Year 3 Interim Report

2017-2018

Annual Report

The University of Liverpool

Harris-Wellbeing Preterm Birth Centre

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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 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

Genome – *Scientific Update*

DNA extracted from blood of 127 high risk preterm birth patients, recruited from Liverpool Women's Hospital Preterm Birth Clinic was run on the UK Biobank Axiom array (Thermo Fisher/Affymetrix) and imputed against the Haplotype Reference Consortium (HRC). Genome-wide association studies (GWAS) array data on this pilot cohort has now been analysed using PLINK software. No significant difference in frequencies of any single nucleotide polymorphisms (SNPs) were identified in this cohort. Some regions of the genome are suggestive so further investigations are planned.

DNA from the main cohort (N=440) comprising further 50 high risk women with preterm birth, 181 high risk women with term birth and 259 healthy controls has been extracted and the DNA quality and quantity verified. The samples are being genotyped at the Oxford Genomics Centre using the Affymetrix UK Biobank Axiome array. The same analysis pipeline will be used as the pilot cohort and we will perform a meta-analysis of GWAS data.

In addition, TaqMan Allelic Discrimination assays were carried out on 433 patients to detect SNPs (EBF1 and EEFSEC) associated with the Selenium protein. Statistical analysis is underway to determine genetic differences between PPROM, sPTB and term births.

Transcriptome – *Scientific Update*

RNA was extracted from blood samples from 80 high-risk patients and ran on the Clariom D array (Thermo Fisher/Affymetrix). 31 paired samples were further investigated, comparing the term births with preterm births (PPROM and sPTB) at 16 weeks and 20 weeks of gestation. Current analysis using differential expression analysis (logistic regression) has indicated the role of the infection and immunological pathway in mediating preterm birth and selenoaminoacid pathway. These findings will be presented at the upcoming Edinburgh Spontaneous Preterm Birth Congress, May 2018. We are now collaborating with the Muglia lab part of the March of Dimes Centre, Cinncinnati, USA in investigating the role of selenium and its associated proteins in preterm birth. Results of this collaboration will be available in June 2018. Further plans are to investigate options for transcriptome analysis of the large cohort.

Metabolomics – Scientific Update

Serum samples from 129 patients, at 16 weeks and 20 weeks, were run on 600Hz NMR (Nuclear Magnetic Resonance) machine at the University of Liverpool's NMR Centre. Analysis of the metabolomics results, using statistical analysis, has shown there to be no significant differences between the metabolites of high-risk patient at 16 weeks and 20 weeks of gestation. Similar analysis was carried out comparing preterm births: PPROM and sPTB. This has suggested some unknown metabolites that may play a role in initiating PPROM or sPTB, indicating a need to further investigate the two different phenotypes.

2.	Progress so far (continued)

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

Proteome – *Scientific Update*

Statistical analysis (Linear Discriminant Analysis and prediction modelling) on placental markers in high-risk patient samples has been carried out. The predicting power for preterm birth was not significant in these placental markers.

Microbiome - *Scientific update*

The complete vaginal microbiota Harris cohort comprises of 710 participant samples from 158 participants at high risk of preterm birth and 221 participants at low risk of preterm birth. The first half of the cohort have undergone 16s rRNA sequencing analysis. The remaining 362 samples have undergone DNA extraction and quality control. These samples will have 16s rRNA sequencing analysis April-June 2018.

Dr Goodfellow has been accepted onto a workshop supported by the Natural Environment Research Council covering the conceptual and practical issues involved in the analysis of the 16s dataset. This will help Dr Goodfellow design the downstream processing pipeline using the DADA2 package. We are aiming to complete primary analysis of the vaginal microbiome in this cohort by the end of November 2018.

System Biology Analysis update

Machine Learning methodology based on the pilot cohort is being developed in preparation for the large cohort by Professor Bertram Muller-Myhsok and Juhi Gupta (PhD student).

Juhi Gupta (PhD student) visited Professor Bertram Muller-Myhsok at the Max Planck Institute in Munich to work on data integration of the multiple 'omics' data collected for the pilot cohort. Integration has now been completed and machine learning techniques are being applied to further analyse the results. The bioinformatics skills gained will be applied to the full cohort too.

2.	Progress so far (continued)

WP2 Developing better tocolytics

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

In antenatal clinics, $MgSO_4$ is currently given for fetal neuroprotection whilst a tocolytic is administered to delay delivery. Our first objective therefore was to investigate which tocolytic would be most promising in combination with $MgSO_4$.

In year one we established that combining $MgSO_4$ and atosiban was more effective in inhibiting contractions than atosiban alone in mouse myometrium. The IC_{50} values in the presence and absence of atosiban were 3.23mM (n= 9) and 9.52mM (n=11) respectively ($P<0.0001$).

In year two, we also explored combining $MgSO_4$ with another tocolytic, indomethacin. We found that the potency of $MgSO_4$ was no greater in the presence of indomethacin than in its absence. The IC_{50} values in the presence and absence of indomethacin were 11.01mM (n= 8) and 9.52 (n=11) respectively (P= not significant). Hence, we suggested that combining indomethacin with $MgSO_4$ did not confer any further tocolytic activity.

In year 3, we were investigating the effect of combining $MgSO_4$ with nifedipine, an L-type Ca channel blocker, in delaying delivery. Firstly, a dose response was carried out to determine the appropriate dose (0.01nM) to use in spontaneous and oxytocin-induced contractions. We found that the effect of nifedipine was time dependent, therefore, dose response experiments in combination with $MgSO_4$ were not carried out. Instead, a single dose of $MgSO_4$ was chosen in the presence (12mM) and absence (4mM) of oxytocin, combined with 0.01nM of nifedipine.

In comparing the results, we show that combining $MgSO_4$ with nifedipine (n=7-8) did not result in any further inhibition of contractions compared to each drug alone. The combination of $MgSO_4$ and atosiban inhibited contractions to the greatest extent compared to $MgSO_4$ plus indomethacin or $MgSO_4$ plus nifedipine. The order of potency for the combinations were $MgSO_4$+atosiban > $MgSO_4$+nifedipine > $MgSO_4$+indomethacin.

2.	Progress so far (continued)

WP2 Developing better tocolytics

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

Previous animal and human studies suggest the increase of calcium channel density and expression with increased gestation. Since magnesium's main action is on calcium channels, we then asked, could these changes in calcium channel density and expression affect the effectiveness of magnesium at different gestational ages? Our second objective therefore was to investigate whether the tocolytic effect of $MgSO_4$ could be altered by gestation.

Undoubtedly, the effect of $MgSO_4$ has been well studied in term pregnant, however its effect at different gestational time points has not been previously studied. These experiments are difficult to perform on human myometrium, as we do not have access to tissues at defined gestational stages, hence, experiments were done using mouse model.

We investigated the effect of $MgSO_4$ in the presence of oxytocin at different gestational time points. The time points investigated were non-pregnant 14-day pregnant (equivalent to about 30 weeks in human) and 19-day or term pregnant (which is also term in human). We found that there was a decrease in the potency of $MgSO_4$ as gestation increased. The IC_{50} values for $MgSO_4$ significantly increased.

To gain understanding of the mechanisms underlying these gestational changes, we further investigated the expression of L-type Calcium channels at these different gestational time points. Very little is known on changes in the expression of the L-type Calcium channel subunits in any species, and no studies has been carried out in mouse, hence the need for this research. The time points studied were non-pregnant, 14-day pregnant, 18-day pregnant, 19-day pregnant, and postpartum. We found there to be significant difference in the expression of L-type calcium channels across the gestations compared to non-pregnant.

2.	Progress so far (continued)

Other research activity

Oxytocin and vasopressin signalling in the uterus

Oxytocin receptor antagonists (ORAs) are one class of tocolytics used to treat and stop preterm labour contractions. It has long been recognised that the oxytocin receptor (OTR) shares high sequence homology with the three subtypes of vasopressin receptors; V1aR, V1bR and V2R. In addition, the native hormones, oxytocin and vasopressin can each activate all 4 receptor subtypes creating significant cross-talk between the two signalling systems. There has been a long standing interest in the contribution of these different receptors towards contraction following stimulus with oxytocin or vasopressin. Our understanding into which receptors are responsible for mediating the effects of these hormones however, has been significantly hampered by the lack of receptor-selective agonists and antagonists.

In year's one and two, we examined the effect of custom-designed, vasopressin and oxytocin receptor subtype-selective peptides (from the Muttenthaler laboratory) on human myometrial contractions. A vasopressin 1a/1bR-selective agonist demonstrated that the V1aR is present and is functional in human myometrium. We also demonstrated that a OTR-selective peptide could elicit similar responses (amplitude, AUC) to oxytocin in human myometrium but with altered frequency of contraction.

Using a selective inhibitor of the OTR, the activity of our OTR-selective ligand but not that of oxytocin, was completely abolished indicating some involvement of the AVPRs in the response to oxytocin. The effect however, was observed at supraphysiological concentrations (≥100nM) and hence suggests that, similar to rodents, the effect of oxytocin in vivo is likely to predominate via the OTRs and not the AVPRs. In year 3 we examined this further by using other antagonists to OTRs and V1aRs to confirm findings and to establish which receptors also mediate the response to vasopressin.

In paired experiments, using a second OTR antagonist, L371,257, oxytocin's effect was reduced (EC50 shifted significantly to the right) whilst concentration-response curves to AVP were not altered significantly. Selective pre-blocking of the V1aR (SR49059) inhibited the contractile response to vasopressin but also reduced oxytocin's effect. This suggests that in human myometrium, AVP functions predominately via its receptor and not via the OTR. This is the opposite to rodents where the principle receptor through which both oxytocin and vasopressin operate is the OTR. That the effect of oxytocin was reduced in the presence of a V1aR inhibitor also confirms our previous findings which suggests some involvement of the V1aR in mediating the oxytocin response.

This work is currently in preparation for publication for the British Journal of Pharmacology (planned submission December 2018). Data obtained from the oxytocin-selective peptide in this study has also been published in Science Signalling (see below). The work also attracted attention from a number of news and media outlets including The Independent (14 December 2017)

https://www.independent.co.uk/news/science/love-hormone-mental-illness-treatment-health-oxytocin-university-queensland-a8108596.html

2.	Progress so far (continued)

Other research activity

Oxytocin and vasopressin signalling in the uterus

In addition, our collaboration with Dr Gruber in year 2, investigating the effect of the insect orthologue of vasopressin, inotocin, Dr Arrowsmith has published a paper and produced a video –

Link to video: Contractility Measurements of Human Uterine Smooth Muscle to Aid Drug Development

in the Journal of Visualised Experiments (see publications below), highlighting the importance and scope of the human myometrial contraction model in drug development.

2.	Progress so far (continued)

Other research activity

Nanomedicine techniques

Encapsulating Indomethacin in solid lipid nanoparticles as innovative tocolytic technology

Andrew Sharp, Sarah Arrowsmith and Jessica Taylor

Premature birth is defined as the birth of a child prior to 37 weeks gestational age and accounts for 85% of perinatal morbidity and mortality.[1] Globally an estimated 15 million babies each year are born prematurely, with up to 1 million infant mortalities resultant of preterm birth complications.[2] Not only is preterm birth emotional burden, it is also hugely costly for healthcare systems costing over 59% of the budget allocated for neonatal care.[3] Consequently, there is a clear demand for further tocolytic drug development to enhance medicinal efficacy and ultimately reduce maternal-fetal implications and mortality.

Our aim is to develop Indomethacin containing solid lipid nanoparticles (SLNs) with enhanced tocolytic efficacy, whilst simultaneously reducing clinical side effects. We have chosen to develop solid lipid nanoparticles (SLNs) as the colloidal carrier for our tocolytic agent. Solid lipid nanoparticles are a new pioneering area of nanomedicine research which has previously been shown to have advantages over other colloidal systems including polymeric nanoparticles, liposomes and nanoemulsions.[4] SLNs are new attractive alternatives to the drug market as their excipients are biodegradable, biocompatible and are all generally considered as safe (GRAS) by governmental regulations. In addition, development of an SLN system gives us the capability to improve active targeting to the uterine myocytes by engineering PEG-lipid conjugates as stabilisers to target uterine receptors. As a result we can improve clinical uses of the drug, reduce dosage regimens and reduce any side effects caused by unwanted placental transfer; therefore lessening the potential for perinatal morbidity and mortality.

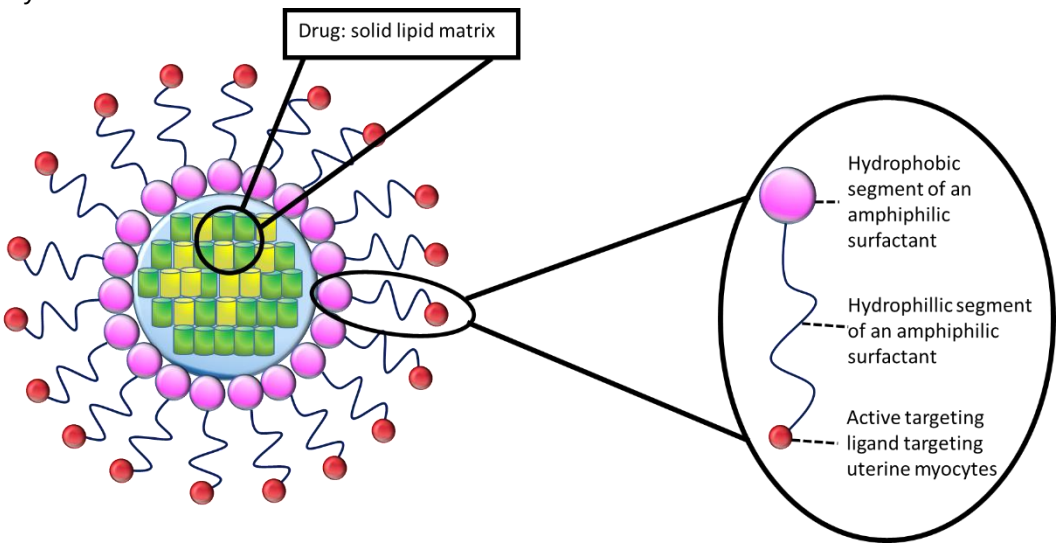

Figure 1: Solid lipid nanoparticles contain a lipid matrix which consists of a solid lipid excipient and the drug. The lipid matrix is stabilised by amphiphilic surfactants, enabling stability in a physiological aqueous environment.

2.	Progress so far (continued)

Other research activity

Nanomedicine techniques

Encapsulating Indomethacin in solid lipid nanoparticles as innovative tocolytic technology

Thus far, we have managed to encapsulate Indomethacin at 30 wt% drug loading using a blend of surfactants in the aqueous phase. We are currently optimising the solvent injection method; a simple, scalable and rapid synthetic procedure which enables us to synthesise SLNs with low shear mixing and temperature control. We have had previous proof of concept studies on mouse myometrial tissue which has promisingly shown no biological effect from the non-drug excipients. However, with loaded Indomethacin we have seen a complete prevention of contraction or a decrease in frequency and intensity as shown in Figure 2.

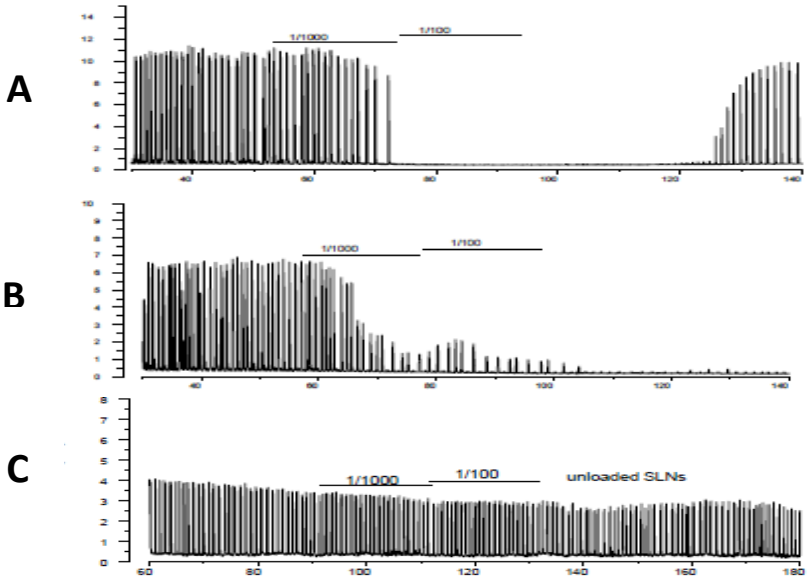

Figure 2: Graphs A and B show the contractile tissue dissected from the uterus of a mouse initiating a response to Indomethacin loaded SLNs at 30 wt% drug loading in physiological saline solution. The Y axis corresponds to the contractile force in mN against time in minutes on the X axis. Graph (A) showed a complete prevention of contractions between 50 and 120 minutes. Graph (B) showed a decrease in frequency and intensity of contractions. Graph (C) shows that unloaded SLNs have no effect on the contractility of the tissue.

Our future work primarily concerns optimising the SLN synthetic procedure in order to obtain the most physically stable environment with the highest drug loading possible. We will then further explore ex vivo biological testing to identify lead SLNs to take forward for incorporation of targeting ligands.

1 E. R. Norwitz and A. B. Caughey, Rev. Obstet. Gynecol., 2011, **4**, 60–72.
2 World Health Organization (WHO), Preterm birth factsheet, http://www.who.int/mediacentre/factsheets/fs363/en/ , (accessed 8 April 2018).
3 J. Morrison, J. Perinatol., 2005, **25**, 1–3.
4 P. Naseri, N, Valizadeh, H. Zakeri-Milani, Adv. Pharmaceuitcal Bull., 2015, **5**, 305–313.

2.	Progress so far (continued)

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 as well as developing better modes of treatment and prevention. Key to this is determining the differing pathophysiological mechanisms of preterm birth in these different populations including the role of the myometrium and premature uterine contraction. In controlled *in vitro* conditions, we have shown significant differences in contractility between myometrium from singleton and twin pregnancies and shown reduced potency of some tocolytics in twins. We hypothesise that differences in gene expression underlie these changes.

Dr Sarah Arrowsmith and Dr Andrew Sharp have 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.

We aim to determine the differentially expressed gene sets between sample groups, perform enrichment analysis based on functional roles of genes identified and use pathway analysis software to determine the functional significance of our findings.

The methodology we have chosen is based upon the expert guidance of the Centre for Genomic Research at the University of Liverpool (www.liv.ac.uk/cgr). Funding will enable the construction of 24 RNASeq libraries from 4 patient groups (6 individual replicates): (A) twin and (B) singleton pregnancy at term (>37 weeks gestation) and (C) twin and (D) singleton pregnancy preterm (<37 weeks gestation). A recent publication found a significant number of novel transcripts in myometrium with labour onset using a sample size of just 5. RNASeq libraries will be constructed for sequencing on the Illumina HiSeq 4000 platform, reference genome mapping performed with TopHat2 and differentially expressed genes identified using edgeR.

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2.	Progress so far (continued)

Other research activity

***Multiple pregnancy* -** Examining the myometrial transcriptome in twin pregnancy continued

During year 3, sufficient myometrial samples from women with twins and singleton pregnancy delivering preterm or term have been sought and processed for RNA sequencing at the University's Centre for Genomic Research (CGR). Quality control steps have shown RNA integrity (RIN numbers) to be of suitable quality (<7) for sequencing. We aim to have the samples sequenced and gene ontology analysis performed by October 2018 with outcomes ready for publication in 2019, following Dr Arrowsmith's return from maternity leave (October 2018).

The study will be the first to examine for changes in the myometrial transcriptome between singleton and twin pregnancies which will confer the much-needed scientific insights into the physiology of the myometrium with twin pregnancy. The outcomes of this study will be used to drive the formulation of new hypotheses for testing and informing new therapeutic targets aimed at improving the management of preterm birth in multiple pregnancy. We aim to present the findings at the BMFMS annual conference in 2019.

In addition, using samples from singleton and twin myometrium, Dr Arrowsmith has carried out a reference gene analysis of genes most commonly used for the normalisation of expression in qPCR studies. This is to assist in the validation of the results from the sequencing study as it is important that reference gene expression is stable between patient groups e.g. singleton and twin myometrium and across a range of gestations i.e. term and preterm samples.

Interestingly, we have found that those genes used most frequently as reference genes in studies of expression in human myometrium (e.g. 18sRNA, ACTB and GAPDH, as identified in published studies) are not suitable reference genes when comparing twin and singleton myometrium gene expression across a range of gestations. We found novel reference genes, CYC1 and YWHAZ to be more stably expressed than those used most often in the literature, with little variation in gene ranking between sample subsets. Pairwise variation analysis showed that the optimal number of reference targets for qPCR data normalisation is two. We anticipate that these findings will form a short publication in Reproductive Sciences (planned submission December 2018).

2.	Progress so far (continued)

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)

In January 2016, NMA of studies on treatment of short cervix was published as an abstract (Jarde A, AJOG) very similar to one proposed in our WP3. In collaboration with statisticians from Liverpool and Bristol, we are currently writing a protocol to update this work. Our NMA will incorporate new trial data for cervical pessary, cervical cerclage and progesterone but will also include additional treatments such as prophylactic antibiotics, bed rest and home uterine monitoring. To date there has been no such formal systematic review and network meta-analysis of all relevant interventions to prevent preterm birth in high risk pregnant women.

Keeping systematic reviews up-to-date is difficult, but researchers have proposed new strategies to improve the reviews of important clinical topics. These "living systematic reviews" integrate new trial evidence into the review as it becomes available. This model of online publication and continuous updating of evidence has been extended to NMA as 'living network meta-analysis'. Our NMA will be the first 'living NMA' to consider clinical interventions relevant to pregnancy and childbirth. We will jointly evaluate all treatments to prevent preterm birth in high risk pregnant women with singleton pregnancy and rank the treatments for their safety and their effectiveness to prevent preterm birth. We will update the findings at regular intervals to capture new trial evidence, with the first iteration anticipated by spring 2019 and published in the Cochrane Library.

Cochrane overview of systematic reviews

In February 2018, together with a collaborator at the World Health Organisation, we submitted a completed draft of a Cochrane overview of interventions to prevent preterm birth. Cochrane overviews of systematic reviews are high-impact, descriptive syntheses able to present trial evidence for multiple interventions targeting a single health problem. Our overview summarised 70 included systematic reviews of diverse interventions such as antenatal dietary education, vitamin supplements or reduced smoking during pregnancy. We assigned graphic icons to communicate both the effectiveness and the quality of trial evidence. We found clear evidence of benefit that five interventions reduced preterm birth; three further interventions reduced perinatal death. The overview will undergo peer review before its publication in the Cochrane library by summer 2018.

Systematic review of guidelines

N Medley and Z Alfirevic recently published a systematic review of the practice recommendations found in clinical guidelines relevant to preterm birth, BJOG (February 2018). From 49 clinical guidelines (including World Health Organisation guidance), the review identified several clinical practices of benefit to pregnant women, including cervical length screening for women of high risk of preterm birth; antibiotics for women with preterm prelabour rupture of membranes; and steroids for women at risk of preterm birth. The review also identified multiple guideline recommendations against clinical practices deemed ineffective; treatments such as universal cervical length screening, or the use of cerclage in women with multiple pregnancy were not of benefit. Finally, the review highlighted important clinical questions for which guidelines had few or no recommendations, such as whether or not to use cervical pessary or emergency cerclage.

2.	Progress so far (continued)

Progesterone trials individual patient data (IPD) repository

N Medley and Z Alfirevic, with the University of Liverpool and the University of York, were awarded an NIHR Cochrane Program Grant to establish an online repository of individual patient data from clinical trials of progesterone to prevent preterm birth. The two year project is based in Liverpool (start date December 2017) and will create an online platform, management structures and governance to facilitate responsible data sharing to enable better understanding of the effects of progesterone to prevent preterm birth.

Tasks during year one of the grant include: recruiting an advisory committee, establishing management and governance to structure the transfer, hosting and onward sharing of IPD datasets, and creating a website with a search function for the interested public, trial investigators and researchers. The website front page is shown below. An advisory committee, comprising diverse experts met on April 4, 2018, to discuss proposed management and governance of the repository.

Additional publications, presentations and funding awards are listed in the appropriate sections below.

3.	Impact

PUBLICATONS IN PRINT

Alfirevic Z, Stampalija T, **Medley N**
'Cervical stitch (cerclage) for preventing preterm birth in singleton pregnancy'
Cochrane Database of Systematic Reviews 2017, Issue 6. Art. No.: CD008991. DOI: 10.1002/14651858.CD008991.pub3

Medley N, Poljak B, Mammarella S, **Alfirevic Z**
'Clinical guidelines for prevention and management of preterm birth: a systematic review'
BJOG: 20 Feb 2018. DOI: 10.1111/1471-0528.15173

Van 't Hooft J, **Alfirevic Z**, Asztalos EV, Biggio JR, Dugoff L, Hoffman M, Lee G, Mol BW, Pacagnella RC, Pajkrt E, Saade GR, Shennan AH, Vayssière C, Khan KS
'CROWN initiative and preterm birth prevention: researchers and editors commit to implement core outcome sets'
BJOG. 2018 Jan;125(1):8-11. doi: 10.1111/1471-0528.14987. PubMed PMID: 29055092.

Berghella V, Palacio M, Ness A, **Alfirevic Z**, Nicolaides KH, Saccone G.
'Cervical length screening for prevention of preterm birth in singleton pregnancy with threatened preterm labor: systematic review and meta-analysis of randomized controlled trials using individual patient-level data'
Ultrasound Obstet Gynecol. 2017 Mar;49(3):322-329. doi: 10.1002/uog.17388. Epub 2017 Feb 8. Review. PubMed PMID: 27997053.

Arrowsmith S, Keov P, Muttenthaler M, Gruber CW.
Contractility Measurements of Human Uterine Smooth Muscle to Aid Drug Development.
Journal of visualised experiments: JoVE, January 2018, issue 131.

Link to video : Contractility Measurements of Human Uterine Smooth Muscle to Aid Drug Development

Muttenthaler M, Andersson Å, Vetter I, Menon R, Busnelli M, Ragnarsson L, Bergmayr C, **Arrowsmith S**, Deuis JR, Chiu HS, Palpant NJ, O'Brien M, Smith TJ, **Wray S**, Neumann ID, Gruber CW, Lewis RJ, Alewood PF
Subtle modifications to oxytocin produce ligands that retain potency and improved selectivity across species.
Science Signalling: December 2017, volume 10, issue 508.

S Arrowsmith
'Oxytocin Receptor'
Encyclopedia of signaling molecules (2nd Edition), pg 3694-3704 Ed. Sangdun Choi
ISBN 978-3-319-67198-7 Springer International Publishing

3.	Impact

PUBLICATIONS SUBMITTED

Medley N, Vogel JP, **Care A, Alfirevic Z**
'Interventions during pregnancy to prevent spontaneous preterm birth: an overview of Cochrane systematic reviews'
Cochrane Database of Systematic Reviews. Submitted February 2018.

Care A, Jackson R, O'Brien E, Leigh S, Cornforth C, Haycox A, Whitworth, M, Lavender T, **Alfirevic Z**
'Cervical cerclage, pessary or vaginal Progesterone in high-risk pregnant women with short cervix: a randomised feasibility study'
Ultrasound in Obstetrics and Gynaecology. Submitted March 2018/

Care A, Muller-Myhsok B, Olearo E, Todros T, Caradeux J, Goya M, Palacio M, Carreras E, **Alfirevic Z**
'Can Previous Preterm Birth Classification Influence Treatment of a Short Cervix in a Subsequent Pregnancy? A Comparison of Vaginal Progesterone and Arabin Pessary'
Ultrasound in Obstetrics and Gynecology, UOG-2018-0087. Accepted March 2018.

3.	Impact

Other funding obtained

Medley N, Alfirevic Z et al. Progesterone for preterm birth prevention: how to establish a Cochrane-affiliated individual patient data (IPD) repository. NIHR Cochrane Programme Grant award, Dec 1 2017- Nov 30 2019.

Care A, pitched for “Genomes for Life” funding for NMR Metabolomics 24/07/2017 and was awarded funding for NMR Metabolomics and assistance with data analysis and/or training. The funding was awarded by the Tech Directorate, University of Liverpool.

A Sharp and S Arrowsmith ‘Examining the myometrial transcriptome in twin pregnancy’ BMFMS/TAMBA, May 2017-Mar 2019

Awards

Professor Z Alfirevic – RCOG Academic Prize 2018

Angharad Care - Prize Winner - 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 Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)

Juhi Gupta – First Prize Winner - Poster presentation “Genome-wide association study of high-risk spontaneous preterm birth” – Institute of Translational Medicine Research Day, University of Liverpool (06/07/2017)

Collaborations

Angharad Care – Visit to March of Dimes Prematurity Research Centre Ohio Collaborative (Vanderbilt University, Nashville, USA) 29/04/2017-04/05/2017. Visit funded by The Bernhard Baron Travelling Scholarship.

WP1 team collaborating with Professor Louis Muglia Lab in Cinncinnati Childrens Hospital part of the March of Dimes Preterm Birth Prevention Centre, USA to establish levels of selenium and associated proteins in preterm birth patients.

Collaboration with Little Heartbeats national patient group to investigate the prevalence and current management of previable PPROM (preterm prelabour rupture of membranes) through UKOSS study application.

WP2 team ongoing collaborations with Ass Prof Markus Muttenthaler, and Ass Prof Christian Gruber, University of Vienna.

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 and raising awareness of preterm birth.

March 2017
One of our patients (Christine) was interviewed and spoke movingly about the loss of her baby boy as a result of preterm birth. Christine's interview can be viewed on our website and the Wellbeing of Women video 'how we are making a difference'.

2 November 2017
Lady Harris visit and Public & Patient Involvement Meeting
Lady Harris met with staff and members of our PPI group in Liverpool and members of the Harris-Wellbeing PTB Centre team provided an update about our research.

17 November 2017
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.

December 2017
Prima women's magazine - Christmas edition
'Our miracle babies are a Christmas wish come true'
Using social media, we identified a number of women who were willing to help by sharing their stories to help raise awareness of preterm birth. One of our patients (Liza) was invited to be interviewed in London and her story was published in the Christmas edition of Prima the monthly women's magazine.

February 2018
Preliminary project application written with national patient group “Little Heartbeats” to UKOSS, NPEU Oxford, to investigate the role of previable prelabour rupture of the membranes. Results of application will be available May 23rd 2018.

Social Media

Facebook – 5* rated and 115 likes
https://www.facebook.com/harriswellbeing/

Twitter – 86 followers
@WellbeingHarris
https://twitter.com/WellbeingHarris

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

3.	Impact

PRESENTATIONS

Scientific meetings attended and presented

Harris-Wellbeing Preterm Birth Research Centre - **2nd Annual Invited Lecture** – Friday 15 December 2017
Speaker: Professor Phillip Bennett, Imperial College London
"Bugs, biomarkers and babies. A systems medicine approach to preterm labour"
(25 attendees)

Zarko Alfirevic

- 1st World Congress on Maternal Fetal Neonatal Medicine (WMFNM), London (23-26/04/2017)
- FMF World Congress, Slovenia (24-29/06/2017)
- 3rd Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)
- SASOG Congress 2018, Durban (01-08/03/2018)
- RCOG 2018 Singapore (21-24/03/2018)

Sue Wray

- Symposium Keynote "Hypoxia and myometrial function: something old and something new" Society Reproductive Investigation, Orlando, USA (03/2017)
- Invited Lecture "Hypoxia and uterine function: something old and something new" Walter & Matilda Research Institute, Pittsburg, USA (03/2017)
- Invited Lecture "Hypoxia and uterine function: something old and something new" Department of Physiology, Case Western University, Ohio, USA (04/2017)
- Invited lecture "Hypoxia and myometrial contractions" Department of Physiology, Cologne, Germany (05/2017)
- Keynote Lecturer, "Myometrial Physiology – the rhythms of contraction and life" International Union Physiological Sciences Conference, Rio, Brazil (07/2017)
- 3rd Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)
- Invited lecture "Myometrial physiology and pathophysiology", Australian Physiological Society Annual meeting, Melbourne, Australia (11/2017)
- Invited lecture "Hypoxia and uterine contractions: Something old and something new" Department of Physiology, Heidelberg, Germany (12/2017)

Angharad Care

- Visit to March of Dimes Prematurity Research Centre Ohio Collaborative - Vanderbilt University, Nashville, USA. Visit funded by The Bernhard Baron Travelling Scholarship (29/04/2017-04/05/2017)
- Oral Presentation & Prize Winner "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 Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)
- Invited speaker "Preterm birth prevention: what we know and what we don't" - Fetal and Maternal Medicine Study day, Corniche Hospital, Abu Dhabi (16/12/2017)
- Oral presentation "Using NMR for Prediction of Spontaneous Preterm Birth" - University of Liverpool Metabolomics Symposium (28/03/2018)

3.	Impact

PRESENTATIONS

Scientific meetings attended and presented

Andrew Sharp

- Oral presentation and Prize - ISSHP-September Berlin-international society for study of hypertension in pregnancy
- Oral presentation - ISUOG-September Vienna-International society for ultrasound in obstetrics and gynaecology
- TAMBA (twins and multiple birth association) study day-December Liverpool (included a focus on research into preterm birth prevention)

Laura Goodfellow

- 3rd Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)
- Oral presentation in 'Stump the experts' session- RCOG National Trainees Conference, Leeds (16-17/11/2017)

Sarah Arrowsmith

- Oral presentation *"Reference gene analysis for gene expression studies in singleton and twin myometrium"* - 3rd Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)

Nancy Medley

- Oral presentation "Clinical practice recommendations to prevent or manage preterm birth: a systematic review of practice guidelines" - 3rd Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)

Blessing Osaghae

- Oral presentation "The dual tocolytic effect of magnesium plus nifedipine on mouse myometrial contractility" - 3rd Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)

Juhi Gupta

- Poster presentation "*Genome-wide association study of high-risk spontaneous preterm birth*", first prize winner – Institute of Translational Medicine Research Day, University of Liverpool (06/07/2017)
- Poster presentation "*Placenta protein marker study of high-risk spontaneous preterm birth in Caucasian population*" – Early Career Researchers Symposium, University of Liverpool (04-05/09/2017)
- Oral presentation *"Genome-wide association study of high-risk spontaneous preterm birth in Caucasian population"* - 3rd Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)

Seham Alsaif

- Oral presentation *"The effect of obesity on uterine contractility"* - 3rd Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)

Jenny Gomersall (student)

- Oral presentation *"A comparison of three methods of diagnosing preterm labour (PTL) <30 weeks"* - 3rd Annual UK Preterm Birth Research Conference, Leeds (07-08/09/2017)

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).

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

Progress so far

Genes
Researchers have collected samples of genetic materials from 127 women with and 433 without preterm birth; the samples are ready to be sent for further analyses. We have collected similar samples including plasma and serum from women at risk of preterm birth at 16 and 20 weeks in their pregnancy; the initial results of this comparison show that women's immune systems may contribute to whether or not women go on to experience preterm birth with a particular focus on a pathway involving a common dietary antioxidant. Examination of blood serum from 129 women is ongoing and as a result of our findings, we are looking to see if certain minerals and their binding proteins are found at decreased level in preterm birth samples compared to term sample. The testing and comparison of micro-organisms found in samples of vaginal fluids from over 350 women with and without preterm birth is ongoing.

Muscles
Researchers continue to explore the timing and dosage of different drugs applied to uterine tissues. As experience has increased the rate of obtaining useful data has increased. Our paper on magnesium and its efficacy at decreasing uterine contractions when given on different hormonal backgrounds and gestational age, is about to be submitted. The first author will be the Harris PhD student, Blessing Osaghae. 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 Fellow.

Drug delivery
Scientists hope to better understand how to get an effective dose of a drug directly to the uterine tissue to stop contractions by testing different microscopic particles to carry the drug and different targets on the uterus.

Summarising evidence
Researchers are planning a new study that will compare all treatments for pregnant women at high risk of preterm birth, in order to produce a ranking of which treatment is best for both safety and effectiveness. Treatments to be compared included cervical cerclage, cervical pessary, progesterone, prophylactic antibiotics and bed rest.

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

Implications for future prevention, treatment or cure and future work

Genes
Personalised medicine may allow healthcare providers to tailor treatments to fit an individual woman. The more we know about how (and if) genes contribute to preterm birth, the better we will be in identifying women who are at higher risk. Women who are not at higher risk will also be able to avoid unnecessary treatments. The same logic applies to a better understanding of the microscopic organisms that live in all women's bodies; if we can understand why and how these contribute to various problems pregnant women face, we will be better able to know which pregnant women will benefit from which treatments.

We are currently investigating a specific antioxidant mineral that has been linked to preterm birth from our transcriptomic work and other group's genetic work. If we can establish if there is a genuine link to preterm birth, it is possible that we could target specific dietary supplements as a next step towards preterm birth risk reduction.

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 starts to identify how genes may contribute to preterm birth, we can then test our treatments on uterine samples matched to the different genes expressed by women.

Drug delivery
The potential to deliver drugs more effectively to the target organ via nanomedicine techniques may have significant benefits for patients. This effect could be either by improving the effectiveness of these drugs in preventing preterm labour, or by reducing potential side effects allowing more effective medications to be used safely.

We anticipate that improved approaches to delivery of tocolytics could prevent babies being born early, reducing complications to the baby's health and saving money in both the short and long term.

Summarising evidence
There is a large body of work of all kinds evaluating different causes of and treatments for preterm birth. It is important for all researchers to know what evidence is out there, in order to avoid wasting research money on questions that have been answered well and to target research efforts and funding on problems that we believe are a priority to healthcare providers and to women.

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

What future research might emanate from your work?

Genes
We are currently investigating the role that a common mineral with antioxidant properties found in diet might contribute to spontaneous preterm birth. If we find that there is a link between this substance and preterm birth, then the next direction of research would be to try and understand how this substance is linked. We are currently measuring levels of this antioxidant in our cohorts' blood samples. If too little of this mineral is being absorbed then future research direction would investigate clinical trials of dietary supplements in pregnant women.

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
We aim to develop approaches to drug delivery to be effective and safe for mothers. This will involve ensuring that enough drug is carried and that it reaches the target organ. We will then confirm that the effect on the womb is more than if the drug were given normally. Once we are happy with these features we will look to use these techniques in a trial in pregnancy.

Summarising evidence
Summarising evidence helps women and their doctors identify treatments that work best. Pregnant women and doctors can then make decisions to avoid treatments that are not suitable or helpful. A new direction in summarising evidence involves continuously updating summaries of evidence online, so that any very recent work can be incorporated into decisions about treatments straight away; no one has yet done this for all treatments for pregnant women at high risk of preterm birth. Another new direction involves hosting clinical trial datasets online, so that researchers can share trial data to answer more questions about preterm birth. Harris Preterm Birth researchers are working in both of these directions, to provide pregnant women and their healthcare providers with a ranking of which current treatments work best for which women, and to further data sharing amongst researchers by creating a website to house clinical trial.