductions for PPROM at term?" Oral presentations by three medical students supervised by Dr Goodfellow and Professor Alfirevic.

Sarah Arrowsmith

Poster presentation – "Comparing the myometrial transcriptome in singleton and twin pregnancies by high throughput RNA-seq" **Arrowsmith S**, Fang Y and Sharp A. Society for Reproductive Investigation, Paris, France, 12-16 March 2019.

Juhi Gupta

3rd European Spontaneous Preterm Birth Congress *'One Problem, One Congress, One Session at a Time'*, Edinburgh (16-18/05/2018)

Poster presentation: **Faculty of Health & Life Sciences - Faculty Poster Day**, University of Liverpool (19/06/2018)

Invited speaker – "Using Machine Learning techniques in Preterm Birth Prediction" **Machine Learning Event**, University of Liverpool (17/10/2018)

4.	Summary in terms suitable for the non-medical reader

Aims of our research

We aimed to bring together different kinds of experts to better understand the reasons why some women experience preterm birth; our research teams look at preterm birth from several different vantage points.

Looking closely at genes

We have scientists who look closely at the different types of women who have experienced preterm birth, in order to find out if differences at the level their genes might explain why they delivered their babies early. Using computer software, the group compared genetic (DNA and RNA) samples from women who delivered early with genetic samples from women who did not delivery early. In addition to genetic material, the team also analysed metabolites found in women's blood and micro-organisms found in women's serum and vaginal secretions.

Looking closely at muscles

Another research team consists of laboratory scientists who are interested in the way women's muscles react to various drugs, with the logic that stopping the muscles in the uterus from contracting will prevent preterm labour and birth. This team experiment by applying drugs used for pregnant women to sample muscle tissue from pregnant mice. In the lab, it is possible to generate contractions in mouse tissue and then eliminate these contractions with different drugs, or different doses of the same drug. It is also possible to examine how the uterine tissue 'talks' amongst itself to start a contraction or spread it across the uterus.

Getting drugs to the right place in women's bodies

A third group of scientists want to better understand why drugs seem to work in the laboratory to stop contractions in mice tissue, but don't seem to work in the hospital to stop contractions in pregnant women. One answer may have to do with whether or not the drug is able to get to the correct place (that is, to the woman's uterus) without getting diluted along the way or causing women unwanted side effects. Rather than taking a pill that goes to the stomach and then through the bloodstream, scientists aim to build a delivery system to send the drug directly to the target of the uterine tissue. Creating a microscopic particle to carry a drug is new and exciting work that may improve the effectiveness of drugs to prevent preterm birth.

The bird's eye view – summarising what we know about preterm birth

A final group of researchers consider the bigger picture of many different kinds of preterm birth research, in order to summarise answers to the questions we know fairly well and to identify the questions for which we need better answers. For example, this team has summarised clinical practice guideline recommendations, so that doctors and pregnant women can better understand which treatments for pregnant women are endorsed across the globe (such as steroids for women at risk of preterm birth) and which treatments are not helpful (such as cerclage for women with twin pregnancy). This team has also characterised the usefulness of all treatments to prevent preterm birth according to a 'traffic light' schema, so that diverse audiences can better understand the impact of specific treatments for pregnant women.

4.	Summary in terms suitable for the non-medical reader

Progress so far

Genes

This project has finished recruitment and is now in the analysis stage. In total we have 566 participants who have kindly given medical information and samples of blood, urine and vaginal secretions in pregnancy. Participants had two study visits: the first at 15-18 weeks gestation and the second at 19-22 weeks gestation, and they gave permission for the study team to review their medical records to obtain pregnancy outcome data.

Fifty-five of our participants had a preterm birth or preterm premature rupture of membranes before 34 weeks of pregnancy. The pregnancy outcome data have now been carefully looked at by two doctors to determine whether there were any 'contributing factors' that led to the preterm birth, such as infection or too much fluid around the baby. Previous work trying to predict preterm birth has struggled because of the many causes of preterm birth, and so we are trying to carefully link our data to the specific outcomes to prevent this problem.

We are currently in the data analysis stage of linking our detailed pregnancy information to the results obtained from blood and vaginal secretions samples to assess how the data interact, and whether they give us an indication of better ways to predict, and prevent, preterm birth.

Muscles

Researchers continue to explore the timing and dosage of different drugs applied to uterine tissues. Our paper on magnesium and its efficacy at decreasing uterine contractions when given on different hormonal backgrounds and gestational age has been published in Reproductive Sciences (March 2019). The first author is Blessing Osaghae, a former Harris-Wellbeing student and now Research Associate. Her second paper investigating the combination of different drugs is being written up for publication and will be submitted soon. Our work investigating how different hormones work at the cellular level on the uterus has progressed very well with two very good publications, featuring Dr Sarah Arrowsmith, a Harris-Wellbeing Fellow. Another publication with Dr Arrowsmith as first author is currently in preparation and due to be submitted for publication soon.

4.	Summary in terms suitable for the non-medical reader

Drug delivery

A PhD student in the Department of Chemistry is working on this project.

The aim of this ongoing project is to develop a range of nanoformulations with active targeting capabilities to the myometrium tissue, in order to reduce the incidence of preterm birth. We endeavour to develop library of nanoformulations through the optimisation of materials chemistry. These formulations will enable us to incorporate a tocolytic agent that otherwise causes clinical implications.

The key benefits of nanomedicines are attributed to lower dosage requirements for the same or enhanced therapeutic effects, site specific delivery, reduced toxicity and biodegradability. Additionally, for to the nanoformulation of molecules that have already received approval by the Food and Drug Administration (FDA), the translation from laboratory research to clinic is somewhat quicker in comparison to the approval of new medicines. As a result, the nanomedicine field has received a huge amount of interest for new formulations for both acute and chronic clinical implications; with intense focus on drugs with poor pharmacokinetic parameters. Moreover, this opens a door towards further development within obstetrics, with nanomedicine at the heart of this exciting, novel research. Within the field of obstetrics, there is an enormous requirement for new developments of drug formulations, with our main focus being the prevention of preterm birth.

To date we have developed an indomethacin loaded formulation containing 0.8 wt% drug that can be lyophilised and stored as a powder. Current optimisation is ongoing to enhance the drug loading through developing a library of different formulations through the usage of different lipids and varying the chemical stabilisers.

Simultaneously, there are other projects underway looking at alternative nanoformulations including solid drug nanoparticles, in order to discover a different lead candidate that may also be used with potential higher drug loading capabilities. The different nanoformulations may be compared through *ex vivo* studies and the most appropriate candidates will be taken forward for further testing.

Summarising evidence

As mentioned above, we have published two summaries of evidence relevant to preterm birth prevention aimed at two different audiences – a summary of clinical guideline recommendations and an overview of the effectiveness of treatments to prevent preterm birth. A research team is currently preparing a review of all treatments relevant to women with singleton pregnancy and high risk of preterm birth – whether this risk is due to pregnancy history or to the presence of infection with no symptoms during pregnancy. This new study will compare treatments formally in a network meta-analysis; statisticians will be able to rank order different treatments, such as cerclage or progesterone, in order to be able to say which treatment is best at lowering women's risk of preterm birth, baby death or other complications of pregnancy. A very different sort of project is also ongoing for WP3. Researchers have had funding via an NIHR Cochrane Programme grant to explore methods for establishing a data sharing repository at Liverpool University, to enable researchers to share prepared datasets of trials of progesterone to prevent preterm birth. The milestones and deliverables for this project are well-underway, including collaboration with organisations with expertise in data sharing (such as the UK Data Service) and delivery of specialist training sessions for clinicians, in order to create a community of clinicians who understand how to facilitate responsible sharing of clinical trials data.

4.	Summary in terms suitable for the non-medical reader

Implications for future prevention, treatment or cure and future work

Genes

A specific antioxidant seems to be related to preterm birth within our cohort. We are collaborating with the University of Ohio to verify this result in our full cohort. We have discussed this result with the scientists working on the 'muscles' component of our research and they are assessing how this could be integrated into their work.

Our 'biomarkers of preterm birth' project has created a database with detailed medical information and results of many readings from blood and vaginal swab samples. We are now analysing whether, and if so how, we are able to link these results a woman's chance of preterm birth.

Once this analysis is complete we aim to work with other scientists to verify our findings in other cohorts, and if our results hold true then we can assess ways of using this information to reduce a woman's chance of preterm birth.

Muscles

As we understand more about how healthcare should be personalised, we also learn that it is unreasonable for a single drug treatment to be useful in every case of threatened preterm delivery. Our work investigating how different drug combinations can affect the uterus will ultimately be useful in providing a range of preferred options. As the genes project, described above has now started to yield important information about how different genes may contribute to preterm birth, we will soon be starting to test our treatments on uterine samples matched to the different genes expressed by women.

Drug delivery

Developing safe methods of delivering medicines to the organ of effect could be critical to developing new and more effective tocolytic drugs to prevent preterm birth or alternatively to ensuring that a higher dose of tocolytic reaches the target organ (the myometrium of the uterus) rather than being lost within the body. More effective drugs and better targeting may reduce the dose required to be effective and thereby reduce the potential side effects of therapy.

Summarising evidence

An important conclusion of our systematic review of guideline recommendations was that guideline developers must avoid duplication across guidelines to avoid wasting scarce research funding.

A second important conclusion of both the guideline review and the overview of preterm birth clinical trials was the clear lack of information relevant to women at high risk of preterm birth due to multiple pregnancy. No guideline recommended an effective strategy for women with multiple pregnancy, because no clinical trial has found definitive evidence of effectiveness in multiple pregnancy. Research on preterm birth in multiple pregnancy must become a priority for clinical researchers and funders.

Overall, more basic research into the mechanisms contributing to preterm birth is needed (in both singleton and multiple pregnancy). A clinical expert noted recently, "Continually updated secondary analyses are important to direct (clinical) practice." Clinicians and pregnant women want to make decisions based on evidence. However, we also need more, good-quality clinical trial evidence for important prevention and treatment strategies such as cervical pessary and cervical cerclage (Shennan 2019. DOI: 10.1111/1471-0528.15587).

4.	Summary in terms suitable for the non-medical reader

What future research might emanate from your work?

Genes

Many of the technologies used in the 'biomarkers of preterm birth' project are new. For example the technology used to assess the vaginal swab samples was first used in pregnancy only five years ago. The ways to analyse these results are therefore also new, and we have developed a new classification system that we feel optimally describes the types of microbiome seen in pregnancy. Now that this has been developed it can easily be applied to other cohorts, to allow meaningful comparisons. We have had initial contact with other research groups about combining our findings to assess similarities, and differences between our cohorts. We plan to perform our analysis thoroughly, and then use the work developed through this project to collaborate meaningfully with other groups about our analysis methods and important findings.

Muscles

In the future we will want to investigate how we can manipulate and alter both natural hormones and drugs to work even better on the uterus. The ideas to be tested will stem from our on-going studies of both signalling pathways in the uterus, and of how the hormones signal to the uterine cells.

Drug delivery

It is conceivable that developments in this field will lead to new drug delivery systems that may be more effective and stable than those currently used. We anticipate that at some point we will be able to test some of these drug delivery systems against current, routine management in a randomised clinical trial.

Summarising evidence

The overview of clinical trials evidence relevant to preterm birth pointed to specific Cochrane systematic reviews that were out of date. Cochrane reviews of important interventions such as cervical pessary should be updated regularly, and we hope that our overview will prompt this work.

The overview also concluded that important work should be done to standardise the methods and conclusions of Cochrane overviews. Overviews of Cochrane reviews are highly accessed documents that provide summaries of clinical evidence for a diverse audience, and yet there is very little guidance for authors to complete these projects to agreed standards.

Our grant to pilot methods for Cochrane to host and enable sharing of individual patient trials' data aims to pin down the most effective means to pull together clinical trials data owners and eligible researchers who want to reuse datasets. Not only will this project improve the quality of Cochrane systematic reviews (because individual patient data are important for some research questions), reuse of data improves value for money of all research projects. To this end, funders increasingly require applicants to have a clear plan for data sharing once a project is completed. Cochrane want to know more about what is required to host data and enable onward sharing, and we anticipate our project recommendations will shape the decisions that Cochrane take forward.