Network analysis of genomic regulators of labour using *in silico* techniques

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences

Year of submission

2015

Sylvia Lui

School of Medicine

CONTENTS

FIGURES

LITERATURE REVIEW AND METHODS

3.0 Results chapter

4.0 Results chapter

5.0 Results chapter

TABLES

Literature review and methods

3.0 Results chapter

4.0 Results chapter

5.0 Results chapter

Final word count: 75947

Abbreviations

Terminology

Symbol	Identifier
ACTH	Adrenocorticotropic
AODIL	Arrest of development in labour
BioGRID	Biological General Repository for Interaction Datasets
CRH	Corticotropin-releasing hormone
Dec	Decidua
DHEA	Dehydroepiandrosterone
ER	Estrogen receptor
HPA	Hyphothalamic pituitary axis
IHC	Immunohistochemistry
IPA	Ingenuity Pathway Analysis
LPS	Lipopolysaccharide
mir-	MicroRNA
MONO	Monocytes/ mononuclear cells
Myo	Myometrium
NBF	Neutral buffered formalin
PBMC	Peripheral blood mononuclear cell
PCA	Principle components analysis
PPLR	Probability of postive log ratio
PR	Progesterone receptor
PTB	Preterm birth
PTL	Preterm labour (<37 weeks)
PTNL	Preterm not in labour
PUMA	Propagating uncertainty in microarray analysis
RMA	Robust multi-array analysis
TL	Term labour (>37 weeks)
TNL	Term not in labour

Genes

Symbol	Identifier
ABL2	C-abl oncogene 2, non-receptor tyrosine kinase
ABP1/AOC1	Amine Oxidase, Copper Containing 1
ACTG2	Actin, Gamma 2, Smooth Muscle, Enteric
ACVRL/ ALK-	Activin receptor-like kinase 1
ACVRL1	Activin A Receptor Type II-Like 1
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1, motif 9
AGTPBP1	ATP/GTP binding protein 1
ALDH2	Aldehyde dehydrogenase 2
ANGPTL4	Angiopoietin-like 4
AP-1	Activator Protein 1/ c-JUN
APP	Amyloid beta (a4) precursor protein
AR	Androgen receptor
ATOH1	Atonal Homolog 1 (Drosophila)
ATP-	ATPase, Class II, Type -
BATF	Basic Leucine Zipper Transcription Factor, ATF-Like
BCL-	B-cell CLL/ lymphoma
BDKR-	Bradykinin Receptor-
BDNF	Brain-derived neurotrophic factor
BMP-	Bone morphogenetic protein-
BRCA1	Breast cancer type 1 susceptibility protein
C18orf1/ LDLRAD4	Low density lipoprotein receptor class A domain containing 4
CALM1	Calmodulin 1
CatSper-	Cation Channel, Sperm Associated-
CBS	Cystathionine-Beta-Synthase
CCL-	Chemokine CC motif ligand
CCND1	Cyclin D1
CD2AP	CD2-associated protein
CD3E	CD3e Molecule, Epsilon (CD3-TCR Complex)
CDC34	Cell Division Cycle 34
CDH1	E-Cadherin
CDKN1A	Cyclin-dependent kinase inhibitor 1 A
CDX	Caudal type-

CEBP-	CCAAT/enhancer binding protein (C/EBP)
CHST12	Carbohydrate (chondroitin 4) sulfotransferase 12
COPB	Coatomer Protein Complex, Subunit Beta 1
CPI-17	17kd protein kinase C–potentiated inhibitor of protein phosphatase 1c
CRIM1	Cysteine rich transmembrane BMP regulator 1 (Chordin-Like)
CRLF2	Cytokine Receptor-Like Factor 2
CRYB-	Crystallin, beta -
CSF2/GM-CSF	Colony Stimulating Factor 2 (Granulocyte-Macrophage)
CSNk1a1	Casein Kinase 1, Alpha 1
CTGF	Connective Tissue Growth Factor
CTNNB1	Beta-catenin
CTSB	Cathepsin B
CXCL-	Chemokine motif C-X-C ligand
CYR61	Cysteine-Rich, Angiogenic Inducer, 61
DCP2	Decapping MRNA 2
DCUN1D1	DCN1, defective in cullin neddylation 1, domain containing 1
DDAH-1	Dimethlyarginine dimethlyaminohydrolase 1
DLG2	Discs, Large Homolog 2 (Drosophila)
DNM2	Dynamin 2
DNMT	DNA methyltransferase
DST	Dystonin
DUSP	Dual Specificity Phosphatase-
EDNRB	Endothelin Receptor Type B
EGF	Epidermal growth factor
EGR-	Early growth response-
ELK1	ELK1, member of ETS oncogene family
EMX2	Empty Spiracles Homeobox 2
ENG	Endoglin
ER-	Estrogen receptor
ERBB1IP	Erbb2 interacting protein
EREG	Epiregulin
ERK/ MAPK	Extracellular signal-regulated kinase/ Mitogen-Activated Protein Kinase
ERK1/2	Extracellular-signal-regulated kinases (ERKs) or classical MAP kinases
FBN2	Fibrillin 2
FCER1A	Fc Fragment Of IgE, High Affinity I, Receptor For; Alpha Polypeptide
FCGR3A	Fc gamma RIIIA/CD16
FGF-	Fibroblast Growth Factor-
FKBP5	FK506 binding protein 5
FLT1/VEGFR-1	Fms-related tyrosine kinase 1/ Vascular Endothelial Growth Factor Receptor 1
FOS-	FBJ Murine Osteosarcoma Viral Oncogene Homolog
FOX-	Forkhead box protein-
FRYL / KIAA0826	FRY-like
G1P3	Interferon alpha-inducible protein
GALNT14	Polypeptide N-acetylgalactosaminyltransferase 14
GJA1	Gap junction protein 1 / Connexin 43
GLRB	Glycine Receptor, Beta
GNG- T1	Guanine Nucleotide Binding Protein (G Protein), Gamma-
GOLGA8A	Golgin A8 family, member A
GPR-	G protein-coupled receptor
GPX-	Glutathione Peroxidase-
GRIA2	Glutamate Receptor 2
GSTT2	Glutathione S-Transferase Theta 2
HBEGF	Heparin-binding EGF-like growth factor
HDAC9	Histone Deacetylase 9
HGF	Hepatocyte growth factor
HIF1A	Hypoxic ischemic factor
HLF	Hepatic Leukemia Factor
HMGB-	High Mobility Group Box-
HNT/NTM	Neurotrimin
HOXA9	Homeobox A9
HPGD	Hydroxyprostaglandin dehydrogenase 15 -(NAD)
HSD17B4	Hydroxysteroid (17-Beta) Dehydrogenase 4
HSPA1B	Heat Shock 70kDa Protein 1B
ICAM-	Intercellular adhesion molecule-
IDO1	Indoleamine 2,3-dioxygenase 1
IER-	Immediate early response-

IFGIP-	Interferon, Gamma-Inducible Protein
IFITM1	interferon-induced transmembrane protein 1
IFNAR-	Interferon (alpha, beta and omega) receptor-
IFNGR-	Interferon gamma receptor-
IGF-	Insulin like growth factor-
IGFBP-	Insulin like growth factor binding protein-
IKBK-	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase
IL-	Interleukin-
IL6ST	Interleukin 6 Signal Transducer
INHBA	Inhibin, Beta A
INSR	Insulin receptor
IRAK-	Interleukin-1 receptor-associated kinase-
IREB-	Iron-responsive element binding protein-
IRF-	Interferon regulatory factor-
ITG-	Integrin- (Fibronectin Receptor, Beta Polypeptide, Antigen CD29)
JAK3	Janus kinase 3
KCNAB2	Potassium Channel, Voltage Gated Subfamily A Regulatory Beta Subunit 2
KRIT1	Krev Interaction Trapped Protein 1
LIF-	Leukemia inhibitory factor-
LILR-A5	Leukocyte immunoglobulin-like receptor subfamily A, member 5
LMCD1	LIM And Cysteine-Rich Domains 1
LMO4	LIM Domain Only 4
LONP	lon peptidase
LRAP/ERAP2	Leukocyte-Derived Arginine Aminopeptidase/Endoplasmic Reticulum Aminopeptidase 2
LXR/RXR	Liver X receptor/retinoid X receptor
MaFF	V-Maf Avian Musculoaponeurotic Fibrosarcoma Oncogene Homolog F
MEL/RAB8a	Oncogene C-Mel/Ras associated protein RAB8
METTL18	Methyltransferase like 18
MFGE8	Milk fat globule-EGF factor 8 protein
MIF	Macrophage migration inhibitory factor
MnSOD	Manganese superoxide dismutase
MORC3/ ZCWCC3	MORC family CW-type zinc finger 3/ Nuclear matrix protein NXP2
MSC	Musculin
MT-	Metallothionein-
MX1	Homolog of murine myxovirus resistance 1
MYCB	V-Myc Avian Myelocytomatosis Viral Oncogene Homolog
NDRG4	N-Myc downstream-regulated gene 4 protein
NFAT	Nuclear factor of activated T-cells
NFE2L2	Nuclear factor erythroid 2-like 2
NFkB	Nuclear factor of kappa light chain gene enhancer in B-cells
NFKBI-	Nuclear factor of kappa light chain gene enhancer in B-cells, inhibitor
NFXL	Nuclear transcription factor X box binding
NID1	Nidogen 1
NKX3-1	NK3 Homeobox 1
NLRC4	Nod-like receptor family, CARD domain containing 4
NNMT	Nicotinamide N-methyltransferase
NOS-	Nitric oxide synthase-
NR1H2	Nuclear receptor subfamily 1, group H, member 2/ Liver X Nuclear Receptor Beta
NR3C1	Glucocorticoid receptor
OBSL1	Obscurin-like 1
OPLAH	5-Oxoprolinase (ATP-Hydrolysing)
OSM	Oncostatin M
OXTR	Oxytocin receptor
PAI/SERPINE-	plasminogen activator inhibitor type/ Serpin peptidase inhibitor
PAPPA	Pregnancy-Associated Plasma Protein A, Pappalysin 1
PBEF1/ NAMPT	Pre-B-Cell Colony Enhancing Factor 1/ nicotinamide phosphoribosyltransferase
PCDH1/ ARH	Protocadherin
PCP4	Purkinje cell protein 4
PDE1B	Phosphodiesterase 1B, Calmodulin-Dependent
PDGF	Platelet-derived growth factor
PDI	Protein disulfide isomerase -
PELI1	Pellino E3 ubiquitin protein ligase 1
PGDH	Prostaglandin Dehydrogenase
PGF2α	Prostaglandin F2α
PGR	Progesterone receptor
PGRMC- / PMBP	Progesterone receptor membrane component -

PHLDA1	Pleckstrin homology-like domain family A -
PI3K/ PIK3C-	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit -
PIK3R1	Phosphoinositide-3-Kinase, Regulatory Subunit 1 (Alpha)
PKC	Protein Kinase C
PLA1A	Phospholipase A1 member A
PLA2G-	Phospholipase A2, group -
PLC E1	Phospholipase C, epsilon 1
POLR2I/CDK19	Polymerase II polypeptide I / Cyclin dependent kinase 19
PPARG	Peroxisome proliferator-activated receptor gamma
PRKAA1	Protein Kinase, AMP-Activated, Alpha 1 Catalytic Subunit
PROK2	Prokineticin 2
PSCDBP/CYTIP	Cytohesin 1 Interacting Protein
PTEN	Phosphatase and tensin homolog
PTGDS	Prostaglandin D2 Synthase
PTGER4	Prostaglandin E Receptor 4 (Subtype EP4)
PTGS2	Prostaglandin synthase 2/ cyclooxygenase-2
PTPN2	Protein tyrosine phosphatase, non-receptor type 2
PTPRK	Protein Tyrosine Phosphatase, Receptor Type, K
PTPRZ1	Protein Tyrosine Phosphatase, Receptor-Type, Z Polypeptide 1
PTX3	Pentraxin 3
PURA	Purine-Rich Element Binding Protein A
QRSL1	Glutaminyl-TRNA Synthase (Glutamine-Hydrolyzing)-Like 1
RCN2	Reticulocalbin 2, EF-Hand Calcium Binding Domain
RelA	p65 Subunit of the NFkB complex
RelB	Subunit of the NFkB complex
RGS12	Regulator Of G-protein signaling 12
Rnd-	Rho GTPase
ROK-	ATP-Dependent RNA Helicase
RRAD	Ras-related associated with diabetes
RUNX1/ AML1	Runt-related transcription factor 1
S100A-	S100 calcium binding protein A
SCD1	Stearoyl-CoA desaturase-1
SERPINA3/AACT	Serpin Peptidase Inhibitor, A3 /Alpha-1 Antiproteinase, Antitrypsin)
sFRP4	secreted frizzled-related protein 4
SHC1	(Src homology 2 domain containing) transforming protein 1
SLC16A-	Solute Carrier Family 16 (Monocarboxylate Transporter), Member-
SMAD-	SMAD Family Member -
SNAI1	Snail family zinc finger 1
SOCS-	Suppressor of cytokine signalling
SOD-	Superoxide dismutase
Sp-1	Specificity protein 1
SQSTM1	Sequestosome 1
STAM2	Signal Transducing Adaptor Molecule (SH3 Domain And ITAM Motif) 2
STAT-	Signal transducer and activator of transcription
STUB1	STIP1 homology and U-box containing protein 1
SYN1	Syncytin-1
TAGLN-	Transgelin-
TCL1-	T-cell Leukemia/Lymphoma 1
TDRD9	Tudor Domain Containing 9
TEX1	Testis expressed gene 1
TFAP2A	Transcription factor AP2A
TGF-	Transforming Growth Factor-
THBS1	Thrombospondin 1
THSB1	Thrombospondin
TIMP1	Tissue inhibitor of metalloproteinases 1
TIRAP	Toll-interleukin 1 receptor domain containing adaptor protein
TLR-	Toll-like receptor
TNC	Tenascin C
TNF-	Tumour necrosis factor-
TNFAIP-	Tumor necrosis factor alpha-induced protein-
TNFRSF-	Tumor necrosis factor receptor superfamily -
TNFSF-	Tumour necrosis factor, superfamily
TP53	Tumor Protein P53
TRAF	TNF receptor-associated factor
TREM1	Triggering receptor expressed on myeloid cells 1
TUBB-	Tubulin, Beta Class I

TWIST-	Twist Basic Helix-Loop-Helix Transcription Factor 1
TXA2R/ TBXA2R	Thromboxane A2 receptor
UBC	Ubiquitin
UBE2D3	Ubiquitin-conjugating enzyme E2D 3
UPF2	UPF2 Regulator Of Nonsense Transcripts Homolog
VCAM	Vascular cell adhesion protein 1
VCP	Valosin Containing Protein
VEGF	Vascular endothelial growth factor
VIM	Vimentin
VMP1	Vacuole membrane protein 1
WNT-	Wingless-type mmtv integration site family
XRCC5	X-Ray Repair Complementing Defective Repair In Chinese Hamster Cells 5
ZDHHC-	Zinc finger, DHHC-type containing
ZEB	Zinc finger E-box binding homeobox

Abstract

Preterm birth is a worldwide health issue, with increased incidence of fetal mortality and risk of morbidities. Inhibition of myometrial contractions is the only therapeutic intervention available, but this strategy remains limited in effectiveness as labour is initiated by a network of processes. The regulation of these mechanisms, and the coordination between different uterine tissues, is poorly understood. In order to develop effective therapies, we must first define and understand the processes occurring prior to and during labour. We wished to explore the interactions that occur in the decidua and myometrium, identify the master regulators within the global system of labour and select potential therapeutic candidates for suppression of multiple labouring processes. The detailed investigation of the role of the decidua in labour is critically important, as it is the site of significant inflammatory, progesterone and prostaglandin activity during labour. Using a global approach of detection and analysis, this study used high through-put microarrays, pathway and network analysis, an *in silico* prediction workflow, and *in vitro* inhibition of candidate proteins in human and mouse cell lines to identify molecular fingerprint of labour, characterise the changes occurring near the end of pregnancy that lead towards activation of labour, and identify master regulators of these pathways. In human choriodecidua, the most enriched pathways of global gene expression during term labour were inflammatory-associated. Network analysis identified vimentin, TLR4 and TNFSF13B as master regulators of labour in the decidua and identified MT2, TLR2 and RelB in the myometrium. Preliminary *in vitro* inhibition experiments indicated blockade of individual master regulators could moderately decrease expression of multiple downstream labour-associated genes. The mouse decidua was also characterised by the presence of a significant inflammatory response during active term labour. However, major changes in decidual function and regulators were evident prior to labour; these processes were initiated as early as 60 hours preceding labour. The mouse myometrium was enriched for chemokine pathways, but the majority of gene changes occurred only 12 hours prior to labour in this tissue. In the mouse, *in silico* predicted master regulators included IRAK4, TIRAP in the decidua and the TLR2/4 complex in the myometrium. In conclusion, this study has shown for the first time that in normal term labour, the human choriodecidua is highly functional with active sterile inflammatory processes occurring. The findings of widespread preparatory events in the murine decidua, that precede changes in the myometrium, suggests integral roles in the initiation of labour. There are highly conserved similarities in the inflammatory receptors, ligands and transcription factors between human and mouse tissues. The master regulators identified govern multiple downstream genes are that not directly associated with contraction, indicating there are multiple important processes occurring during labour as well as the contractile factors. Although some of the regulators have been previously identified as being associated with labour, this study was the first to identify their network regulatory characteristics. Instead of targeting a single and potentially late-stage event such as myometrial contraction, targeting multiple processes with the inclusion of processes in the closely-related and highly functional decidua, may be a more effective strategy in preventing progression of the labour process.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright Statement

Acknowledgements

To my supervisors Dr Rebecca Jones, Dr Lynda Harris, Dr Adam Stevens and Dr Clare Tower, I am sincererly thankful for this opportunity, but also for the confidence they instilled in me. The encouragement, guidance and support they provided has helped me to achieve more than what I had ever expected. This journey has been amazing. A special thank you to Rebecca and Lynda whose advice, friendship and support has been valued greatly. They have been incredible, and I aspire to provide the same kind of leadership, knowledge and enthusiasm they have shown me.

Without my family I could not have done this. Thanking them doesn't seem enough, but I feel blessed to have such a large and wonderful family. My Mum and Dad, my siblings Betty, Nancy, and Bobby, my little sisters Velia and Yvonne, Aunty Annie and Uncle Harry, Grandma, Aunty May and Uncle John, Arthur and Carol, all of my cousins and Aunty Cherry. Everyone has been so very generous and supportive. I would like to thank them all for their encouragement, and also, putting up with my long absences and sudden appearances. I can honestly say that I feel I have a home on opposite sides of the world. I hope I make them proud and will always try to do so.

To my fellow PhD-ers and colleagues, this experience would not have been as enjoyable (though at times, it may have just been shared hysteria) or achievable without them. To my darling friends close and to those far away, thank you so much for being there, it has made all the difference. I am truly grateful to surrounded by such great people.

To my partner, Paul Bannon, who has experienced the ups and downs of the last few years with me. The bringer of many cups of tea, supplier of treats, purveyor of cat pictures and source of strength. Thank you, for keeping me going and making me laugh, even when I really didn't want to.

This is dedicated to my wonderful Mum.

Who has been missed dearly.

1.0 Literature review

The World Health Organisation recognises that birth before 37 weeks is a major world-wide health issue, and despite this premature birth (PTB) has been on the rise over the last 20 years in most countries. Advances in medical care in developed countries have greatly improved survival for infants born prematurely, especially for extremely premature infants (< 28 weeks). However, there have been no advances in treatment that decrease risks of prematurity, and thus prevent neonatal morbidity and infant death (Lawn et al. 2010). Investigating the biological pathways involved in triggering spontaneous term labour (TL) is crucial in understanding the preterm and term pathology. Specific interventions targeting earlier-stage biological signals, in addition to targeting the late myometrial excitation state, would potentially give rise to a treatment with a greater efficacy in the cessation of idiopathic premature labour processes, and so prevent PTB and improve neonatal outcomes.

1.1 Preterm labour

1.1.1 Incidence of preterm labour

Many pregnancies presenting with contractions before 37 weeks will self-resolve. However, approximately 20% of women with preterm contractions will progress to premature labour (PTL) and PTB. This rate significantly increases when also presenting with a short cervix or cervical dilatation (Gomez et al. 2005; Romero et al. 2012). There are a number of markers and measurements used to aid the diagnosis of true PTL (Table 1). Cervical length in combination with the presence or absence of fetal fibronectin, has been the most commonly used in recent years for risk of PTL. Other traditional and relatively non-invasive tests include nitrazine pH strips or an absorbent pH pad for alkalinity of vaginal fluid for amniotic leakage, and the Ferning test of vaginal fluid for pattern formation, associated with salt crystallisation and high estrogen levels. Biomarkers of PTL in cervicovaginal fluid include: insulin growth factor binding protein 1 (IGFBP-1, decidua related protein) (Conde-Agudelo & Romero 2015a; Ruanphoo & Phupong 2015), alpha fetal protein (AFP) (Di Renzo et al. 2011) and placenta alpha microglobulin 1 (PAMG-1) (Abdelazim et al. 2014; Ehsanipoor et al. 2015). These markers are highly concentrated in amniotic fluid, compared to maternal serum or urine; elevated levels are associated with true TL and PTL, but their ability to accurately predict PTL is variable. Their use as a negative indicator can reassure women with high probability of PTL. Many markers associated with labour and PTB have been identified but there is no single non-invasive test that can positively predict PTL that results in PTB. Clinically, at least two indicators are used to ensure a higher sensitivity and specificity of detection, allowing hospital admission and treatment to be managed more accurately.

Table 1. Common tests for threat of preterm labour and delivery.

The range in sensitivity and specificity result in a degree of uncertainty in interpreting risk of actual preterm delivery. At least two indicators are used to ensure an accurate assessment. SS= sensitivity, SP= specificity, PPV= positive predictive value and NPV= negative predictive value. Table modified and updated from (Di Renzo et al. 2011).

Test	Test methods	Cut-off	SS (%)	SP (%)	PPV (%)	NPV (%)	Problems
Cervical length	Ultrasound of cervical length. Symptomatic women with PPROM.	≤ 2cm	51	71	62	61	Symptomatic women delivering within 7 days with PPROM. A short cervix in asymptomatic women does not always result in PTB (Mehra et al. 2015).
Fetal fibro-nectin	Multiple kits available. Immuno-chromatographic assay kit that detects fetal fibronectin, in cervicovaginal fluid.	> 50 ng/ml	97-98	70-97	74-93	98-100	Effective for negative prediction. Positive prediction in multiple pregnancies and symptomatic threatened PTL and delivery within 7 days. Semen and blood may affect the results (Watson et al. 1998; Leitich et al. 1999; Abdelazim et al. 2014).
Nitrazine (pH)	A sensitive pH strip test of amniotic fluid leakage in cervicovaginal samples (alkaline)	+ve/ -ve pH>5.2	90-97	16-70	63-75	80-93	Alkaline urine, blood, vaginal infection, semen, and hygiene products can alter the colour of the pH strip (Bennett et al. 1993; Tagore & Kwek 2010).
Ferning test	Sample of vaginal fluid that forms a fern-like pattern indicates amniotic fluid and raised estrogen levels. Absent with progesterone.	+ve/ -ve	51-98	70-88	84-93	87-97	Fingerprints, semen, or cervical mucus can produce the same fern-like effect (Garite & Gocke 1990; Abdelazim et al. 2014).
Absorb-ent pad pH test	**AmnioSense.** Non-invasive pH test using an absorbent pad to detect amniotic fluid leakage (alkaline)	pH > 5.2	98.3	70	65-70	98	Mild infection, blood, semen and hygiene products can alter pH tests. This has not yet been documented for the Amniosense (Mulhair et al. 2009).
IGFBP and AFP	**ROM plus Check** and **Amnioquick Duo+.** Kits that detect high amniotic fluid protein levels in vaginal fluid. (ng/ml) IGFBP-1 (5 and 10). AFP (150 and 5) respectively	IGFBP1 and AFP	80-99	75-100	85-100	74-94	Protein low in semen, urine and blood. High levels of blood may affect the results (Ruanphoo & Phupong 2015).

AFP	Presence of high levels of alpha fetal protein in vaginal secretions. Indicates leakage of amniotic fluid	>30µg/l	90-94	95-100	94-100	91-94	Presence of blood may affect the results (Garite & Gocke 1990; Waller et al. 1996; Ho et al. 2012).
IGBP-1	**Actim PROM-TEST** and **AMNI Check**. Kits that detect decidual protein levels in vaginal secretions. It is undetectable from semen, urine and blood.	>3µg/l	74-97	74-98	73-97	56-95	Positive and negative predictive value variable across different studies. Symptomatic women delivering within 7 days with PPROM (Lembet et al. 2002; Ruanphoo & Phupong 2015)
PAMG-1	**AmniSure Test ROM** (AmniSure International LLC, MA). Immunoassay for a placental glycoprotein protein in cervicovaginal fluid.	>5 ng/ml	98-99	87.5-100	98-100	91-99	Can be positive in asymptomatic women without ROM. Associated with PTB and adverse outcome in symptomatic women, with or without ROM (Lee et al. 2012a; Ng et al. 2013; Ehsanipoor et al. 2015)

1.1.2 Incidence of preterm birth

PTB can be divided into three categories according to gestational age. Infants born extremely preterm are before 28 weeks, very preterm are between 28 and 32 weeks, and late preterm are between 32 and 37 weeks. The latter makes up 84% of all PTBs (Goldenberg et al. 2008; Beck et al. 2010; Lawn et al. 2010; Blencowe et al. 2012; Zeitlin et al. 2013). Low-income countries experience high rates of PTB and many high income countries report an increase in number of cases. High income countries also experience higher rates of iatrogenic PTBs, including multiple births and those caused by pregnancy other complications such as pre-eclampsia. The European rate of PTB is 5.5% to 11.1%. The overall rate in the U.K has an average trend of 7.7% (Zeitlin et al. 2013). However, these rates include multiple births and iatrogenic births. In other a high income countries such as the US, the incidence of PTB has fluctuated, slowly decreasing over the last ten years but remains at 11.5%, which is comparable to that of PTB rates in Southeast Asia (Beck et al. 2010; Blencowe et al. 2012).

1.1.3 Consequences of PTB

1.1.3.1 Mortality and morbidity of premature delivery

Prematurity at birth increases risk of mortality. According to the World Health Organisation (updated November 2015), PTB is the highest cause of infant death in the US (Lawn et al. 2010; Blencowe et al. 2012), and the second leading cause of infant death after pneumonia in the UK (Blencowe et al. 2012). Morbidity in premature infants is increased, compared to those born at

term, and even in infants born close to term. Birth before 35 weeks increases risk of lung-related diseases such as respiratory distress syndrome, apnoea of prematurity and chronic lung disease from medical intervention. There are also cardiovascular and neurological effects. These morbidities are less common and are associated with degree of prematurity, health condition at birth and weight at birth. Some neurological effects persist well into adulthood and the degree of severity rises with greater prematurity. These include damage to the brain causing cerebral palsy, and decreased concentration and hyperactivity in childhood (Talge et al. 2010). In extreme prematurity, infants are exposed to risks of reduced cognitive function, and additional cardiovascular complications can exacerbate these symptoms. In many cases cardiovascular complications can be mild, but poor circulatory function in premature infants is associated with hypothermia, brain haemorrhage, hearing loss and retinopathy of prematurity. Under-developed fetal immunity in the gut can result in infection in the gastrointestinal tract or necrotising enterocolitis, and in severe cases lead to infant death (Goldenberg et al. 2008).

1.1.3.2 Economic costs of preterm delivery

Survival of infants born extremely premature has dramatically improved, but it places a considerable financial burden on available medical services. In the UK, advanced neonatal care has increased the survival of infants born between 22 - 26 weeks from 40% in 1995, to 53% in 2006. However, hospital stay was on average three and a half months, and 59% of those infants had continuing major health complications (Costeloe et al. 2012). Although the majority of PTBs occur after 32 weeks, late premature infants (35-36 weeks) still require greater access to medical care, with a four-fold risk of an adverse medical condition, compared to an infant born at term (Wang et al. 2004). As a consequence, there is a 5.9 fold increase in medical costs in the UK for preterm infants (born at 32 to 33.6 weeks), and even in late preterm infants (born at 34 to 36.6 weeks) there is a 2.8 increase compared to infants born at term in their first 2 years of life (Khan et al. 2015). Applying these statistics to the annual PTB rate, the study estimated an extra £122 million annually for 32 to 36.6 week born preterm born infants up to the age of 2 years old (Khan et al. 2015). This expenditure is reflected in the exceptional survival rates however, the economic consequences of PTB birth are variable depending on hospitalisation length and gestational age. The considerable costs of PTB are due to the health implications after birth, which increase with decreasing gestational age (Mangham et al. 2009).

The issue remains that there are very few effective approaches for preventing spontaneous PTL and PTB. In low income countries with fewer neonatal medical services, very PTB infants have a survival rate of approximately 10% compared to almost 90% in the UK and US (Blencowe et al. 2012), further highlighting the need for an understanding of labouring processes and an effective approach towards prevention of PTBs and PTL.

1.1.4 Causes and risk factors of preterm labour and birth

There are a several causes of PTL leading to PTB, but a major proportion of PTL is defined as spontaneous or idiopathic. A third of women experiencing true PTL will deliver prematurely with no prior indication or detectable cause for preterm delivery (Goldenberg et al. 2008). There are also iatrogenic PTBs resulting from elective or medical caesarean delivery. Elective caesareans are an increasing concern in high-income countries, but emergency caesareans are also performed for pregnancy complications such as preeclampsia, fetal growth restriction, fetal anomalies and obstructed labour. Multiple pregnancies also increases risk of PTL, as physical stretching of the uterine tissues promotes contractile signals (Loudon et al. 2004; Shynlova et al. 2007). Sustained maternal infection from sexually transmitted diseases, bacterial vaginosis, and chorioamnionitis increases the risk of PTL (Goldenberg et al. 2008). Other risk factors include tobacco smoking (Ahern et al. 2003; Cnattingius 2004), black ethnicity (Ahern et al. 2003; Blencowe et al. 2012), obstetric history (Sadler et al. 2000), short cervix (Gomez et al. 2005; Smith et al. 2008; Romero et al. 2012), uterine and placental abnormalities (Miller et al. 1997; Airoldi et al. 2005; Belfort 2011; Fishman & Chasen 2011), maternal age under 19 and over 40 years of age (Astolfi & Zonta 1999; Tough et al. 2002; da Silva et al. 2003; Pennell et al. 2009), low body mass index (Hickey et al. 1997; Hendler et al. 2005), low socioeconomic status (Peacock et al. 1995; Glinianaia et al. 2013; Hsieh et al. 2015), and maternal health conditions such as diabetes, hypertension and asthma (Rush 1979; Goldenberg & Culhane 2005; Peterson et al. 2015). There is a distinct disparity of fetal gender in very preterm cases (<32 weeks), with males out numbering females (Challis et al. 2013) by more than 50% (Ingemarsson 2003). However the effect of fetal sex is contentious as idiopathic PTB and the risk of recurrent idiopathic PTB, has been shown to be greater when the first pregnancy is a female infant (Kamphuis et al. 2015). Though the study found a pattern of difference, the statistical differences are low (15.8 vs. 15.2%; adjusted odds ratio 1.2; 95% confidence interval 1.05–1.3) (Kamphuis et al. 2015).

Risk factors for PTB analysed by cluster network analysis, subsequently identified PTL as possessing five major clinical phenotypes including maternal stress, PPROM, familial factors, maternal comorbidities and a group of infection, decidual haemorrhage and placental dysfunction (Esplin et al. 2015). The risk factors are summarised in Table 2.

Table 2. Risk factors for idiopathic preterm labour and delivery

Maternal infection	Prolonged infection increases risk of PTB; diseases include sexually transmitted diseases, bacterial vaginosis, and also chorioamnionitis of the fetal membrane (Goldenberg et al. 2008).
Tobacco smoking	Smoking increases risk by 1.5 fold compared to non-smokers (Ahern et al. 2003; Cnattingius 2004). Rates of PTB are significantly decreased, even if presenting with other high risk factors, if smoking is ceased (Kyrklund-Blomberg & Cnattingius 1998).
Black ethnicity	African American women have significantly higher rates of premature labour and delivery in comparison to Caucasian American women. This higher risk with ethnicity remains even when there are other PTB high-risk factors are involved such as smoking (Ahern et al. 2003; Lu & Chen 2004; Blencowe et al. 2012). The rates of PTB were similar between American Indian/Alaska Native, or Asian/Pacific Islander women compared to Caucasian women in the cohort (Lu & Chen 2004).
Obstetric history	History of PTB. Previous induced abortions are associated with PTB (Moreau et al. 2005; Klemetti et al. 2012). It is debatable whether a history of cervical surgery increases the risk of PTB (Sadler et al. 2000; Nam et al. 2010; Bevis & Biggio 2011; Farinelli et al. 2012). However women with a short cervix have a much greater risk (Gomez et al. 2005; Smith et al. 2008; Romero et al. 2012). Abnormalities in the uterus such as bicornate shape are known to result in PTB (Airoldi et al. 2005).
Maternal age	PTB is increased in mothers <19 years old, and those who also experienced late menarche (Hediger et al. 1997; da Silva et al. 2003). Pregnancy at >40 years old are twice as likely to experience PTB compared to women 20-35 years of age (Astolfi & Zonta 1999; Tough et al. 2002; Pennell et al. 2009)
Body mass index (BMI)	Low BMI (<19) before pregnancy increases PTB risk, especially combined with black ethnicity (Hickey et al. 1997). A high BMI is not independently associated with PTB but, risk of PTB increases with another adverse factor such as smoking or low socioeconomic status (Hendler et al. 2005).
Socioeconomic factors	Unemployment, low income, lower education, and single relationship status are associated with idiopathic preterm delivery (Peacock et al. 1995; Glinianaia et al. 2013; Hsieh et al. 2015).
Placental abnormalities	Placental abnormalities including placental accreta (attachment to the myometrium) increase the incidence of PTB. However as placental accreta is associated with previous caesarean delivery, it may be a consequence of caesarean delivery rather than cause (Belfort 2011; Fishman & Chasen 2011). Placenta previa (partial/full attachment over the cervix) (Miller et al. 1997), placental abruption and preterm premature rupture of membranes (PPROM) also increase PTL risk (Mackenzie et al. 2004; Lockwood et al. 2005; Buhimschi et al. 2010; Han et al. 2011; Lockwood et al. 2012).
Maternal conditions	Maternal health condition such as diabetes, hypertension and asthma are associated with increased risk of PTB (Rush 1979; Goldenberg & Culhane 2005; Peterson et al. 2015).
Fetal gender	Male infants significantly outnumber female infants by 55-60% in very premature cases (< 32 weeks) with better survival in female infants (Ingemarsson 2003). Upon lipopolysaccharide (LPS) stimulation, male placentae produce more prostaglandins and cytokines than female placentas (Challis et al. 2013).

1.1.5 Treatments for preterm labour

The goal in treating pregnancy complications is to improve outcomes for the infant without detrimentally affecting the health of the infant or mother. There are several recommendations from the World Health Organisation to reduce the incidence of premature labour and PTB: cessation of smoking, cervical cerclage - however the length of cervix and intact fetal membranes determines the effectiveness of this technique (Hassan et al. 2001; Groom 2007; Owen et al. 2009), reduction in the number of embryos transferred during *in vitro* fertilisation, progesterone therapy for PTB high risk women, administration of antenatal corticosteroids to improve fetal development with tocolysis to slow labour progression.

Tocolysis is one of the few therapies available to slow PTL. These drugs aim to suppress myometrial contraction and include: oxytocin receptor antagonists, non-steroidal anti-inflammatory (NSAID) therapies, calcium channel blockers, beta receptor agonists, nitric oxide donors and magnesium sulphate, all of which have varying efficacies and side effects to the mother and/or fetus (Haas et al. 2012a; Vogel et al. 2014). In the UK, the Royal College of Obstetricians and Gynaecologists guidelines recommend the calcium channel blocker, nifedipine, and oxytocin receptor antagonist, Atisoban. Both of these are associated with the least number of side effects in comparison to other available tocolytics (Conde-Agudelo et al. 2011; Flenady et al. 2014; Vogel et al. 2014); however, studies comparing the therapies show little effectiveness in delaying labour more than 48 hours against a placebo in singleton pregnancies (Flenady et al. 2014; Danti et al. 2014). The use of tocolytics does little to increase gestational age at delivery and there is no evidence of improved fetal outcomes. Due to the difficulty in assessing idiopathic PTL and lack of therapies for pregnancy complications, current tocolytic treatment allows a window of 48 to 72 hours for corticosteroid treatment to accelerate maturation of the fetal lungs, thus decreasing neonatal morbidity. Evidence indicates that blocking myometrial excitation pathways is relatively ineffective, and yet they are the target of all PTL therapies (Haas et al. 2012a; Roos et al. 2013; Mackeen et al. 2014; Vogel et al. 2014). In addition, tocolytic study data often doesn't reflect efficacy due to biased study design and reporting (Roos et al. 2013).

In a recent meta-analysis of tocolytics, none of the available treatments demonstrated improved neonatal outcome, and withdrawal of treatment was common due to severity of maternal and fetal side effects (Haas et al. 2012a; Jørgensen et al. 2014; Vogel et al. 2014). The most maternal side effects were caused by beta receptor agonists, magnesium sulphate and calcium channel blockers (Haas et al. 2012a; Vogel et al. 2014). However the top three tocolytics that consistently delayed labour for 48 hours were prostaglandin inhibitors, magnesium sulphate and calcium channel blockers (Abramovici & Jenkins 2013; Klauser et al. 2014; Vogel et al. 2014). The least number of side effects with moderate rates of delaying PTB was observed with atosiban (Jørgensen et al. 2014; Vogel et al. 2014). Of the most commonly used tocolytics, indomethacin (a non-steroidal anti-inflammatory (NSAID) prostaglandin inhibitor) (Panter et al. 1999), ritodrine (beta-receptor antagonist) (Leveno et al. 1990), nitric oxide donors (Duckitt & Thornton 2002) and magnesium sulphate (Crowther et al. 2002) often result in minor maternal and fetal side-effects, and there

were no advantages between the therapies, which were ineffective for very PTL and PTB before 32 weeks. Nifedipine, a calcium channel blocker (King et al. 1998) and atosiban, an oxytocin receptor antagonist (Tsatsaris et al. 2004; Vrachnis et al. 2011; Jørgensen et al. 2014) delayed premature labour for up to 48 hours with fewer maternal side effects in comparison to beta blockers. However comparing the efficacy of a tocolytic with another tocolytic gives little evidence towards efficacy for delaying PTL and as a result, it is difficult to evaluate the usefulness of tocolytics as a treatment (Conde-Agudelo et al. 2011; Abramovici et al. 2012; Haas et al. 2012b; Abramovici & Jenkins 2013). Antibiotics are also used for PTL therapy, as prolonged infection during gestation has been linked with neurological defects. The use of antibiotics such as erythromycin and amoxicillin-clavulanate, however, has also been associated with a small, but increased risk of cerebral palsy (Gilbert et al. 2005; Kenyon et al. 2008).

Prophylactic treatment with progesterone can delay the onset of premature labour in high risk cases, and is currently the most effective PTB treatment (Norman et al. 2012); the long term outcomes of progesterone prophylaxis including infant health are due to be published 2015/2016 from the OPPTIMUM study. From available data, the actual effectiveness of progesterone as a prophylactic is unclear. There have been reports on improvements in infant health in asymptomatic women with a short cervix (<2.5mm) (Meis 2005; Romero et al. 2012) but the data are inconsistent, with no evidence of efficacy for PTL in singleton (Likis et al. 2012; Martinez de Tejada et al. 2015) or multiple pregnancies (Brizot et al. 2015). A systematic review demonstrated no reduction in fetal morbidities such as respiratory distress syndrome, and no effect on PTL in using progesterone compared to placebo, in either moderate or late preterm infants (Eke et al. 2015). Confounding factors included route of administration and drug type, as not all cases of premature labour responded well to progesterone therapy. Vaginal progesterone appears to be the most effective formulation with the least side-effects for early treatment of singleton high risk pregnancies with PTL, history of prior PTB and short cervix. Evidence for developmental advantages for the infant is sparse (Norwitz & Caughey 2011; Likis et al. 2012; Haram et al. 2014; Romero et al. 2014; Conde-Agudelo & Romero 2015b; O'Brien & Lewis 2015) but birth weight of infants are increased when women with PTL were treated with vaginal progesterone compared to nifedipine (Kamat et al. 2014) or placebo (Eke et al. 2015). The evidence presented here however are from reviews (Norwitz & Caughey 2011; Haram et al. 2014; Romero et al. 2014; Conde-Agudelo & Romero 2015b; O'Brien & Lewis 2015). Two were meta-analyses with contradictory reports of progesterone as being effective (Likis et al. 2012) or not effective (Eke et al. 2015) in delaying PTL. Of the three experimental studies, two indicated no effect with progesterone treatment (Brizot et al. 2015; Martinez de Tejada et al. 2015) and one with a significant postponement of PTL (Kamat et al. 2014), but this was achieved with different doses at 200mg vs 400mg/day, respectively. Comparison of equivalent doses and administration route, length and effect of treatment on PTL and fetal outcome are required.

Targeting therapeutic treatments towards earlier or multiple events within the labouring process may be more effective in preventing premature delivery. To develop therapies that have greater

effectiveness, a better understanding of the mechanisms and functions of activated intracellular pathways upstream of active labour is required. The long term objectives of this project are to target pathways that regulate the processes of PTL, but firstly, the pathways that regulate normal TL must be investigated and understood. Uterine activation is a necessary component of contraction and labour, and there are established events associated with the labour cascade (as discussed below). However, a more complex understanding of the interplay between cellular pathways, intercellular signalling and regulation of the switch from uterine quiescence to uterine tissue activation is yet to be elucidated.

1.2 Promoters of labour and mediators of contraction

1.2.1 Promoters of labour

1.2.1.1 Fetal Hypothalamic Pituitary Adrenal (HPA) axis

The fetal hypothalamic pituitary adrenal (HPA) axis describes a complex endocrine pathway originating from the fetal hypothalamus, anterior pituitary and the adrenal glands. The adult HPA axis has roles in many signalling pathways, but during pregnancy, it is thought that the additional signalling from the fetal HPA axis triggers pathways favouring parturition (Figure 1). At term, maturation of the fetal HPA axis initiates production of corticosteroid releasing hormone (CRH) from the fetal hypothalamus. The placenta also produces CRH, increasing as the pregnancy develops towards term. Towards the end of pregnancy, the combined concentration of CRH from the fetal hypothalamus and placenta signals the fetal anterior pituitary gland to synthesise adrenocorticotropic hormone (ACTH). Stimulated by the production of fetal ACTH, the fetal adrenal glands commence synthesising cortisol and dehydroepiandrostone (DHEA). Cortisol and DHEA are regulators of sex steroids, prostaglandin synthesis, oxytocin receptor and connexin 43 expression, and hence regulate a wide range of molecules involved in the initiation of contraction and labour.

Studies in sheep have shown that disruption of the fetal HPA delays normal labour. The silencing of fetal anterior pituitary gland function eliminates ACTH production, depleting cortisol and DHEA signalling and their downstream factors. Despite available circulating CRH, the loss of ACTH extended pregnancies significantly beyond term (Antolovich et al. 1990). Administering ACTH to pregnant sheep induces spontaneous PTL, where myometrial activation is similar to TL, with elevated myometrial gap junction associated protein 1 (GAP1) mRNA and protein expression with exogenous ACTH (McNutt et al. 1994). Due to the experimentally invasive nature of these techniques, it is difficult to investigate the timing and function of the HPA axis in human parturition. However, there are reports of elevated cord plasma levels of ACTH in labour at TL and PTL (Bagnoli et al. 2013), maternal plasma levels of CRH, ACTH and cortisol in labouring women are raised, with no change in DHEA levels (Ochedalski et al. 2001; Ochedalski & Lachowicz 2004).

Extended gestation is associated with low fetal plasma ACTH, attenuated cortisol production, and lowered urinary estrogen in cases of human anencephalic fetuses. In a primate model of fetal

anencephaly, similar low plasma changes were observed, with 40% of pregnancies delivering beyond term dates (Novy 1977). In a study, treatment using the anti-inflammatory glucocorticoid steroid betamethasone, suppressed primate fetal production of ACTH precursors, adrenal ACTH receptor mRNA expression and fetal adrenal weight (Pepe & Albrecht 1995). Hypermaturation of the fetal HPA axis has been noted in male fetuses (Ingemarsson 2003; Challis et al. 2013). This suggests there is a higher expression of steroidal genes, a higher stimulated production of prostaglandins, pro-inflammatory cytokines and anti-inflammatory mediators promoting preterm maturity and labour by male placentas (Challis et al. 2013). There are glucocorticoid receptor isoforms in the placenta, with 8 differentially expressed in the placenta of women presenting with PTB. The glucocorticoid receptor expression of GRαC was greater in PTL compared to TL, and GRαD2 in male placentae compared to female (Saif et al. 2015), providing further support to potential differences in fetal sex, and cortisol production in the fetal HPA axis maturation, to timing of parturition.

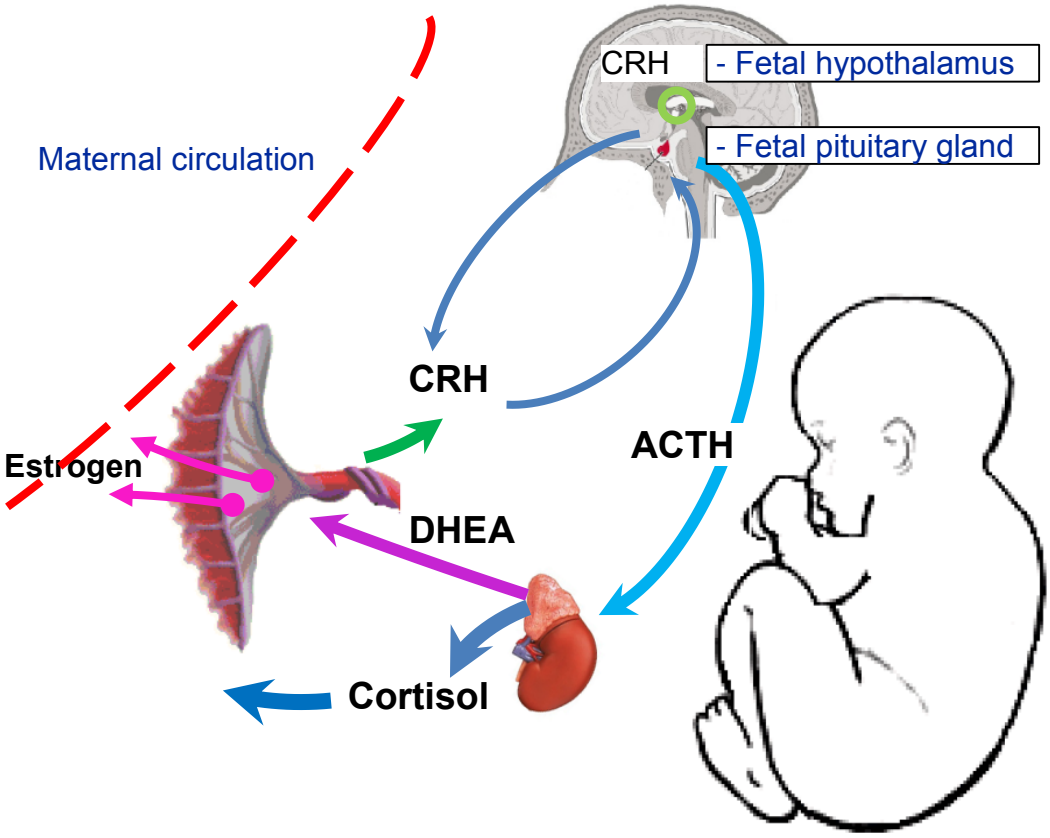


Figure 1. Summary of the fetal hypothalamic pituitary adrenal axis.

The matured fetal brain releases corticotropin-releasing hormone (CRH) in the hypothalamus (green circle). Fetal release of CRH also triggers secretion of CRH from the placenta. CRH induces the anterior pituitary (red dot) to secrete adrenocorticotropic hormone (ACTH). ACTH stimulates the fetal adrenal glands to produce dehydroepiandrosterone (DHEA) and cortisol. DHEA is converted to estrogen in the placenta and cortisol is released into the maternal serum affecting the maternal HPA axis and contributing towards the onset of labour. Images modified from www.braintumour.org.uk; www.chw.org; www.getmedicalmodel.com; http://www.chop.edu/healthinfo/fetal-circulation.html

1.2.1.2 Progesterone

Progesterone function is regulated by progesterone receptor B (PRB) and receptor A (PRA) and is highly conserved across mammalian species. The exact biological mechanisms underlying the functional switch from PRB to PRA dominance during the onset of labour are still in the early stages of investigation. Overall PR expression is maintained in the myometrium and decidua during pregnancy and TL (Haluska et al. 2002). There exists a threshold of PRA/B expression for the commitment towards labouring processes. It is classically thought that the role of progesterone in maintaining myometrial quiescence is promoted when PRB expression is dominant over PRA (Chen et al. 2008). When the relative expression of PRA is higher, progesterone blocks the immuno-regulatory actions of PRB by stimulating pro-inflammatory activity (Merlino et al. 2007; Tan et al. 2012). Term decidua cultured *in vitro*, showed a supressed immune response to progesterone in the presence of prostaglandins (Goldman et al. 2005). PRA expression in the placenta and is upregulated at onset of labour, with higher expression in TL compared to PTL; a corresponding down-regulation of PRB is observed in both TL and PTL (Zachariades et al. 2012).

Throughout pregnancy, myometrial quiescence is partly maintained by high levels of progesterone produced by the placenta. In most mammals, a decline in maternal serum progesterone drives parturition. Spontaneous fetal loss or PTL in the rat can also be simulated by inhibiting progesterone receptors (Evans et al. 1982). In primates, serum progesterone levels remain elevated throughout pregnancy and parturition. Rather than a hormone withdrawal, a block in progesterone function signals the end of pregnancy, disrupting the myometrial quiescence (Chen et al. 2008). Progesterone maintenance is crucial in myometrial quiescence, such that it is a target of tocolysis, as described earlier. Decreased PRB expression occurs concurrently with a placental conversion of DHEA to estrogen, further shifting the balance from progesterone to estrogen dominance.

In a study investigating PRA to PRB dynamics, the dominance of PRA did not simply suppress PRB activity but acted via upregulating NFκB activity, increasing expression of pro-inflammatory cytokines, increasing COX-2 (cyclooxygenase-2/ prostaglandin synthase 2, PTGS2) expression, and upregulating genes involved in cellular homeostasis (Tan et al. 2012), thus contributing to incremental rise of myometrial sensitivity towards contractile action. When acting through PRB, progesterone promotes NFκB inhibitor activity, decreases myometrial pro-inflammatory cytokines and PTGS2 expression (Lei et al. 2012; Tan et al. 2012) and down-regulates calcium ion homeostasis genes (Tan et al. 2012). Functional withdrawal of progesterone in the myometrium involves the promoter binding of PRA peaking just prior to TL, with PRA expression gradually decreasing with onset of labour, in conjunction with reduced transcriptional activity downstream of PRB. This occurs in both PTL and TL (Chai et al. 2014). Epigenetic modifications of the PRA promoter are found in the term myometrium (Chai et al 2012), with epigenetic related metal-binding nuclear transcriptional activator enzymes possessing a higher affinity for the PRA promoter.

1.2.1.3 Estrogen

Estrogen is responsible for a wide variety of endocrine functions in sexual health in both males and females. Estrogen has two main receptors (ER) α and β with three ligands: 17-β-estradiol, estrone and estriol, and the ratio between these ligands determine the functional effect (Mesiano et al. 2002). In women, estrogens are produced by granulosa cells in the ovaries, induced by follicle-stimulating hormone from the pituitary gland. During pregnancy, they are synthesized by the placenta by conversion of fetal DHEA, increasing as the fetal HPA axis matures. ER expression in the myometrium also increases at term, where greater ER activation is associated with the rise in PRA dominance (Mesiano et al. 2002). The surge in estrogen production and ER expression by the myometrium strongly promotes contractile events in the lead up to labour. Abnormal elevation in estrogen levels, and especially a high ratio of estriol to estradiol, has been associated with PTL (Smith et al. 2009). Estrogen has been demonstrated to increase the frequency of myometrial contraction *in vitro* mediated through the relaxation phase of smooth muscle activity (Jiang et al. 1991). Smooth muscle tone in myometrial tissue is higher when exposed to a combination of progesterone and estrogen, compared to progesterone only (Fu et al. 1996). Interaction between progesterone and estrogen receptors also contributes to the control and coordination of uterine activating factors. Treatment with estradiol alone in rat myometrium elicits expression of prostaglandin receptors EP2 and 4 through ERβ, however estradiol and progesterone increases contractile EP1, EP3 and via ERα (Blesson et al. 2012). The roles of prostaglandins and their receptors are detailed in section 1.2.2.1. Promoter regions of PR receptors are also regulated by estrogen but they do not contain estrogen transcription responsive elements (Kastner et al. 1990). In humans, high estrogen to progesterone ratio contributes to higher myometrial sensitivity to contractile signals. Estrogen stimulates myometrial mRNA expression of GJA1 (connexin 43, CX-43)(Di et al. 2001), PTGS2 (Mesiano et al. 2002) and prostaglandin receptor expression (Boos et al. 2006), gap junction formation (Petrocelli & Lye 1993; Rezapour et al. 1996), oxytocin receptor expression (Mesiano et al. 2002) and leukocyte recruitment via progesterone receptor (Tibbetts et al. 1999). Estrogen signalling is crucial but the molecular mechanisms are numerous and the regulation between labour-associated signals unclear. In breast cancer cells, ERα and β activity induced by inflammatory chemokines CXCR4 and CXCL12 are mediated through estrogen and AP-1 transcriptional activity (Sauvé et al. 2009). Myometrial AP-1 and cFOS activity increases GJA1 signalling (Mitchell & Lye 2001) and is promoted by CRH (Wu et al. 2007). The activity of AP-1 also upregulates inflammatory signalling and contractile genes associated with preterm and TL (MacIntyre et al. 2014).

1.2.1.4 Uterine stretch

Pressure or stretch on the uterus from the developing fetus acts as a physical stimulus, releasing molecules that activate the uterus in preparation for labour and delivery. Uterine stretch applied artificially with an expanding balloon in the first trimester of pregnancy induces myometrial contractility and leads to fetal loss (Manabe et al. 1981). This simulated uterine expansion also induces myometrial contraction in women at term (Manabe et al. 1983). Increased contraction by

uterine stretch is facilitated by, and increases OXTR mRNA expression, NFκB signalling (Terzidou et al. 2011), leukocyte infiltration (Zhao et al. 2013), cytokine expression (Loudon et al. 2004; Shynlova et al. 2007a; Shynlova et al. 2008; Hua et al. 2012) and PTGS2 mRNA expression and synthesis (Sooranna et al. 2004). Artificial stretch has shown to elicit human cervical softening by significantly increasing prostaglandin secretion (Manabe et al. 1982), PTGS2 gene expression and pro-inflammatory gene activation (Lei et al. 2011). Uterine stretch in the rat also increases expression of matrix metalloproteinases (MMP) 2 and 9 (Yin et al. 2012), alters fibronectin localisation (Shynlova et al. 2007b) and extracellular matrix expression (Shynlova et al. 2009), which facilitates the coordination of labouring signals.

1.2.2 Mediators of contraction

1.2.2.1 Prostaglandins

Prostaglandins have multiple roles in the body including inflammation, neurotransmission, endothelial permeability and coagulation. During parturition, prostaglandin levels increase in the maternal serum and uterine tissues, inducing sensitivity of the myometrial to signals that induce contractility, cervical ripening, uterine involution and changes in tissue structure to promote ROM (Keelan et al. 2003a; Olson et al. 2003; Makino et al. 2007). Prostaglandins are synthesised through the eicosanoid pathway from phospholipids and arachidonic acid (Figure 2). The enzymes PTGS1 and 2 catalyse the conversion of arachidonic acid into the inactive intermediate prostaglandin H2 (PGH2), which is further converted into a number of active prostaglandins that act in an autocrine or paracrine manner. The PTGS enzymes have different expression patterns and induce different functions; PTGS1 is expressed in most tissues and PTGS2 expression is induced by inflammatory mediators. Active prostaglandins include prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, PGD_2, Prostacyclin (PGI_2), 6-keto-$PGF_{1\alpha}$ (stable product of PGI_2) and also thromboxane, which causes platelet aggregation. In uterine tissues, the most abundant forms of prostaglandins are $PGF_{2\alpha}$ and PGE_2, which exert their actions through specific G-protein-coupled transmembrane receptors. $PGF_{2\alpha}$ activates the prostaglandin FP receptor (FP) and PGE_2 has four main prostaglandin E receptors: EP1, EP2, EP3, and EP4. PGD_2 binds to prostaglandin D receptor (DP) and PGI_2 binds to prostaglandin I receptor (IP). Thromboxane binds to the prostaglandin thromboxane receptor (TP) (Figure 2). Each receptor activates a specific signalling pathway which exhibit distinct tissue-specific localisation and temporal expression. Activated receptors EP2, EP4 and IP act as smooth muscle relaxers, while FP, EP1 and EP3 increase calcium influx, thus driving and intensifying myometrial contractions (Jabbour and Sales, 2004). In the rat, there are isoforms of EP2 expression in cervical epithelium and smooth muscle, which alter with exogenous progesterone, or progesterone receptor antagonism (Hinton et al. 2010). Recent studies have shown that at least 15 prostaglandin-synthesis related genes are involved in labour and are dependent on the uterine tissue type (Philips et al 2011; Alzamil et al 2014) and phenotype of labour (Phillips et al. 2014). At term, there is abundant myometrial prostaglandin synthesis of PGI_2, PGD_2 and $PGF_{2\alpha}$. The choriodecidua and placenta are also sites of high prostaglandin production and contain high concentrations of $PGD_{2\,\alpha}$, PGF_2 and PGE_2 at term. PGE_2 is characteristic of the

amnion, as is PGI_2 in the umbilical cord (Phillips et al. 2011). Many inflammatory factors also induce the expression of prostaglandins including interleukin (IL)-1β, IL-6 and IL-8, tumour necrosis factor (TNF) α and toll-like receptor (TLR) 2 (Phillips et al. 2014). The prostaglandin $PGF_{2\alpha}$ can upregulate the myometrial expression of the converting enzyme PTGS2, receptor OXTR, and the protein GJA1 and their transcription is promoted through different signalling pathways such as ERK, NFAT, NFκB and PKC (Xu et al. 2015).

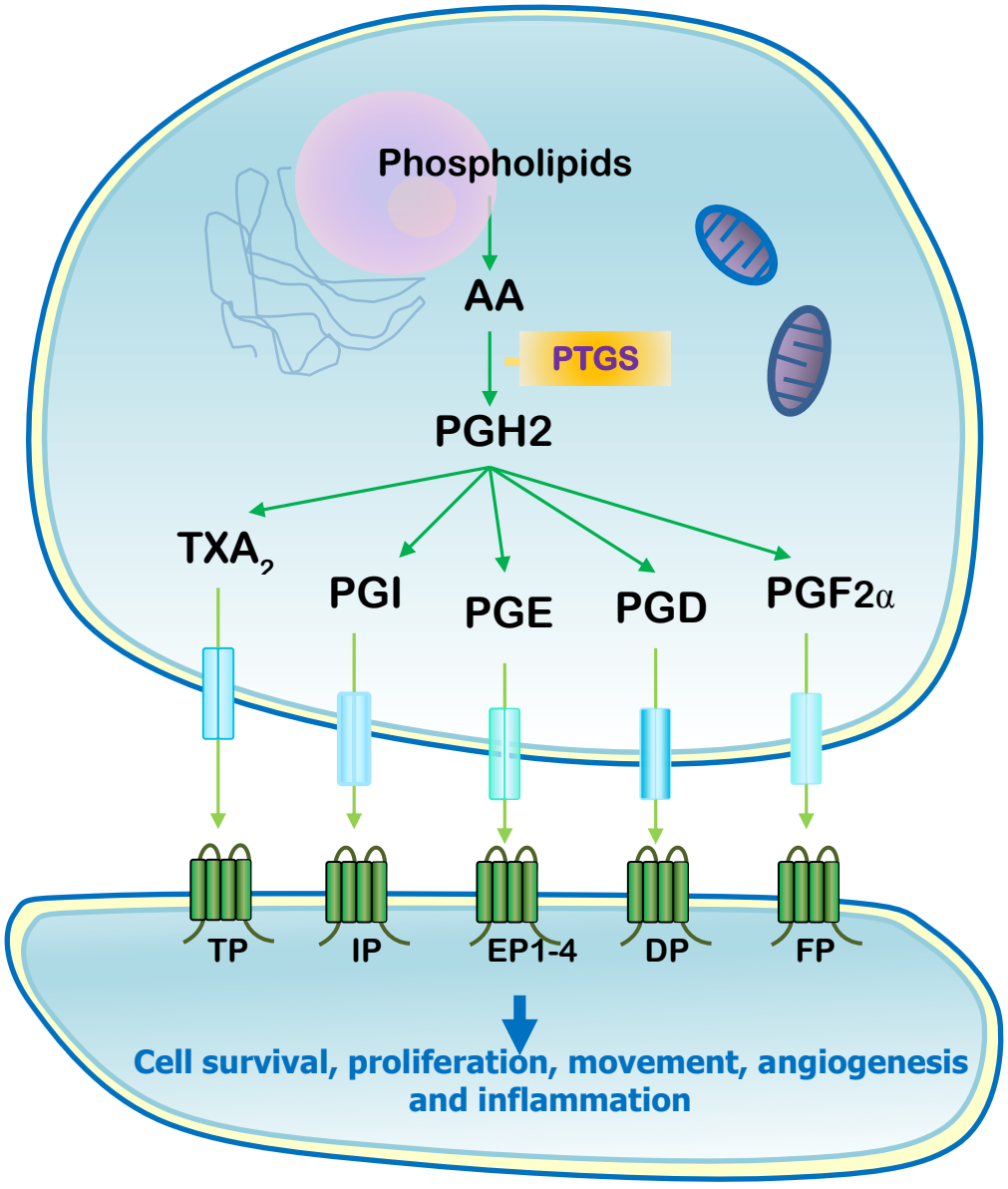


Figure 2. Diversity of intracellular prostaglandins produced through the eicosanoid pathway.
Phospholipids are converted into arachidonic acids (AA), which are reduced to prostaglandin H2 (PGH2) by PTGS2 (yellow box). The PGH2 precursor is converted by prostanoid enzymes into bioactive prostaglandin I2 (PGI2), PGE_2, PGD_2, $PGF_{2\alpha}$ and thromboxane A2 (TXA2). These are transported out of the cell to their G-coupled membrane receptors: prostaglandin I_2 receptor (IP), Prostaglandin E1-4 receptors (EP1-4), prostaglandin D_2 receptor (DP), prostaglandin $F_{2\alpha}$ receptor (FP) and thromboxane receptor (TP), respectively, which are expressed on the cell surface. Image adapted from (Fletcher et al. 2010).

1.2.2.1.1 Prostaglandins in the Myometrium

Myometrial tissues express PTGS enzymes, transporters and contain active prostaglandins during labour. Immune cells are present in the uterine tissues and constitutively express PTGS1 enzymes and PTGS2 when stimulated by inflammatory events (Seibert & Masferrer 1994). Expression of PTGS2 is increased in human term labouring tissues in the myometrium, promoting NFκB activation (Poligone & Baldwin 2001) and thus local cytokine levels such as TNFα (Poligone & Baldwin 2001; Ackerman et al. 2005), IL-1β (Belt et al. 1999; Bartlett et al. 1999), IL-8 (Elliott et al. 2000), and TGFβ (Brown et al. 2000). Activation of FP by $PGF_{2\alpha}$ is also crucial in stimulating smooth muscle contraction in the myometrium (Cook et al. 2000). Inhibition of PTGS-2 expression, using a PTGS-2 selective inhibitor, delays the onset of labour by decreasing conversion of $PGF_{2\alpha}$, but not PGE, and reduces response to inflammatory products (Olson & Ammann 2007).

Prostaglandins have been shown to produce anti-inflammatory actions. IL-1β stimulation of prostaglandins has been shown to suppress *in vitro* human myometrial release of chemokine motif CXC ligand 8 (CXCL8), chemokine motif CC ligand 2 (CCL2) and granulocyte-macrophage colony-stimulating factor (GMCS) (Mosher et al. 2012) most likely via the inhibitory action of cyclopentenone prostaglandins on IκB inhibitors (Poligone & Baldwin 2001). These specific prostaglandins also lower the cytotoxicity of macrophages and natural killer cells (Goodwin et al. 1983).

Mice completely lacking the PTGS1 enzyme demonstrate dysfunctional parturition. These mice have reduced uterine contractions resulting in extended pregnancy. The PTGS1 knockout mice eventually give birth successfully, but have higher rates of pup morbidity and mortality (Lim et al. 1997). Female mice homozygous for PTGS2 knockout are infertile due to abnormalities in ovary development, fertility, and decidua formation (Lim et al. 1997). FP knockout mice also demonstrate dysfunctional parturition (Tsuboi et al. 2000). Mid-gestation treatment with prostaglandins $PGF_{2\alpha}$ and PGE_2 rescues normal parturition, but importantly $PGF_{2\alpha}$ treatment did not benefit pup survival rate (Sugimoto et al. 1997).

1.2.2.1.2 Prostaglandins in the cervix

Prostaglandins are also involved in cervical ripening (Witter 2000). They promote glycosaminoglycan synthesis, an important factor regulating the breakdown of collagen in the cervix (Schmitz et al. 2001). A lack of FP signalling is associated with a reduction of cervical macrophage infiltration, suggesting a role of cervical FP expression and inflammation in cervical ripening (Yellon et al. 2008). Macrophages and prostaglandins appear to interact, but the signalling and regulation of this crosstalk is not clear. Clinically, low dose application of a synthetic PGE_2 is an effective inducer of labour, promoting cervical ripening, compared to no treatment (Toplis & Sims 1979; Norstrom 1982; Ekman et al. 1983; Lopes et al. 1991; El Fekih et al. 2009)

1.2.2.1.3 Prostaglandins in the decidua and fetal membranes

In the decidua, PTGS1 is expressed throughout pregnancy (Gross et al. 1998). In humans, PTGS2 expression levels peak at labour (Fuentes et al. 1996; Reese et al. 2000), but is stably expressed through labour in mice (Gupta et al. 2001). The prostaglandins synthesised in humans include $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{2\alpha}$ where concentrations are the most abundant at labour (Skinner & Challis 1985; Norwitz et al. 1991). Fetal membranes from women at term produce more PGE_2 and $PGF_{2\alpha}$ than women with preterm delivery (Reece et al. 1996). This suggests that prostaglandin levels and specific receptors have a role in the timing of normal labour. Exogenous addition of inflammatory cytokines such as IL-1β and IL-6 also induces decidua prostaglandins (Dudley et al. 1993; Mitchell et al. 1995; Ishihara et al. 1996). In addition, anti-inflammatory IL-10 regulates fetal membrane prostaglandin synthesis (Brown et al. 2000) demonstrating the prostaglandin production and inflammatory stimuli relationship.

1.2.2.2 Oxytocin

Oxytocin is a hormone secreted by the posterior pituitary, affecting behaviour, inflammation and in pregnant women, promotion of labour and lactation. Oxytocin receptor (OXTR) mRNA expression increases at term in the human myometrium and is stably expressed across pregnancy in the decidua (Wathes et al. 1999).

The myometrium and decidua also locally secrete oxytocin at term, and during labour oxytocin secretion intensifies and sustains myometrial contractility (Terzidou et al. 2011). Rat studies have shown the myometrium is sensitive to oxytocin at term and during labour, but not at earlier gestational stages (Garfield & Beier 1989; Arthur et al. 2008). Oxytocin contributes to the increasing frequency of action potentials and thus the excitability of the smooth muscle. This facilitates strong myometrial contractions and drives the final stages of birth. The effectiveness of oxytocin as a contractile stimulator is such that it generates a response in electrically-stimulated post-term myometrial tissue, with a response similar to that of normal term myometrial tissue (Arrowsmith et al. 2012). Oxytocin has been shown to increase myometrial tone during the active contraction-relaxation cycle. Higher intracellular calcium concentration and improved myometrial tension are enhanced by oxytocin during the active phase of contraction and relaxation (McKillen et al. 1999).

In an equine endometrial model, oxytocin treatment promoted the expression of PTGS enzymes (Penrod et al. 2012). Oxytocin has also been demonstrated to stimulate the release of prostaglandins from the cervix in bovine models (Fuchs et al. 2002), suggestive of a synergistic action of oxytocin and prostaglandin in myometrial activation. The dilatation and structural changes in cervical ripening involves inflammatory processes, that facilitate tissue remodelling, and these are tightly regulated by oxytocin. In an *in vivo* experiment with LPS gram-negative bacteria stimulation, volunteers treated with oxytocin had lower levels of ACTH, cortisol, IL-1RA, IL-4, IL-6, CCL2, CCL3, CCL4, CXCL10, and TNFα compared to placebo controls (Clodi et al. 2008). In human term myometrium and fetal membranes, oxytocin can stimulate the production of IL-6, IL-8, CCL5,

and PTGS2 (Kim et al. 2015). Stimulation of amnion cells with IL-1β increases oxytocin secretion (Terzidou et al. 2011). This strongly associates oxytocin in the regulation of inflammatory factors. After birth, oxytocin continues to be produced in an autocrine and endocrine manner, assisting in maternal-infant bonding, promotion of uterine vascular constriction, blood clotting, remodelling of the uterus postpartum and lactation. Expression of OXTR and interactions with labour-associated genes are complex; the expression of OXTR upregulates GJA1 mRNA expression and contributes to formation of gap junctions, though overstimulation can cause desensitisation of the OXTR pathway and a reduction in activity (Vrachnis et al. 2011).

1.2.2.3 Gap junction proteins and formation

Gap junctions (connexins) are transmembrane intercellular communicating channels that allow exchange of ions, hormones, signalling peptides and electrical signalling between neighbouring cells (Sakai et al. 1992). They are composed of connexin protein complexes that are transiently formed and degraded, resulting in fast and coordinated intercellular communication. Myometrial gap junctions increase during pregnancy, allowing rapid contractile synchronization over the whole uterus during the progression of labour (Willecke et al. 2002).

In the murine myometrium, gap junction formation in humans and rats greatly increases during term labour (Lye et al. 1993; Chow & Lye 1994) and also in PTL (Tabb et al. 1992) demonstrating a role in both normal and abnormal labour. Over 20 types of vertebrate connexin proteins exist, where GJB2 (connexin 26; CX26) and GJA1 (CX43) are the most highly expressed in reproductive tissues. In the rat, both proteins are expressed in the myometrium and decidua. However, GJB2 is expressed throughout pregnancy, whereas GJA1 is increased the day before parturition (Winterhager et al. 1991), peaking during the labour process (Lye et al. 1993; Khanam & Burnstock 2007). The importance of coordinated cell-cell communication for amplified signalling through GJA1 gap junction formation has been demonstrated in transgenic mouse models, where the ablation of GJA1 prolonged pregnancy and term labour (Doring et al. 2006; Tong et al. 2009). This GJA1 knockout had no effect on prostaglandin receptor FP, oxytocin or progesterone receptor expression (Doring et al. 2006) but resulted in decreased gap junction formation in the myometrium and reduced response to oxytocin (Tong et al. 2009). This suggests that GJA1 expression and gap junction formation have a major role in myometrial contractions via enabling the cellular to cellular communication.

Hormones have a major influence on the formation of GJA1 gap junctions. Progesterone receptor signalling is associated with GJA1 mRNA expression in the human myometrium. In humans, myometrial GJA1 protein expression was decreased in late pregnancy and during labour (Chow & Lye 1994), but analysis of gap junction formation revealed a significant increase in labouring tissues (Tabb et al. 1992; Chow & Lye 1994). A study using immunocytochemistry, found that a ratio of high estrogen to progesterone induced more GJA1 protein expression in myometrial cells, compared to progesterone alone (Kilarski et al. 1996; Kilarski et al. 2000). An antagonist of progesterone significantly increases GJA1 mRNA expression in rats (Petrocelli & Lye 1993) and estrogen stimulates an increase (Petrocelli & Lye 1993; Xie et al. 2012). Rat myometrial increases

in GJA1 mRNA and protein expression has been demonstrated to be transitory and specific to labour. There are negligible levels during pregnancy, an increase during labour, and rapid regression postpartum (Lye et al. 1993).

1.2.2.4 Labour as an inflammatory process

As previously described, promoters of labour and contractile mediators are actively involved in normal parturition. In addition, many inflammatory activating events have also been described during labour and participate in the processes of parturition. In particular, macrophages, neutrophils, T helper cells, mast cell and natural killer cells were present in the absence of signs of infection.

1.2.2.4.1 Evidence that macrophages are involved in parturition

Macrophages are associated with wound healing and debris removal by phagocytosis to maintain tissue homeostasis. Circulating monocytes are induced to differentiate into macrophages by local pro-inflammatory signals and once differentiated, further amplify inflammatory activity by affecting local prostaglandin levels (Norwitz et al. 1991) and hormone production (Challis et al. 2009). Maturation of macrophages is influenced by natural killer cells, T cells and neutrophils; secretion of cytokines such as interferon-γ (IFN-γ), CCL2 and TNF-α. Macrophage release of IL-1β promotes inflammation by altering vascular permeability, endothelial adhesion and migration of neutrophils, eosinophils, and T lymphocytes (Bless et al. 2000), and MMP production (Haque et al. 2004). PTGS2 expression are upregulated by macrophages and they stimulate T- lymphocytes to adopt a Th2, rather than a Th1, subtype (Taylor et al. 2006; Nagamatsu & Schust 2010; Gomez-Lopez et al. 2014a). T cell features are described in chapter section 1.2.2.4.3 below. Subsets of macrophages predominantly produce anti-inflammatory factors such as IL-10 and IL-4, thus inducing an autocrine reduction in cytotoxic response, which is characteristic of the alternatively activated M2 phenotype (Xu et al. 2006; Makita et al. 2015).

At the onset of parturition, macrophages make up the highest population of immune cells in the uterus (Bulmer et al. 1988; Norwitz et al. 1991; Thomson et al. 1999; Osman et al. 2003). They are significantly increased during labour across many species including humans (Thomson et al. 1999; Hamilton et al. 2012), mice (Mackler et al. 1999) and rats (Hamilton et al. 2012). Characterisation of macrophage infiltration of the rat uterus demonstrated that macrophages were present at least 12 hours prior to normal term labour, suggesting a role in priming uterine tissues for parturition (Hamilton et al. 2012). Macrophage infiltration was also significantly higher in the decidua than in the myometrium in both rat and mouse models (Hamilton et al. 2012; Shynlova, et al. 2013b). Macrophages have also been shown to increase in numbers during term human labouring myometrium (Thomson et al. 1999; Hamilton et al. 2012), cervix (Osman et al. 2003), decidua (Vince et al. 1990; Hamilton et al. 2012) and maternal blood (Yuan et al. 2009). Similarly, leukocyte infiltration has been observed at term labour in the murine myometrium (De et al. 1993; Mackler et al. 1999; Shynlova et al. 2013b), decidua (Mackler et al. 1999; Hamilton et al. 2012; Shynlova et al. 2013a) and cervix (De et al. 1993; Mackler et al. 1999; Yellon et al. 2009). The

increase in uterine macrophages during labour is paralleled by a corresponding increase in cytokines and chemokines at the onset of parturition (Sennstrom et al. 2000; Winkler et al. 2001; Keelan et al. 2003a; Hamilton et al. 2013). Though these factors have been identified at onset of contraction and initiation of labour, their roles as upstream mediators or downstream effectors are unclear.

1.2.2.4.2 Evidence that neutrophils are involved in parturition

Neutrophils are short lived, and like all immune cells originate from the bone marrow and are found in the circulation. As the first line of defence, neutrophils engulf pathogens, release digestive proteases, reactive oxygen species, and inflammatory cytokines and chemokines. Neutrophils can deactivate their cell surface receptors and alter the response of T and B lymphocytes. Neutrophils release angiogenic factors such as vascular endothelial growth factor (VEGF) as well as TNF-α, IL-1β, CXCL10, CXCL8 and CCL3, a similar cytokine release profile to that of macrophages (Kasama et al. 2005). The production of these cytokines suggests neutrophils play a role in regulating the pro-inflammatory or regulatory actions of macrophages. Neutrophils are suggested to be the administrators of the innate inflammatory response, quickly attracting other innate immune cell types with the ability to mediate adaptive immunity. Neutrophils co-localise with T-lymphocytes, assisting in T-cell activating by acting as a pathogen presenter to dendritic cells.

An abundance of decidual and placental neutrophils is associated with pregnancy pathologies, however, there are elevated numbers in the blood in normal term pregnancies (Yuan et al. 2009), in the cervix at the onset and during labour (Bokstrom et al. 1997) and during labour in the myometrium (Thomson et al. 1999). Neutrophils are attracted by the chemokine CXCL8, which is abundant in myometrial and cervical tissue at term labour (Osman et al. 2003).

1.2.2.4.3 Evidence that T cells are involved in parturition

T lymphocytes are a leukocyte subtype that aid in generating long lasting immunity through humoral antibody and cellular responses. Under the influence of the cytokine IL-2, T cells transform from a $CD3^+$ naive T cell to antigen- and MHC class II-exposed effector $CD4^+$ helper or $CD8^+$ (cytotoxic) T cells. $CD8^+$ cells are cytotoxic, whereas $CD4^+$ helper cells interact with B cells to produce memory T-cells and facilitate innate immune cell intercommunication via cytokine production. There are three subtypes of effector CD4 helper cells and two are known to be involved in pregnancy: Th_1 and Th_2; a third exists in autoimmunity: Th_{17}. CD4 Th_1 helper T cells mount a pro-inflammatory response driven chiefly by the cytokines IFNγ, TNF-β, IL-2, and IL-12. Th_1 are responsible for cell mediated immunity and inflammation. $CD4^+$ Th_2 helper T cells are involved in antibody mediated responses and inhibit phagocyte functions in response to IL-4, -6, -9, -10, and -13. Helper T cells interact with antigen presenting macrophages, neutrophils and mast cells, as well as memory T cells (Abbas et al. 1996; Duhen et al. 2012). Th_2 cells regulating inflammation by decreasing inflammatory response (Schmidt-Weber et al. 1999; Chen et al. 2004; Gilmour & Lavender 2008). IL-4 is responsible for the conversion of neutrophils, macrophages and

NK cells into an antigen presenting phenotype rather than toward a phagocytotic or cytotoxic phenotype (Gilmour & Lavender 2008; Sykes et al. 2012a).

There are also non-effector regulatory T-cells (T-regs). T-regs are formerly known as suppressor T cells, identified by their lack of IL-2 and expression of CD4, CD25 and FOXP3 (Fontenot et al. 2003). As their name suggests, they have a highly specialised role in regulation of immunity by immuno-suppression and tolerance by regulating the immune reaction (Abbas et al. 1996; Fontenot et al. 2003; Duhen et al. 2012). In pregnancy, the major T cell type present are the Th_2 helper $CD4^+$ T cells and T-reg cells, both of which play a major role in maintaining pregnancy and tolerance of the fetal allograft (Alijotas-Reig et al. 2014; Jiang et al. 2014; La Rocca et al. 2014) .

In general, the T-cell population is increased in the maternal serum during pregnancy through to postpartum (Wegienka et al. 2011). In the myometrium, T-cells increase during labour (Thomson et al. 1999). In the cervix, T-cell numbers are significantly higher at term and throughout labour compared to non-labouring tissue (Bokstrom et al. 1997; Osman et al. 2003). In the human decidua, T-cells are present at term (Osman et al. 2003) and significantly increased after spontaneous term labour (Gomez-Lopez et al. 2013). In mice, reducing the number of T-regs induced PTL in mice. The study then demonstrated that co-culturing exogenous T-reg cells from normal pregnant mice, with T-cells isolated from the T-reg deficient PTL mouse model, reduced abnormal pro-inflammatory secretions (Zenclussen et al. 2005).

Mouse models have shown that T-reg development in normal pregnancies are driven by paternal antigen priming of the maternal tissues towards cell expansion and tolerance of the fetal allograft, supporting the successful maintenance of pregnancy to term (Zenclussen et al. 2005; Leber et al. 2010). The pattern of elevated T-cell in mice and humans during pregnancy, and the reduction in T-regs in abortion induced mice, suggest they are required for pregnancy and appropriate timing of labour via the alteration of immune tolerance contributing to uterine tissue activation. The mechanism and control of their transformation at the end of pregnancy towards a labour-promoting phenotype is unclear.

1.2.2.4.4 Evidence that NK cells are involved in parturition

NK cells are able to recognise stressed cells, foreign bodies and synthesise cytotoxic cytokines without the presentation of antibodies. Specific to uterine tissues, is a specialized lineage of NK cells known as uterine NK cells (uNK). These uNK cells interact with monocytes, stimulating release of cytokines IL-12, IL-15, and IL-18. These cytokines also stimulate the uNK cells to produce IFN-γ, IL-10, CCL-2 and TNF-α (Fehniger et al. 1999). These cytokines influence the maturation of infiltrating macrophages and T cells, stimulating the production of prostaglandins, MMP release and facilitate vascular remodelling (Winkler et al. 1997; Zhang et al. 2011a).

The origins of uNK cells are unknown, but they possess regulatory characteristics and reduced cytotoxic ability (Murphy et al. 2009). They are positive for CD56, in contrast to CD56 'low' peripheral blood NK cells, and do not express the cytotoxicity marker CD16 (Manaster et al. 2008;

Zhang et al. 2011a). uNK cells are abundant in the decidua and are important mediators of angiogenesis and vascular remodelling during implantation and pregnancy (Wu et al. 2004; Yagel 2009; Zhang et al. 2011a). There are more activated uNK cells in cord blood (Perez et al. 2007) and maternal peripheral blood at term (Mosimann et al. 2013), and $CD56^+$ uNK cells are also higher in blood collected from women during labour compared to non-labouring women (Yuan et al. 2009). The MHC/KIR receptor expression profile of uNK cells in the decidua is essential in maintaining a normal pregnancy, suggesting a role for NK cells in regulating immune recognition of the fetus (Chao et al. 1999). In T-cell and NK cell deficient mice (NOD/SCID), the absence of functional NK and T cells prevented the onset of preterm labour in the prescence of LPS, which normally induces PTL (Lin et al. 2006). In mice with progesterone-delayed parturition, uNK cells were reduced in size and less differentiated compared to normal control mice (Kokubu et al. 2005).

1.2.2.4.5 Evidence that mast cells are involved in parturition

Mast cells respond quickly to cellular injury, complement proteins, or antibodies produced by the adaptive immune system. Actions of mast cells include vasodilatation, endothelium activation and vascular permeability, causing symptoms such as swelling, heat and vascular remodelling (Rodewald & Feyerabend 2012). Like other cells in innate immunity, mast cells secrete cytokines, chemokines, prostaglandins and growth factors (Rodewald & Feyerabend 2012; Gomez-Lopez et al. 2014a). High numbers of uterine mast cells are observed throughout pregnancy, compared to non-pregnant uterine tissues. They significantly increase in the cervix during the onset of labour in mice (Menzies et al. 2012) but no increase is observed in the labouring myometrium (Menzies et al. 2012; Thomson et al. 1999). Human mast cells and histamine release have been shown to contribute to the myometrial sensitivity of contractile processes during labour (Garfield et al. 2006). Allergen priming and histamine treatment of myometrial tissue produces strong myometrial contractions compared to non-primed tissue (Garfield et al. 2006). The mast cell population and histamine secretion in uterine tissues increases towards the end of pregnancy and during labour, and are absent by 8 hours post-partum (Padilla et al. 1990). However, mast cells have been shown to be non-obligatory for labour despite their abundance, temporal-specific expression and function. The depletion of mast cells does not delay the onset of labour, nor does it affect the infiltration of other immune cells (Menzies et al. 2011). However due to the redundancy of immune cell function, when they are present they are likely to be involved in localised priming of uterine tissues to contraction and cervical ripening by their withdrawal (Garfield et al. 2006; Menzies et al. 2011).

1.2.2.4.6 Evidence for cytokines and chemokines during labour

Infiltration of leukocytes into tissues is controlled by cytokines and their respective receptors. Chemokines are chemotactic cytokines that can elicit distinct specific responses by acting via different receptors (Figure 3 and 4). For example, the binding of CXCL12 to CXCR4 increases estrogen receptor activity in breast cancer cells (Sauvé et al. 2009). However when bound to ubiquitin, CXCR4 increases calcium influx and protein degradation (Saini et al. 2010).

The following describes some of the cytokines expressed in uterine tissues. Multiple cytokines are stimulated at TL in the myometrium including IL-1β (Osman et al. 2003; Gorowiec et al. 2011), IL-6, IL-8 (Osman et al. 2003; Mittal et al. 2010; Shynlova et al. 2013b), granulocyte-colony stimulating factor (G-CSF), CCL3 (Gorowiec et al. 2011), CCL2, CCL5 (Kayisli et al. 2002; Mittal et al. 2010; Chaemsaithong et al. 2013) and CXCL6 (Mittal et al. 2010). Conversely, receptors for chemokines such as CCR2, CXCR1 and CXCR2 reduce in expression at time of labour (Hua et al. 2013).

In the cervix, secretion of cytokines corresponded with labour, cervical dilatation and ROM. IL-β, IL-6 (Steinborn et al. 1996a; Sennstrom et al. 2000; Osman et al. 2003), IL-8 (Sennstrom et al. 2000; Osman et al. 2003; Törnblom et al. 2005), CCL2 (Törnblom et al. 2005) and G-CSF (Sennstrom et al. 2000) were associated with term normal labour with intact membranes. The additional presence of cervical TNF-α was associated with ROM in TL and PTL (Steinborn et al. 1996b).

In human fetal membranes IL-1β, CXCR-1, -2, -3, (Gomez-Lopez et al. 2013), CCL2, CCL5, IL-8 (Denison et al. 1998; Hamilton et al. 2013) CCL4, CXCL10 (Hamilton et al. 2013), IL-6 (Osman et al. 2003) and IL-10 (Denison et al. 1998) mRNA and protein were elevated in the choriodecidua at term labour. TNF-α expression was associated choriodecidual leukocytes at term labour (Gomez-Lopez et al. 2013). Many of these cytokines were also expressed in the term placenta (Denison et al. 1998). In the amnion and amniotic fluids, IL-1β (Osman et al. 2003), IL-8 (Saito et al. 1993; Denison et al. 1998; Osman et al. 2003), IL-6, G-CSF (Saito et al. 1993) and CCL5 (Athayde et al. 1999) production peak during term labour. Interestingly, monocytes from the human decidua at term produced IFN-γ, TNF-α, TGF-β, IL-4 and IL-10 but no changes were observed with labour (Gustafsson et al. 2006), suggesting a tissue-based origin of inflammation. Supporting this, the placenta is able to generate pro-inflammatory activity from macrophages (TNF-α) and endothelial cells (IL-1β and IL-6) (Steinborn et al. 1998). The uterine pro-inflammatory activity is not leukocyte-dependent and can originate from multiple sources, indicating a dynamic interaction of leukocytes, inflammation and labouring uterine tissues.

Cytokines clearly play a key role in the progression of labour but whether they are contributing to initiation of the labour cascade or, are simply released as a result of other regulatory processes is still unknown. The complexity of cytokine and immune signalling, which operates amongst other hormonal and endocrine signalling cascades, makes it difficult to delineate the function of individual molecules and determine whether they would make good therapeutic targets.

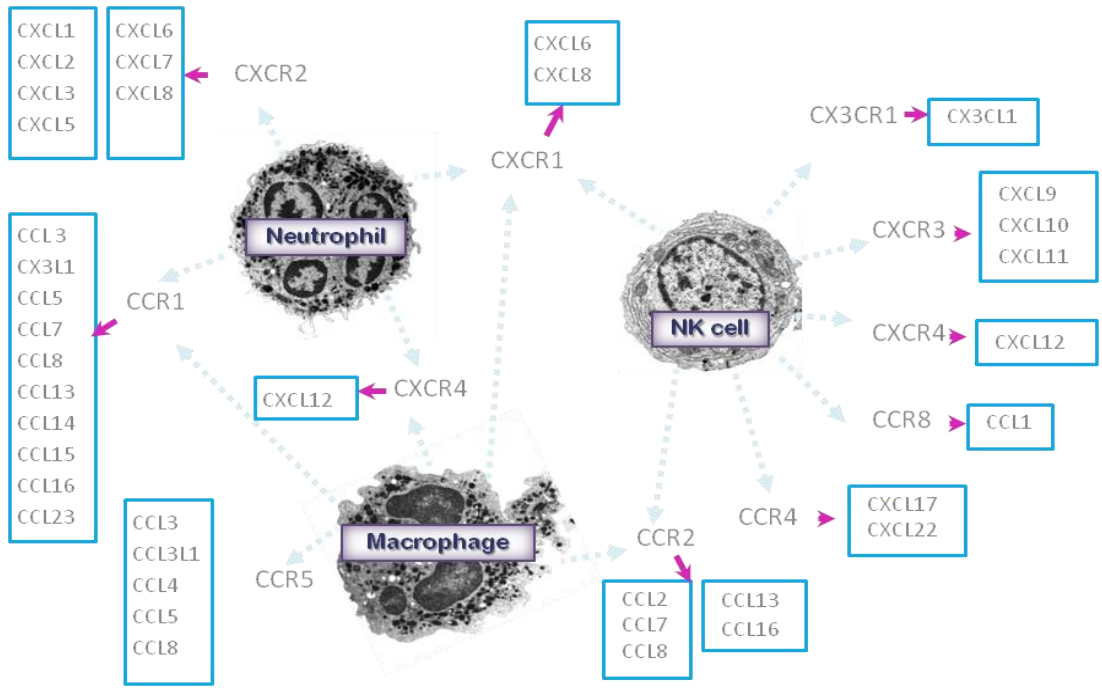


Figure 3. Expression of chemokine receptors on macrophages, neutrophils & natural killer cells.
Each immune cell expresses specific receptors (pale blue dotted arrow) shared with other immune cells. Each receptor has an affinity for several chemokines (blue box) and chemokines may also bind to more than one receptor. Binding is of high affinity regulating a number of biological roles. EM images kindly provided by Dr Carolyn Jones. Modified from (Zlotnik et al. 2006).

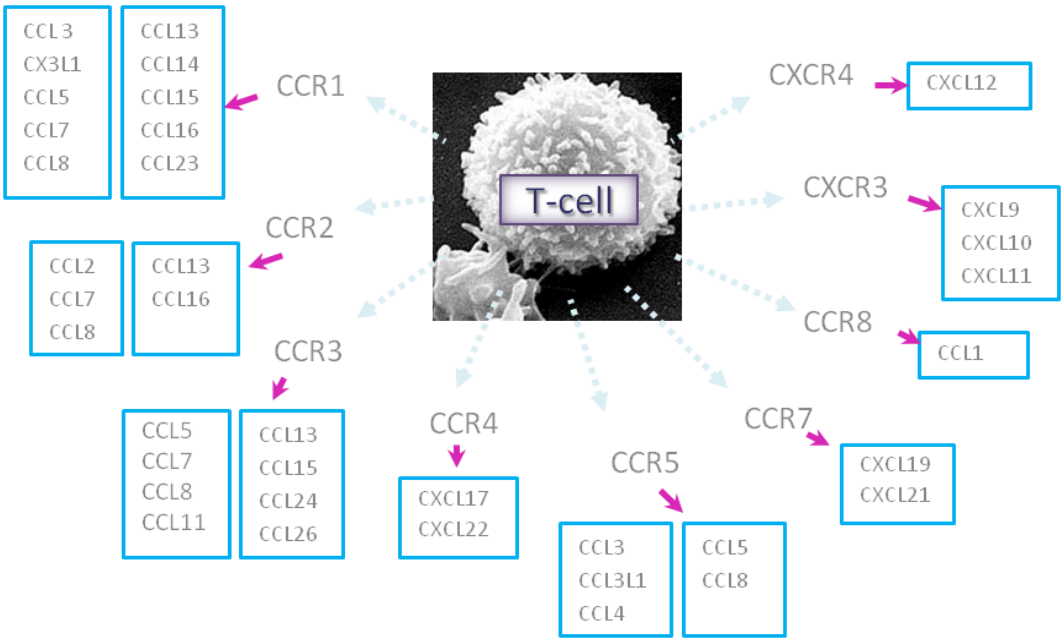


Figure 4. Expression of chemokine receptors on T cells.
T lymphocytes express specific chemokine receptors that may also be expressed on innate immune cells, such as CCR1, 2, 4, 5 and 8; and CXCR3 and 4. Each receptor has an affinity for several chemokines (blue boxes) and chemokines can bind to more than one receptor. Micrograph image from http://en.wikipedia.org/wiki/File:Red_White_Blood_cells.jpg

1.2.2.5 Matrix metalloproteinase

Matrix metalloproteinases (MMPs) are proteases that degrade of extracellular matrix proteins such as fibronectins and collagen; however, they have a wide range of biological roles including releasing apoptotic factors and cytokine release, affecting cell proliferation, cell adhesion and migration, differentiation and apoptosis. There are 24 distinct MMPs, with four naturally occurring inhibitors that regulate MMP function. The balance between expression of MMPs and their inhibitors is suggested to play an important role in driving the timing of tissue separation in labour. During labour MMP1, MMP3, MMP-8, and MMP-9 are expressed by all uterine tissues and tissue resident leukocytes. Pro-inflammatory factors, such as IL-1β, IL-6, IL-8 and TNF-α induce MMP expression and activity in the decidua, cervix, and fetal membranes (Osmers et al. 1995; Roh et al. 2000; Vadillo-Ortega & Estrada-Gutierrez 2005; Christiaens et al. 2008). IL-1β also increases PTGS2 expression in isolated myometrial cells, and stimulates higher MMP production in the cervix (Watari et al. 1999). In a non-human primate model, IL-1β was shown to stimulate prostaglandin production and MMP expression in the fetal membranes (Sadowsky et al. 2006). MMPs function by facilitating ROM during labour, but the coordination of activity in fetal membrane, decidua or cervical activation remains to be clarified. There are hormonal effects, where progesterone dampens MMP production in uterine tissues (Rawdanowicz et al. 1994; Salamonsen et al. 2000). Progesterone, acting through PRB inhibits expression of MMPs (Igarashi et al. 2005), and the switch to PRA dominance facilitates MMP expression (Simian et al. 2009). Extracellular matrix integrity is regulated through PR functions (Lee et al. 2012b). Extracellular matrix breakdown may also be regulated via IL-1β stimulated JNK/AP-1 pathways during parturition (Lappas et al. 2011).

A summary of the promoters and mediators of labour are summarised in Figure 5. The regulatory mechanisms and the order in which the processes occur in the labour cascade are unknown. The hierarchy for promoters and mediators used here is arbitrary.

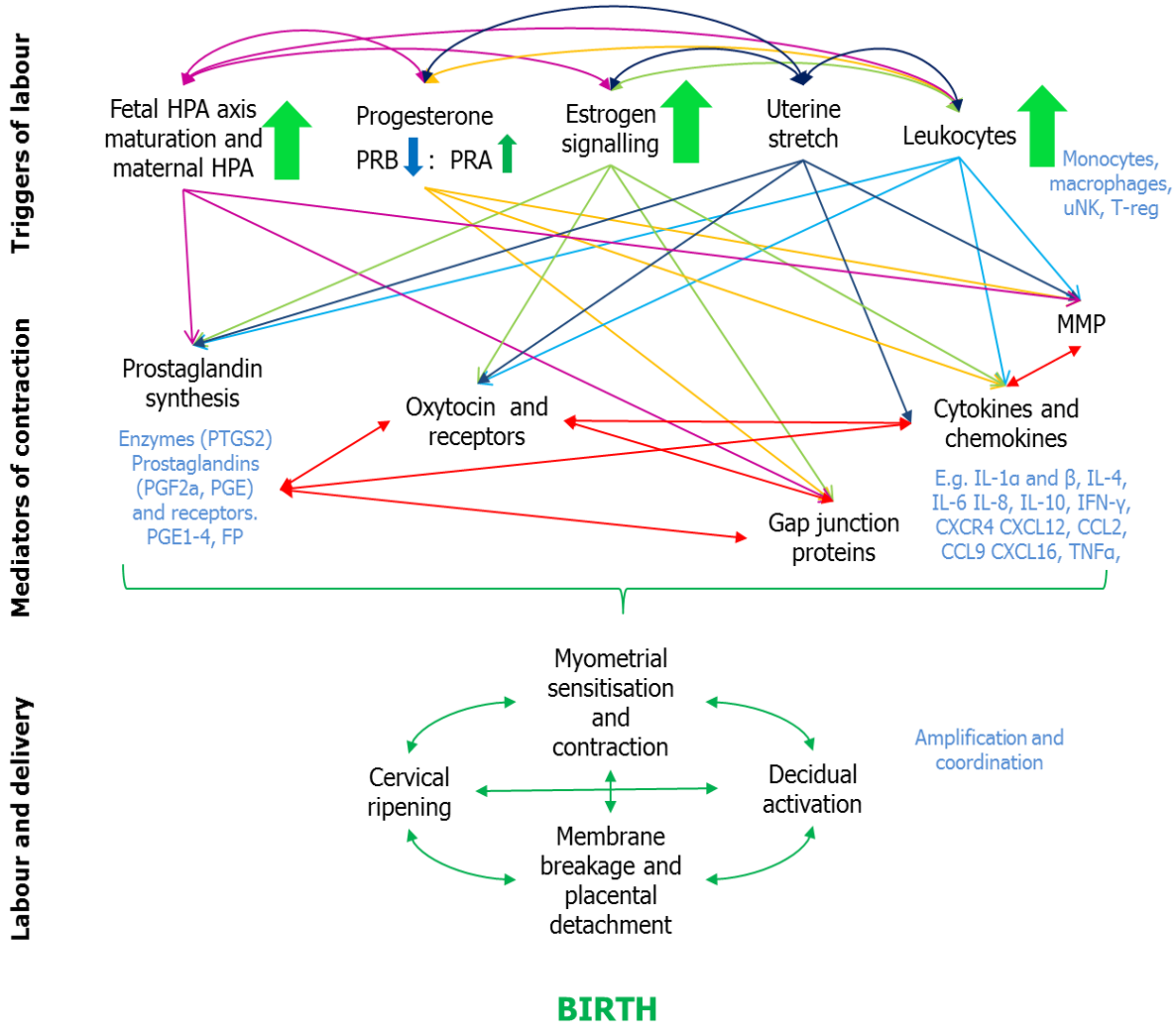


Figure 5. Summary of the promoters of labour and mediators of contraction in successful parturition.

Fetal maturity activates the hypothalamic-pituitary axis resulting in an increase in fetal cortisol and estrogen concentration. Decreased progesterone function with the concurrent increase in estrogen concentration activates infiltration of immune cells and secretion of inflammatory cytokines that trigger prostaglandins, oxytocin and GJA1, as well as MMPs. Immunoregulatory factors are also released by the infiltrating immune cells. The arrows indicate the complexities of coordinating the multiple events required across different uterine tissues for labour to occur.

1.3 Preterm labour

1.3.1 Promoters of preterm labour

In most cases of PTL, the triggers are unknown. However, there is some evidence that some of the same triggers of term labour are involved, but are inappropriately activated prematurely.

1.3.3.1 Fetal hypothalamic-pituitary-adrenal (HPA) axis and preterm labour

There is evidence for a prematurely activated fetal HPA axis in pregnancies presenting with PTL. In women who experience PTL, plasma CRH levels are higher than in women delivering at term (McLean et al. 1999). This elevation can be detected as early as the second trimester of pregnancy. Plasma concentrations of CRH have been linked to the length of pregnancy, not only predicting preterm and normal term delivery, but also post term delivery (McLean et al. 1995). Lower cortisol metabolites and urinary CRH concentrations in obese women were significantly associated with extended pregnancies compared to normal weight women (Stirrat et al. 2015).

It has been suggested that placental release of CRH acts as a 'placental clock' in the HPA axis signalling labour and parturition (McLean et al. 1995; McLean et al. 1999). Placental hypermaturation is associated with pregnancies complicated with preeclampsia and fetal growth restriction (Taché et al. 2011; Morgan et al. 2012). Placental hypermaturation has been observed in women with idiopathic PTL in 84% of the samples, even after controlling for the confounding influence of steroid treatment (Morgan et al. 2012). These symptoms suggest CRH/cortisol signalling in placental, and possibly fetal, maturation contributes towards timing of labour onset. It also implies there is a potential to create further placental deficiencies if treating idiopathic PTL in the presence of placental hypermaturation. Administering treatment for PTL should be approached with caution to ensure only positive benefits to infant health and survival. Heterogeneous maturation is however, characteristic of normal term placentas (Stanek 2013), also the rate of hypermaturity in term placentas was not described to be able to compare the differences between the experimental groups. Syncytial knots were used as a marker of placental hypermaturation, and they are associated with placental dysfunction, but little is known about their formation or function (Coleman et al. 2013).

1.3.3.2 Progesterone and estrogen and preterm labour

The role of progesterone and the use of progesterone as a prophylaxis during PTL has been described in section 1.1.5. It highlights the complexity of the mechanisms of progesterone's action and its use as a treatment, as well as the difficulties in dosing regimen, route of administration and whether there are beneficial outcomes for the infant. The differential expression of progesterone receptor subtype may inhibit progesterone's action and trigger PTL, as progesterone receptor haplotype is significantly different between term and idiopathic PTB (Manuck et al. 2010).

Salivary estrogen to progesterone levels are increased in women with idiopathic PTL, without premature rupture of membranes, compared to the ratios in women who deliver at term (Darne et al. 1987b). Amniotic fluid and maternal plasma estrogen (17 beta-estradiol) concentrations are

elevated in PTL women, and are higher than those of women at term (Mazor et al. 1994). This suggests that spontaneous labour, whether preterm or term is specifically associated with an elevated estrogen levels at the onset of labour. The early activation of the HPA axis in women with PTL also supports the role of estrogen in the untimely activation of uterine tissues: premature release of placental CRH has downstream effects, promoting estrogen synthesis from fetal-derived DHEA.

1.3.3.3 Uterine stretch and preterm labour

As discussed in previous sections, uterine stretch contributes to the upregulation of inflammatory processes and uterine activating proteins that stimulate the myometrium, cervix and fetal membranes into labour. Multiple gestations are a common cause of premature activation of labour, due to the excessive uterine stretch from increased fetal volume, with 42% of twin pregnancies delivering prematurely (Kurdi et al. 2004). CRH levels at 26 weeks are higher in twin pregnancies than in singletons, as are estrogen levels in the third trimester (Smith et al. 2009). Stretch may be involved in PTL, but more likely in multiple gestations, and in combination with other factors such as early activation of the fetal HPA axis and elevated estrogen in the third trimester.

1.3.3.4 Inflammation and preterm labour

There is evidence that some of the inflammatory processes in idiopathic PTL are similar to term labour. The proportion of idiopathic PTBs is significant, occurring in approximately 45-50% of all PTB cases (Pennell et al. 2007; Menon 2008). The inflammatory profile of idiopathic PTL is distinct from infectious PTL. Around 30% of PTL is caused by infection, but the cytokine profile (e.g. IL-1β, IL-6, TNF-α, IFN-γ and CXCL8) is exaggerated (Goldenberg et al. 2008). These cytokines can be stimulated *in vitro* by LPS treatment of fetal membranes, decidua and myometrium (Bowen et al. 2002; Hirsch et al. 2006). Cytokines such as IL-1β are found present in human amniotic fluid at PTL and TL (Romero et al. 1992) and in the choriodecidua at TL (Elliott et al. 2001; Hamilton et al. 2013) and PTL (Hamilton et al. 2013). The expression of cytokines observed in TL and PTL provide evidence that the cytokine profile can be stimulated in the absence of infectious bacterial stimuli.

1.3.3.4.1 Macrophages in preterm labour

Mice deficient in myometrial or decidual macrophages and neutrophils, were observed in the RU486-induced PTL mouse (Shynlova et al. 2013a; Shynlova et al. 2013c). In both human idiopathic PTL and TL macrophage infiltration in the decidua is elevated compared to non-labouring women at term (Hamilton et al. 2012). However, macrophages from maternal peripheral bloodare increased during PTL labour compared to these non-labouring women at term (Yuan et al. 2009).

1.3.3.4.2 Neutrophils in preterm labour

An abundance of neutrophils in uterine tissues has been associated with pregnancy pathologies such as preeclampsia, infection-related PTL (Gervasi et al. 2001; Hamilton et al. 2012) and preterm premature rupture of fetal membranes (PPROM) (Lockwood et al. 2005). There are elevated numbers in the blood of PTL women (Yuan et al. 2009), and in the decidua of women with

chorioamnionitis and PTL, compared to women with idiopathic PTL and TL (Hamilton et al. 2012). In an infectious preterm mouse model induced by LPS, neutrophil infiltration was increased in the myometrium and decidua (Shynlova et al. 2013a; Shynlova et al. 2013b). The pattern of neutrophil infiltration strongly indicates a distinct infiltration of leukocytes, and hence distinct actions in different modes of labour.

1.3.3.4.3 T-cells in preterm labour

T-cells are present in term myometrium (Osman et al. 2003) and are significantly higher in the decidua of women with idiopathic PTL compared to TL and infection-related PTL (Hamilton et al. 2012). T-cells have a significant role in PTL; low T-reg in the thymus and decidua during pregnancy has been associated with spontaneous abortion in mice (Zenclussen et al. 2005). Anti-inflammatory cytokines IL-4 and IL-10 produced by decidual T-cells in humans are lower in preterm pregnancy than in term (Piccinni 2005; Gustafsson et al. 2006), suggestive of a Th1 phenotype. A shift during pregnancy towards a Th_1 cell phenotype is suggested to be a cause of fetal loss and pregnancy related problems, whereas successful pregnancy and labour is predominately associated with a Th_2 response (Sykes et al. 2012b; Alijotas-Reig et al. 2014; Jiang et al. 2014). There are reduced levels of Th2 $CD4^+$ T-cells and elevated Th1 $CD8^+$ T-cells in PTL, compared to women at term but not in labour (Sykes et al. 2012a). Mouse models allow the investigation of PTL, immune activation and states of change throughout the labouring process. Mice deficient in B and T cells (Rag1 knockout) are more susceptible to LPS induced PTL and delivery. This however, can be reduced with the transfusion of purified $CD4^+$, $CD25^+$ T-reg cells during mid gestation (Bizargity et al. 2009). In women with PTL, successful prophalaxis with progesterone demonstrated an augmentation of the T-reg cell population and maintenance of pregnancy (Areia et al. 2015).

1.3.3.4.4 Uterine natural killers cells in preterm labour

During human labour, decidual uNK cells are reduced in women at TL and infection-related PTL compared to idiopathic PTL (Hamilton et al. 2012), suggesting distinct distribution profiles that are specific to term, preterm and non-infection-related labour type. It has been proposed that immune cells are primed in the maternal circulation before transmigration into uterine tissues. Numbers of maternal peripheral blood $CD56^+$ uNK cells are higher in PTL labour compared to non-labouring women (Yuan et al. 2009). Transfer of T-reg cells from normal pregnant mice to a mouse model of PTL reduced PTL incidence and fetal loss (Zenclussen et al. 2005). In IL-10 knockout mice, PTL was associated with the presence of more cytotoxic placental uNK cells compared to the numbers found in wild-type mice. Conversely, deletion of cytotoxic NK cells prevented PTL and prolonged pregnancy (Murphy et al. 2009).

1.3.3.4.4 Chemokines and cytokines in preterm labour

PTL without infection is associated with pro-inflammatory cytokines, which are similar to those found at term labour, but evidence suggests they are abnormally triggered in PTL. Concentrations of IL-1β, IL-6, CXCL8 in the myometrium (Winkler et al. 2001), and IL-1β and IL-8 in cervicovaginal fluids, increase during PTL as cervical ripening progresses and peak at the time of

maximal cervical dilation (Tanaka et al. 1998). In the cervix, cytokine mRNA expression of IL-10, IL-13, IL-1α and β are upregulated in TL and PTL. IL-12 expression is reduced and at a lower concentrations in PTL than in TL (Dubicke et al. 2010a). IL-12 is involved in T cell maturity suggesting that raised IL-12 concentrations may promote higher leukocyte activity during PTL. The cytokines IL-10, TNF-α, IL-1β and IL-6 are elevated in the choriodecidua of women with PTL (Keelan et al. 1999). Though PTL choriodecidua expressed contained 3-6 times greater cytokine concentrations than TL, the cytokine expression profile was similar to normal TL, supporting that there are comparable inflammatory cytokine processes occur in idiopathic PTL and normal TL (Denison et al. 1998). The choriodecidual chemokine expression further indicated distinct profiles between TL, PTL and presence of infection-related PTL. In normal term labour, mRNA and protein expression of CCL2, CCL4, CCL5, CXCL8 and CXCL10 were elevated, CCL8 expression was increased in idiopathic PTL, and an there was an exaggerated upregulation of CCL2, CCL3, CCL4, and CXCL8 in infection-associated PTL (Hamilton et al. 2013). Mitogen stimulation of monocytes obtained from women in PTL resulted in a higher production of the pro-inflammatory cytokines IFN-γ and IL-2, compared to monocytes obtained from women who delivered at term, suggesting a bias towards a stronger pro-inflammatory response (Makhseed et al. 2003).

Individual inflammatory factors have shown to have low predictive value for preterm delivery, whilst a combination of markers demonstrate better predictive markers. In a study using a panel of multiple markers including serum alkaline phosphatase, AFP and GCSF, PTL was predicted with a sensitivity at 81% and specificity at 78% for PTL before 32 weeks gestation. The sensitivity and specificity reduced as PTL gestational age increased (Goldenberg et al. 2001). The study identified that the use of biomarkers in addition to a fetal fibronectin test and/or cervical screening significantly improves positive prediction of PTL. Serum levels of IL-10 and CCL5, in combination with cervical length, possess high predictive levels for spontaneous PTL leading to delivery. The tests had positive and negative predictive values of 76% and 86% respectively (with a sensitivity of 74% and a specificity of 87%) for predicting preterm delivery within seven days (Tsiartas et al. 2012). Second trimester detection of IFN-γ, but not TNF-α or IL-2 or IL-6, in maternal serum has been associated with predicting moderate to late idiopathic PTBs (Curry et al. 2007).

Cytokines have also been shown to directly induce PTL in mice: IL-1β administered to mid to late pregnant mice induced PTL within 24 hours which could be blocked by co-treatment with the IL-1β receptor antagonist (Romero et al. 1991). In a mouse model of IL-6 ablation, parturition is delayed. Systemic treatment with IL-6 from mid-gestation rescued normal term parturition and restored normal oxytocin and prostaglandin expression implicating interleukins in regulating the timing of normal term parturition (Robertson et al. 2010).

1.3.3.4.5 Infection and preterm labour

This study focuses on non-infection related labour, however microbial infection from viruses or bacteria can increase the risk of PTL, especially PTBs at very early gestational ages. Infection can originate in the maternal tissues, fetal membranes, placenta, amniotic fluid, umbilical cord or fetus (Goldenberg et al. 2008). PTL in mice induced by LPS, is associated with elevated myometrial

levels of IL-6 (Witczak et al. 2003; Anbe et al. 2007), CCL2 (Diamond et al. 2007), CXCL8 and TNF-α (Robertson et al. 2006) as well as prostaglandins (Anbe et al. 2007). Women with infectious and non-infectious PTL have high levels of IL-1β, IL-6, CXCL8, and TNF-α in the myometrium (Dudley et al. 1996a) and cervix, leading to the common assumption that infection and inflammation are identical factors in causes of PTL. In the past, studies have described PTL as 'largely subclinical in nature' (Romero et al. 2006), or cytokines are bacterial products (Romero et al. 1992). It was shown however, that concentrations of specific cytokines are discrete between infection and non-infection associated PTL (Hillier et al. 1993; Steinborn, Gunes, et al. 1996; Steinborn et al. 1999; Sadowsky et al. 2006; Hamilton et al. 2013). In particular, IL-1β and TNF-α, upregulated in uterine tissues during term labour and PTL, are significantly higher in the amniotic fluid during infection associated PTL (Sadowsky et al. 2006; Hamilton et al. 2013). As discussed previously, the leukocyte sub-populations in the decidua are distinct between infection and non-infection PTL groups (Hamilton et al. 2012). Infectious PTL may also be defined as related to the existence of a chronic infection that leads to susceptibility to a disproportionate uterine response (Goldenberg et al. 2008).

1.4 Genomic approach to labour

Thus far, the known events involved in the labour cascade have been summarised. The mechanical and hormonal events are numerous, but are frequently investigated separately with the factors consistently demonstraing signalling crossover, between uterine tissues, between triggers of labour and even mediators. This highlights the complexity of signalling during the labour cascade. The interaction, coordination and regulation of each component, as part of the whole system of labour, remains to be identified. Investigating the fundamental molecular mechanisms involved in spontaneous TL is crucial in understanding the abnormal function in preterm and term pathology and hence development of effective therapeutics. Investigating signals that act at an earlier stage in the labour process, elucidating communications between the decidua and myometrium prior to, and at the onset of parturition, and identification of key regulators with multiple downstream targets may reveal novel components that govern the system. In order to achieve this, a global analytical approach is required to examine individual processes in the context of the whole labouring event. The following describes the literature on large-scale molecular analysis of uterine tissues during labour.

1.4.1 Human global gene expression of labour

1.4.1.1 Gene expression profiles in the myometrium and cervix

From the earliest cDNA blot arrays (Aguan et al. 2000; Chan et al. 2002) to large-scale microarrays (Bethin et al. 2003; Hassan et al. 2006; Bollapragada et al. 2009; Weiner et al. 2010; Khanjani et al. 2011; Mittal et al. 2011; Chandran et al. 2014) and RNA sequencing (Chan et al. 2014), it has been demonstrated that the labouring myometrium expresses discrete genes between pregnancy and term labour. These studies are summarised in Appendix Table 1. Aguan et al 2000 undertook one of the earliest studies to identify differential gene expression in the myometrium during labour. The list of upregulated genes included those involved in smooth muscle and uterine contraction, IGFBPs, PKC, and included a platelet aggregation molecule, now known to be a cell adhesion-associated molecule: glycoprotein IIIa/IIb (GPIIIa/IIb) (Aguan et al. 2000). Forming part of the integrin complex, GPIIIa/IIb are expressed by platelets, but are also found on leukocytes, endothelial and smooth muscle cells (Malinin et al. 2012). Down-regulated genes included those involved in myometrial and smooth muscle relaxation and vascular remodelling, and included growth related IGF-2, inflammation/calcium binding-related S100A9 and A8. Within the down-regulated gene list, was the cytokine receptor EB13, which was listed as 'unknown ligand and function' in the myometrium (Aguan et al. 2000). It is now known that EB13 mediates IL27 and IL12 signalling, both of which are associated with the early inflammatory response of CD4+ T helper cells via the JAK/STAT pathway (Carl & Bai 2008). Though the study included a limited number of genes, and the global screening capacity was much reduced compared to available modern microarrays (588 vs 35 800 unique genes), the study clearly demonstrated that labour is a highly unique process and genes not traditionally associated with muscle contraction are also involved.

The pathways that are activated in the myometrium during term labour include a significant number of genes that enriched for inflammation. The cellular pathways associated with these genes include chemokine and cytokine activity, cytokine receptor-receptor signalling, chemotaxis and cell-cell communication (Aguan et al. 2000; Chan et al. 2002; Bethin et al. 2003; Charpigny et al. 2003; Esplin et al. 2005; Rehman et al. 2003; Havelock et al. 2005; Bukowski et al. 2006; O'Brien et al. 2008; Bollapragada et al. 2009; Mittal et al. 2010; Weiner et al. 2010; Khanjani et al. 2011; Mittal et al. 2011; Chaemsaithong et al. 2013; Chan et al. 2014), with high activity associated with NFκB signalling (Mittal et al. 2010; Khanjani et al. 2011; Kim et al. 2015), the MAPK/ERK pathway (Bethin et al. 2003; Lappas et al. 2011; Alexander et al. 2012; Kim et al. 2015), WNT signalling (Rehman et al. 2003), TLR signalling (Hassan et al. 2006; O'Brien et al. 2008); and estrogen receptors (Bethin et al. 2003; Rehman et al. 2003). Though it cannot be ruled out that in some women there may be cases of undetected subclinical infection, as suggested by some studies (Svare et al. 1991; Kara et al. 1998; Ustun et al. 2001; Wu et al. 2009; Genc & Ford 2010; Anon 2011; Castro-Leyva et al. 2012), the samples from women at term labour were normal and non-infection related. The sheer number of investigations involving multiple tissue samples, and studies with strict inclusion/exclusion criteria, also support the premise that non-contractile

related and immune-associated pathways play a significant role in normal term labour in uterine tissues.

Unique patterns of gene expression are also observed in the myometrium in PTL. Myometrial genes common to normal term and infectious PTL myometrial samples (34 genes) were identified in a microarray of 38 500 unique genes (Weiner et al. 2010). From this array, of those that reached the statistical criteria (more than >2 fold of change and present in at least 4 of the 6 arrays), 49 were unique to PTL and 174 were unique to term labour. This analysis indicates that irrespective of the aetiology of labour, there exists a set of genes that control labour, defined as the 'labour activation' or 'effector' set. To elucidate beyond gene-gene pathway functions, Weiner et al also analysed the top ten biological processes associated with labour and their molecular function. Of the labour effector gene set, the genes were primarily categorised into inflammatory-related pathways. In the top 10 highest ranked pathways, 8 out of 10 were inflammation-related, including cell adhesion and chemotaxis pathways. In the logical relationship network analysis of the effector set gene, there were changes predicted in cell cycle and growth (BRCA1, p300, CDK1/P31), apoptosis (p300, p21 and P53) and metabolism (cAMP and pyrophosphate) but also in coagulation and endothelial function (KRK7, tyrRS), components of the complement system (C3Ra and C3a, CD21), and components of the NFκB pathway including RelA (Weiner et al. 2010). Though the microarray comparison between TNL, TL, and PTL indicated that the majority of genes were differentially expressed between the groups, the pathway analysis identified parallels in their biological functions (Weiner et al. 2010).

The myometrium also expresses spatially discrete genes. In a comparison of the fundus and lower segment of the myometrium and cervix, only a small fraction of myometrial genes were commonly expressed across the tissues during labour (28 out of 500 highly significant genes; $p<0.01$) (Bukowski et al. 2006). Genes that were commonly expressed at labour were found in the upper and lower myometrium and cervix, supporting the data from Weiner et al that a particular set of genes has a crucial role in driving the labouring process between tissues. Though the genes expressed were mostly unique in different segments of the uterus, cluster analysis across patient samples demonstrated intercommunicating pathways and co-activated processes (Bukowski et al. 2006). Pathway analysis clustered most of the significantly expressed 500 genes into similar expression profiles. Of those pathways, the highest number of expressed genes included inflammatory associated genes, and those involved in chemotaxis, proliferation, cell migration and tissue formation (Bukowski et al. 2006). These pathways were consistently activated between the individual samples and between the fundus, lower myometrium and the cervix, suggest a coordination of spatially distinct pathways and also potentially, a temporal coordination in the expression of different genes.

Another large scale comparison of myometrial and cervical cDNA identified a profile of 139 commonly expressed genes during term labour. Similar to Bukowski et al. 2006, Bollopragada et al. also identified multiple chemokine transcripts expressed in both the myometrium and cervix. However, functional gene analysis demonstrated at least 40% of these 139 common genes were upregulated, and the functions were categorised into the gene ontology of acute inflammatory

response. Further canonical classical pathway analysis revealed significant activation within the IL-6, CXCL8, IL-1 and TNF-α IP3 cascades (Bollapragada et al. 2009). The study also concluded that the gene profile of fetal membranes demonstrated similarities with the myometrium and cervix, but the fetal membranes data reference was not presented in the methods or results analysis.

These studies have shown selective expression of extensive gene profiles in the myometrium, distinct myometrial compartments and cervix, but the molecular regulation of the mechanical and endocrine processes of labour remain largely unknown. Studies have also indicated that there are potentially greater differences in gene expression between TL and TNL than between PTL and TL (Bethin et al. 2003; Charpigny et al. 2003), indicating that PTL is characterised by premature activation of common signalling pathways, rather than activation of alternative pathways.

1.4.1.2 Gene expression in the fetal membranes

In a study investigating term labouring fetal membranes, the membranes differently expressed 197 genes following labour (Haddad et al. 2006). The gene ontology was enriched for 6 major biological processes: response to wounding, chemotaxis, response to pests, pathogens and parasites, biotic stimulus, abiotic stimulus and viral genome replication. Interestingly in this particular study, the down-regulated genes of the chorioamnion revealed no enrichment for gene ontology categories, but further analysis may have been required. Functional sub-analysis of pathways activated revealed cellular processes in immune-type responses. The genes were highly clustered into functions associated with the haematological system and development including: migration, chemotaxis, immune response, and cell to cell signalling (Haddad et al. 2006). A comparative summary of these studies are in appendix table 1.

During PTL genomic studies, functional analysis identified preterm subgroup profiles in the fetal membranes. In general, the gene ontology was dominated by multiple chemokines and their resepective receptors, anti-inflammatory cytokines, cytokine receptors, chemotactic signals, matrix metalloproteinases, and cell adhesion molecules (Marvin et al. 2002). Infection-related PTL deliveries (iPTL) were associated with extreme NFκB activation. The spontaneous PTL group (sPTL) exhibited upregulation of cytokine/chemokine receptors. The gene ontology of membranes from women with ROM but no infection PTL (aPTL) was predominately associated with the complement cascade and thrombosis (Shankar et al. 2010). All PTL conditions were mediated by TLR signalling (Haddad et al. 2006; Shankar et al. 2010). The gene array also identified unique mechanisms of arachidonic acid conversion within fetal membranes: initial prostaglandin synthesis in iPTL was primarily mediated via aiPLA2γ and PLCXD1, in sPTL it was mediated by PLA2G2A and PLA2G10 and in aPTL through PLCG2, PLA1A and PLCE1 (Shankar et al. 2010).

In a unique sub-analysis, the tissue samples were analysed for inflammatory bias from potential sub-clinical infection by comparing the level of inflammatory gene expression with length of labour. The analysis compared the level of normal term inflammatory activity against time of ROM and duration of labour, as they have both been associated with an increased risk of ascending infection (Haddad et al. 2006). The analysis indicated that neither factor was a determinant of the

inflammatory processes identified from the global analysis, suggesting that the immune profile in normal term labour is a sterile spontaneous inflammatory response.

Distinct changes in fetal membranes have identified during term labour (Haddad et al. 2006) and also for PTL (Shankar et al. 2010). A comprehensive cohort, but small-scale array study detected unique gene profiles in the choriodecidua at term labour and PTL compared to gestational matched not in labour tissues (Marvin et al. 2002). The amnion layer alone expresses unique genes at term labour (Marvin et al. 2002; Lim et al. 2012) and at PTL (Marvin et al. 2002). There are however no large scale studies on the choriodecidua at normal term labour.

During TL and PTL there are also differential expression of microRNAs (miR-) in chorioamnion and isolated decidual cells (Montenegro et al. 2009). Furthermore, labour is such a dynamic event that differential gene expression can be detected in maternal blood (Haddad et al. 2006; Enquobahrie et al. 2009; Peng et al. 2011; Heng et al. 2014), and in fetal tissues in the cord blood (Montenegro et al. 2009; Peng et al. 2011; Davidson et al. 2013) and placenta (Montenegro et al. 2009; Peng et al. 2011). Such transcriptional studies have steered the way into understanding the complexities of labour.

Extensive lists of genes globally expressed during labour have been generated, and more importantly, preliminary analysis of their functions identified. These extensive lists generated by microarray analysis have identified a number of novel genes, suggesting numerous changes occur in the parturition process that encompass more than the contractile events.
However, there is a gap in the transcriptomic data as the gene changes that occur in human choriodecidua during normal term labour have never been investigated. A detail functional analysis of the maternal-fetal interface and the underlying myometrium is required to fully characterise and understand the changing molecular processes of labour. In order to utilise these considerable volumes of data, global unbiased genomic datasets require detailed network analysis to identify not only the key components, but the key high-level, upstream regulators within the biology of labour; identification of these candidates will advance the search for effective therapeutics.

The accessibility of human uterine tissues during gestation presents a challenge to the study of active labour and the stages leading up to active labour. It is these early stages, when priming of the uterine tissues to respond appropriately in labour may be occurring. The ability to examine these early events using mouse models may also help to delineate whether a predominance of inflammation is vital for labour to occur, or whether it is a non-essential but important product of other processes that are occurring. Mouse models provide a useful tool to investigate the temporal changes that during pregnancy, both prior to labour and during active labour, circumventing the difficulties associated with using human tissues. The existing wealth of genetic information of murine models is also useful for experimental investigations in large-scale discovery of potential therapeutic candidates.

1.4.2. Murine global gene expression of labour

1.4.2.1 Gene expression profiles in the myometrium and cervix

It has been shown that there are unique temporal changes in expression of uterotonin genes and non-contractile associated genes from mid-gestation to term and through active labour in the rat myometrium (Arthur et al. 2008; Helguera et al. 2009; Taggart et al. 2012), the mouse myometrium (Cook et al. 2000; Muhle et al. 2001; Bethin et al. 2003; Salomonis et al. 2005; Montalbano et al. 2013; MacIntyre et al. 2014), and in the mouse cervix (Gonzalez et al. 2009). At least two studies included the whole uterus, with only the fetal tissues excised, so could potentially include genomic changes originating from the decidual capularis/parietalis layer (Muhle et al. 2001; Bethin et al. 2003).

Inflammation in the myometrium has been implicated as playing a major role in labour in the mouse. The increased presence of immune cells at term labour is accompanied by an inflammatory gene expression profile that was similar between a non-infection PTL model and term labour (Shynlova et al. 2013c). In a molecular analysis using activation assays and qRT-PCR, a role for NFκB in mid to late pregnancy towards labour in the mouse myometrium was identified by comparing non-gravid animals to those at embryonic day (E)11, E16, and E18 of pregnancy, mice during active labour, and mice treated with RU486 and LPS to induce PTL (MacIntyre et al. 2014). Levels of phosphorylated p65, JNK, and c-Jun remained stable until labour, when activation of JNK/AP-1 complex occurred, with elevated phospho-c-Jun and AP-1 driving the expression of IL-6, -8, -1β, MMPs, PTGS2 and GJA1 (MacIntyre et al. 2014).

Similarly, the uterine transcriptomic profile from a large scale screen of bacterially-induced PTL has a distinct transcriptome to that of non-infection related PTL at mid-late gestation (Muhle et al. 2001). In a SuperArray, analysis of 84 inflammatory genes in the term myometrium, demonstrated that a mouse model ablation of lung surfactant protein A (SP-A) and SP-D compared to wildtype counterparts, significantly delayed parturition in the knockout mice. Normally associated with fetal signalling, SP-A and D negative mice myometrium had down-regulated expression of established labour genes, IL-1β, IL-6, OXTR, and GJA1 (Montalbano et al. 2013).

Normal term and PTL involves a specific signalling profile of prostaglandin converting- and synthesising enzymes in mice (Cook et al. 2000) and in humans (Phillips et al. 2011; Phillips et al. 2014). In the PTGS1 knockout mouse labour model, the myometrial transcriptome exhibited a differential upregulation of several key genes involved in metabolism (ARG1), inflammatory-associated (Immunoglobulin J chain, IgJ, and TNF receptor superfamily 9,) and action potential (troponin, TNNT2) (Zhao et al. 2007). Amongst the down-regulated components were steroid regulated kallikrein-related peptidase 6 (KLK6), epithelial-cell ion transporter (CFTR), signal transducing regulator of Rho-associated protein kinase (GEM) and ion transporter and ATPase activity (Atp8a1) (Zhao et al. 2007). Importantly, they were also differentially expressed between non-labouring mid gestation and at term, suggesting they are contributors to length of pregnancy and the timing of labour (Zhao et al. 2007). In a rat model, a temporal sensitisation of the

myometrium to oxytocin stimulation occurs with pregnancy and during pregnancy progression. There is reduced effectiveness of oxytocin stimulation in non-pregnant samples, compared to mid to late pregnant and active labour (Taggart et al. 2012). Molecular analysis of pregnant rat myometrium showed that levels of mRNA of the immunoregulatory molecule cyclophilin, ERK 1 and 2, Rho-associated kinase-1 and 2, rho family GTPases 1, 2 and 3, and Rho kinases 1 and 2, were consistently expressed throughout gestation, but expression was significantly increased with onset of labour, in both term and preterm cases (Taggart et al. 2012); thus identifying the mechanism leading to enhanced sensitivity of the myometrium to oxytocin signalling as being mediated through the RhoA/ROK pathway. Active labour samples were used in the Taggart et al study, but the tissues were collected on the day of expected delivery and only 7/19 were in active labour, as indicated by the delivery of a pup.

The extensive genomic response profiles of normal, delayed or early parturition require an investigation of large scale gene expression changes and more importantly, the networks and pathways activated by the multiple actions of these altered genes. Only three previous studies included functional pathway analysis of the differentially expressed genes detected. In a normal term mouse model, 41% of the total 12 000 array genes were shown to significantly change in the term uterus (Bethin et al. 2003). The activated pathways were involved in cell cycle, regulator of G protein signalling, immune-related, regulators of cell growth, differentiation, and transformation. Significantly altered genes highlighted at term labour included those associated with apoptosis, signalling with transcription factor SOX4, the JUN/AP-1 transcription molecule cFOS, membrane trafficking zinc finger SR3, matrix metalloproteinases, and transcription factor ELF3 (Bethin et al. 2003). These genes and pathways provide support for the concept of a diverse range of genes involved in the regulation of labour and, in pathways not directly associated with myometrial contraction. In a comprehensive investigation of the 9000 available transcripts in the myometrium between non-pregnant, mid-gestation (E14.5), late gestation (E18.5), and postpartum (24h), over a third were differentially expressed at term (Salomonis et al. 2005). Unsurprisingly, pathway analysis of these genes indicated the myometrium was a site of hormone activity and synthesis, however there was upregulation of cyclic AMP and cyclic GMP stimulation, cytoskeletal cellular transformation, cell junctions, and translational regulators (Salomonis et al. 2005). Many of the pathways not associated with contraction, or contractile sensitisation, have been suggested to play an important, but non-essential role. In a rat model, 43% of 7000 unique genes on a global microarray were altered in the myometrium from late pregnancy (day before birth) to active labour (Helguera et al. 2009). Some of the genes validated from the microarray included upregulation of pro-inflammatory TGFβ-3, retinaldehyde dehydrogenase (ALDH1A2), calcium-binding/extracellular matrix component fibulin, and down-regulation of aquaporin 5 (AQP5), short chain dehydrogenase/reductase (SDR), metabolism associated Solute Carrier Family 5 (Inositol Transporters), Member 3 (SLC5A3/SMIT), thyroid hormone related deiodinase iodothyronine type III (DIO2), and immuno-regulator Fc-γ (Helguera et al. 2009). The genes enriched for pathways in immune-related response, steroid/lipid metabolism, calcium ion maintenance, cell volume regulation, cell signalling, and tissue remodelling (Helguera et al. 2009).

In the mouse cervix, activated genes were enriched for immunity and inflammation pathways in LPS-induced PTB, but genes in the term labour cervix did were not enriched for immune pathways, which were observed in the immediate postpartum cervix. Epithelial cell differentiation pathway was the major group of genes associated with term labour, indicating genes of the cervix at term, term labour and PTL are regulated by differential processes by temporally expressed genes (Gonzalez et al. 2009).

1.4.2.2 Gene expression profiles in the decidua

In a small-scale molecular study of gene changes in the lead up to labour, mRNA expression of contractile-associated genes at term indicated that PGHS1, oxytocin, iNOS and endothelin-1 were primarily expressed in the endometrium. PTGS2, OXTR, PTGS2α FP, GJA1 and endothelin A expression were predominately expressed in the myometrium (Arthur et al. 2008). The pattern of expression of contractile-associated genes indicated a relationship in myometrial function and possibly in regulation of contractility between the 'endometrium' and myometrium at term (Arthur et al. 2008). The endometrium in the study was described as pregnant rat endometrium isolated from the inner layer of the myometrium. As the murine endometrium decidualises during pregnancy in the presence of a fetus, this indicates the tissue analysed was more than likely the decidua. In another study, myometrial upregulation of cFOS was identified, with subsequent in situ hybridisation of cFOS in the decidua (Bethin et al. 2003). This supports the idea of a myometrial–decidual cross talk, with a JNK/AP-1 signalling pathway in both tissues during labour.

A uterine-specific deletion of trp53 led to irregular decidual layer development, reduced layer thickness but increased prostaglandin synthesis activity and increased secretion of PGF2α, which was associated with PTB (Hirota et al. 2010). This over-decidualisation appeared to be facilitated by age-related mTORC1 signalling (Hirota et al. 2011); term labour was restored by PTGS2 inhibition, or with rapamycin (mTORC1 inhibitor), and remarkably, by the creation of another uterine-specific deletion, the trp53 related cell cycle kinase inhibitor Cdkn1a (P21). This study demonstrated that normal decidual development contributes to the regulation of pregnancy duration and onset of labour, whereby cell cycle and apoptosis regulation, and control of prostaglandin synthesis is a causal mechanism (Hirota et al. 2010; Hirota et al. 2011).

The majority of studies using functional genomics and genome-wide analysis focus on comparative analysis of "normal" to "pathological" pregnancy states. Analyses of pathway activation, in conjunction with changes in gene expression during normal pregnancy to labour are lacking. There are no published large-scale microarray investigations of gene expression in the murine decidua at term labour or in the stages leading up to labour to our knowledge. Utilising high through-put genetic approaches and predictive network analyses would help to elucidate the normal progression up to active labour, with detailed upstream analysis of regulatory components, which may provide better insights into more effective therapeutic strategies to block preterm labour.

1.5 Summary

PTB is a worldwide health issue in both developed and developing countries (Blencowe et al. 2012; Norman & Shennan 2013), with increased mortality and risk of morbidities (Lawn et al. 2010; Blencowe et al. 2012). Advances in neonatal care have significantly improved survival for premature infants; however there have been no advances in treatments that effectively suspend labour, thus increasing gestational length and potentially preventing long term health complications. Tocolytic suppression of myometrial contractions is the only therapy available. Meta-analysis of these interventions indicate that these tocolytics have little efficacy in preventing labour, nor do they result in improved health for the infant, but they are administered nonetheless (Haas et al. 2012b). The review of the literature presented above also identifies multiple contributing factors to the labouring process, where myometrial contraction is a late stage event. Whilst many key events involved in mediating labour have already been described, with numerous studies focussing on the changes within the myometrium, we are still in the infancy of understanding the multiple signalling processes that occur, including their interactions, coordination and regulation as a global system. Moreover, a detailed investigation of the role of the decidua in labour is critically important, as it is the site of the myometrium to the decidual maternal- fetal interface and it exhibits considerable inflammatory, progesterone and prostaglandin activity during labour (Makino et al. 2007; Hamilton et al. 2013). Regarding therapeutic interventions, instead of targeting a single and potentially late-stage event such as myometrial contraction, identifying and targeting signals that occur prior to the onset of myometrial contraction, and investigating signals in other closely-related tissues such as the decidua, may be a more effective strategy in preventing the progression of the labour process (Hamilton et al. 2012; Hamilton et al. 2013).

In order to develop an efficacious therapy for PTL, a global investigation of the pathways and regulators of the normal labour cascade is essential. Human genomic studies have identified a large number of genes that are differentially expressed at term labour and PTL (Haddad et al. 2006; Helguera et al. 2009; Mittal et al. 2010). These include many non-contraction-associated genes and the volume of data generated by these studies suggests an important contribution towards labour induction.

Microarray transcriptomics have generated vast quantities of data and identified genes previously not associated with labour. Functional investigations are now required to understand the coordination and interaction between genes, between functional pathways and their upstream regulators, and ultimately, identify key regulators of the labour cascade within the myometrium and decidua at term labour, and potentially in preterm labour, both in human and mouse tissues.

Hypothesis:

Pathway and network analysis of genome-wide transcriptomic data can identify key regulators of the genes expressed during labour. These analyses will also identify the labour-associated genes downstream of the master regulators, and provide therapeutic targets for suppression of multiple labouring processes.

Aims and objectives:

1. To investigate and explore the functions, pathways and molecular characteristics of the human choriodecidua transcriptome expressed at normal term labour, with a focus on understanding the role of inflammation.

2. Using *in silico* analysis, determine the network of interactions within the differentially expressed genes of human choriodecidua and myometrium at term labour. Using this analysis to:

    a. Identify upstream master regulators in both the decidua and myometrium.
    b. Manipulate these master regulators in an *in vitro* model and assess the downstream effects on gene transcription.

3. To phenotype the transcriptome and identify master regulators in a pregnant mouse model to examine the progression of signalling events during pregnancy towards labour. Using this analysis to:

    a. Identify the similarities and differences that define the development of the labour cascade between the myometrium and decidua.

    b. Identify the genes that define the stages just prior to labour and at active labour in the decidua and myometrium.

    c. Identify and manipulate these master regulators and assess the downstream effect for their potential as therapeutic targets.

2.0 Methods

The methods used in this study are described in detail below, however they are also presented in the results chapters in publication format.

2.1 Human tissue collection and sampling

Written informed consent was obtained from women with uncomplicated pregnancies at term (37-41 weeks), undergoing caesarean section without labour (TNL) and from vaginal deliveries (TL), at St Mary's Hospital, Manchester, UK. Placentae were collected within 30 minutes of delivery. The fetal membranes were sampled from the midzone area, distant from the rupture side and placenta (Hamilton et al. 2012; Hamilton et al. 2013). The choriodecidua was removed from amnion by manual separation. Ethical approval was granted by the North West Local Research Ethics Committee (Reference Number: 08/H1010/55 and 08/H1010/55 (+55)).

Strict exclusion criteria were applied to exclude any pathological pregnancies, signs of infection or maternal comorbidity. All women had healthy pregnancies, with no proteinuria, diabetes or maternal hypertension, and infants were of normal birth weight for gestational age (Hamilton et al. 2012; Hamilton et al. 2013). Maternal clinical characteristics for exclusion of infection-related labour were two or more of the following: chorioamnionitis, spontaneous rupture of membranes 48 hours before labour, temperature >37.6^{o}C, white cell count >15, C-reactive protein >10, and a positive high vaginal swab (Hamilton et al. 2013).

Samples from different cohorts of women were used for different experiments. The samples used for microarray analysis (TNL and TL; N=6) and PCR validation (TNL N=10; TL N=11); were collected by Dr Sarah Hornung (nee Hamilton). For choriodecidual explant experiments, tissue was collected from TNL (N=6). For immunolocalisation, fetal membranes were obtained from the Maternal and Fetal Health Research Group Tissue Bank for the following: (i) fetal membranes from TNL (39-40 weeks; N=8); (ii) TL (N=7); and (iii) idiopathic, non-infection-related spontaneous PTL (≤36 weeks; N=8). The samples used were collected based on the exclusion/inclusion criteria specified above.

2.1.2 Human tissue RNA isolation

For the human transcriptome analysis, choriodecidual explants (TNL and TL) were incubated in RNAlater (Ambion, UK) for 24h at 4^{o}C and then stored at -80^{o}C until RNA extraction. RNA was isolated using column extraction methods according to the manufacturer's instructions in the Qiagen RNeasy miniprep kit (UK). After extraction, an extra genomic DNA removal step was performed with DNAeasy kit (Ambion, UK). This kit uses a bead-binding assay to remove contaminating DNA, which is separated by centrifugation, leaving the RNA in the supernatant. Quality of RNA was assessed by measuring absorbance at A260/280 and A260/230. The Quant-iT™ *RiboGreen* RNA Assay Kit (Life Technologies, UK) was used to quantify the RNA. Samples were assayed in duplicate using spectroscopy excitation and emission readings at 480/520nm and compared against a standard curve. Before hybridisation to the microarray, the samples were re-assessed for RNA quality using the Agilent 2200 TapeStation system for a RNA integrity number equivalent (RIN^{e}). The RIN was calculated from electropherogram signal of each sample; the measurement also provides the gel profile of 28s to 18s ribosomal RNA ratio. RIN^{e} scores close to 10 are of the most stable intact RNA and close to 1 are degraded RNA (Schroeder et al. 2006). RIN

scores above 5.5 are acceptable for molecular analysis. Human microarray samples within this study had a RIN[e] of 7 to 8.5. For PCR analysis of individual human samples, RNA was assessed using a NanoDrop 2000 UV-Vis spectrophotometer, measuring absorbance at A260/280 and A260/230.

2.1.3 Human microarray hybridisation

The mRNA samples were hybridised in duplicate onto a Human Genome U133 Plus 2.0 Affymetrix GeneChip according to manufacturer's instructions. Technical quality control and outlier analysis was performed with dChip V2005 (Li & Wong 2001) using the default settings. Normalisation and expression analysis were performed using multi-mgMos (Liu et al. 2005) with a robust multivariate analysis (RMA), a Bayesian method for probe-level measurement error when assessing difference of expression accounting for intensity and spatial labelling of each individual spot (Yang et al. 2002; Liu et al. 2006). Statistical data were analysed with Propagating Uncertainty in Microarray Analysis (PUMA) from the Affymetrix Microarray Suite 5.0 software (Irizarry et al. 2003; Pearson et al. 2009).

2.1.4 Human quantitative Real Time PCR

To reduce non-experimental variation and ensure PCR accuracy, the RNA was transcribed in duplicate. These initial experimental duplicates were then amplified again in duplicate, for technical accuracy using a reference gene YWHAZ. If the expression was similar between technical duplicates, that is, the coefficient of variation of the $2^{(-Ct\ value)}$ was <16.6%, the experimental duplicates were combined for later experiments to analyse of genes of interest.

2.1.4.1 Reverse transcription

After isolation and assessment of RNA quality, the first strand of cDNA was transcribed. An Affinity Script Multiple Temperature cDNA Synthesis Kit (Agilent, UK) was used according to the manufacturer's instructions. Samples were assayed in duplicate; total RNA (100ng per sample) was initially annealed at 65°C with random primers in the first template-primer step. The addition of heat in-activators and reverse transcriptase enzyme converted the annealed primer/RNA mix into cDNA, over a temperature incubation of 25°C for 10 minutes, 50°C for one hour and then at 72°C for 15 minutes to stop the reaction. The cDNA was then stored immediately at -20°C until needed. Two negatives controls were generated with each run; a mock control with template but the exclusion of the reverse transcriptase, and a no template control with water substitution.

2.1.4.2 QRT-PCR

For genes of interest, cDNA was amplified by qPCR using primer sequences referenced from the Primer-BLAST PubMed database (Table 3) and PrimerBank (Wang et al. 2012). The sequences were then purchased from Life Technologies (UK), Eurofin (UK) or Qiagen (UK). PCR fluorescence detection was carried out using the Ultra-Fast SYBRGreen QPCR Master Mix III with 5-carboxy-x-rhodamine (ROX) as a reference dye (Agilent, UK). Samples were amplified in duplicate on a Stratagene QRT-PCR MX3005P machine. Final concentrations of primers were 0.25uM and the annealing temperature of each primer was optimised for 60°C. The amplification cycle was as

follows: single cycle for 3 minutes, then 40 cycles of 95°C for 10 seconds to denature the cDNA, with 60°C for 20 seconds to anneal and extend to the gene of interest; Dissociation curve was 95°C for 1 minute, 55°C for 30 seconds, and to finish at 0.2°C incremental increases up to 95°C. An amplification with single dissociation peak was considered a single product (Figure 6A and B). Relative quantification was used to determine difference in expression of mRNA between samples. Data was expressed as differential expression between two groups using $2^{-\Delta CT}$ formula, or $2^{-\Delta\Delta CT}$ formula for differential expression to a vehicle control, after normalisation to a suitable reference gene.

Before analysis of any of the expression data, a panel of housekeeping genes were assessed for suitability as a stably expressed reference genes (Table 3A). On assessment, it was found that different 'housekeeping' genes altered in expression between each different condition and experiment. The house keeping gene 18SrRNA was the most stably expressed between TNL and TL and chosen for array/PCR validation, RP13L was chosen for the human choriodecidua inhibition studies and YWHAZ for the myometrial. TBP was chosen to normalise the PCR data for the mouse inhibition treatment experiments and GAPDH for the array/PCR validation. For microarray validation studies, amplification was carried out on individual samples (total TNL: n=10 and TL: n=11).

Genes of interest were selected based on differential expression from the microarray and functional significance to labour (Table 3B). The master regulators were identified by the network analysis work flow described in a later section. For the functional study, genes of interest were selected from the causal network analysis prediction and genes associated with labour.

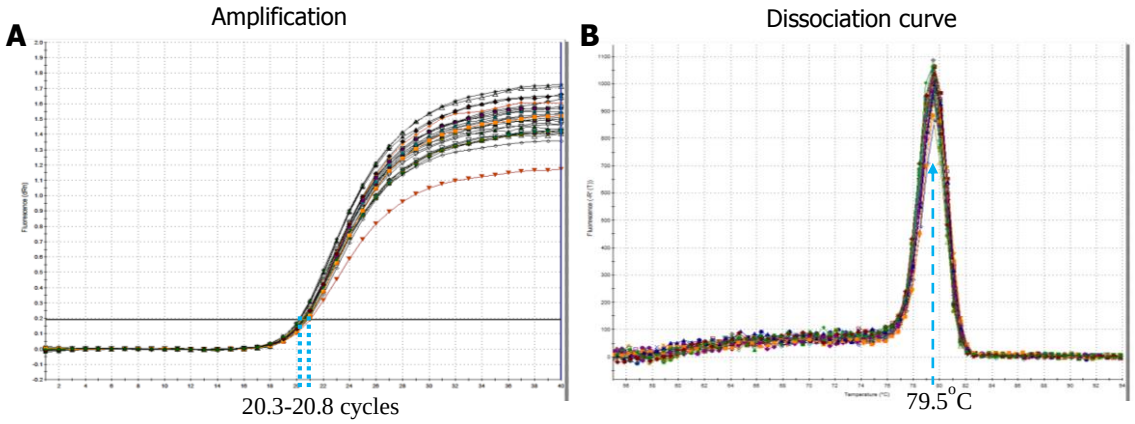


Figure 6. An example of Stratagene MX3005P machine qRT-PCR results.
A) Amplification plot, and B) Dissociation curve. An example of good primer/template amplification at 20.3-20.8 cycles, with a single dissociation peak at 79-80°C, was considered a single product.

Table 3. Summary of human primer sequences used.

A) Housekeeping gene sequences of TBP, YWHAZ, β-actin and 18Sr RNA. B) Genes of interest sequences analysed by qPCR. Gene list was generated from the human affymetrix array, network analysis and genes associated with labour.

A

Gene	Forward 5'	Reverse 3'	Accession number
TBP	CACGAACCACGGCACTGATT	TTTTCTTGCTGCCAGTCTGGAC	NM_003194
YWHAZ	ACTTTTGGTACATTGTGGCTTCAA	CCGCCAGGACAAACCAGTAT	NM_003406
β-actin	ATGTGGCCGAGGACTTTGATT	AGTGGGGTGGCTTTTAGGATG	NM_001101
18SrRNA	GCTGGAATTACCGCGGCT	CGGCTACCACATCCAAGGAA	NR_003286
RPL13	CTTCTCGGCCTGTTTCCGTAG	CGAGGTTGGCTGGAAGTACC	NM_012423

B

Gene	Forward primer 5'	Reverse primer 3'	Accession number
BCL2a	TACAGGCTGGCTCAGGACTAT	CGCAACATTTTGTAGCACTCTG	NM_004049.3
CCL4	CAGCACCAATGGGCTCAGA	CACTGGGATCAGCACAGACT	NM_002984
CD44	CTGCCGCTTTGCAGGTGTA	CATTGTGGGCAAGGTGCTATT	NM_000610.3
CXCR4	ACTACACCGAGGAAATGGGCT	CCCACAATGCCAGTTAAGAAGA	NM_003467.2
FOLR1	GCTCAGCGGATGACAACACA	CCTGGCCCATGCAATCCTT	NM_016725.2
ICAM1	ATGCCCAGACATCTGTGTCC	GGGGTCTCTATGCCCAACAA	NM_000201.2
IDO1	GCCAGCTTCGAGAAAGAGTTG	ATCCCAGAACTAGACGTGCAA	NM_002164.5
IL1-RN	CGGGTGCTACTTTATGGGCA	GGTCGGCAGATCGTCTCTAA	NM_000577
IL-1α	TGGTAGTAGCAACCAACGGGA	ACTTTGATTGAGGGCGTCATTC	NM_000575.3
IL-1β	CTCGCCAGTGAAATGATGGCT	GTCGGAGATTCGTAGCTGGAT	NM_000576.2
IL-6	CCTGAACCTTCCAAAGATGGC	TTCACCAGGCAAGTCTCCTCA	NM_000600
IL-8/ CXCL8	CACCGGAAGGAACCATCTCACT	TGGGGACACCTTTTAGCATC	NM_000584.3
MYLK	CCCGAGGTTGTCTGGTTCAAA	GCAGGTGTACTTGGCATCGT	NM_053025
NFKBIA	CCAACTACAATGGCCACACGTGTCTACA	GAGCATTGACATCAGCACCCAAGG	NM_020529
OSMR	ATGGCTCTATTTGCAGTCTTTCA	CACCCAGATGACATTGGATGTT	NM_003999.2
PLA2G4A	TACCAGCACATTATAGTGGAGCA	GCTGTCAGGGGTTGTAGAGAT	NM_024420
PTGER4	CATCATCTGCGCCATGAGTGT	GCTTGTCCACGTAGTGGCT	NM_000958
PTGS2	CGATGCTCATGCTCTTCGC	GGGAGACTGCATAGATGACAGG	NM_000956.3
SOCS3	CCTGCGCCTCAAGACCTTC	GTCACTGCGCTCCAGTAGAA	NM_003955.4
TLR4	AGACCTGTCCCTGAACCCTAT	CGATGGACTTCTAAACCAGCCA	NM_138554.4
VCAN	GTAACCCATGCGCTACATAAAGT	GGCAAAGTAGGCATCGTTGAAA	NM_004385.4
VIM	AGTCCACTGAGTACCGGAGAC	CATTTCACGCATCTGGCGTTC	NM_003380.3
ZEB2	TCTGTAGATGGTCCAGTGAAGA	GTCACTGCGCTGAAGGTACT	NM_001171653.1

2.1.5 Functional genomics using pathway and network analysis

The workflow for functional and network analysis was developed in order to be able to comprehensively analyse the large scale data sets produced from this work and to analyse previously published data sets. Genomic analysis of term human choriodecidua was carried out using Affymetrix microarray analysis, as described above. The genomic analysis of labouring and non-labouring term human myometrium was undertaken by Weiner et al., 2010. This open-access data set was identified using a literature search, downloaded and analysed using our custom workflow.

2.1.5.1 Human myometrium data extraction and threshold cut-off

The National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) DataSet was searched for published gene array datasets (http://www.ncbi.nlm.nih.gov/gds). The following search terms were applied:

- Labour/labor in combination with myometrium, myometria, myometrial, decidua, decidual, uterine, uterus, cervix and cervices.
- Parturition in combination with myometrium, myometria, myometrial, decidua, decidual, uterine, uterus, cervix and cervices.

Four datasets were found, one on human myometrium, two on mice and one on rat myometrium. No large scale gene studies or microarrays were found on the decidua or choriodecidua or cervix in any species. For this report, the myometrial transcriptomic data PubMed GEO series GSE9159 was used (Weiner et al. 2010). In summary, the data was generated following analysis of myometrial samples obtained from labouring women going caesarean section (TL; N=6; 39 to 41 weeks gestation; emergency), or not in labour (TNL; N=6; 38 to 41 weeks gestation; elective). The myometrial transcriptome was generated using the GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix, UK). To normalise the extracted myometrial GEO data before analysis in this study, ANOVA QLUCOREomics explorer (Lund, Sweden) was used. For statistical significance, only genes with a differential expression of $p<0.02$ were used for functional analysis.

2.1.5.2 Human choriodecidua data threshold cut-off

Probe sets were considered differentially expressed between the two conditions (TL compared to TNL) when they had a PPLR value approaching 0 or 1. The PPLR allows data to be ranked by probability of low certainty (Liu et al. 2006), indicating the likelihood that there are differences in gene expression between labour and non-labour samples (Pearson et al. 2009). We used a threshold cut off of 0.997 and $1x10^{-5}$, indicating a very low probability (between 0.003 and 0.00001%) of uncertainty in the data.

2.1.5.3 Pathway analysis of human tissues

The enriched functions and pathways identified from the differential gene expression were analysed using Ingenuity Pathways Analysis (IPA, Redwood City, CA, USA) and presented according to significance using Right-sided Fishers exact test (hypergeometric test). This software

allows a non-biased global investigation of all known biological systems involved within the parameters of the study groups. These relationships are derived from published literature and multiple database platforms, which are actively curated in the Ingenuity Knowledge Base. Pathway analysis identifies the gene ontology and its downstream events, whereas canonical pathway analysis refers to the classically activated intracellular signalling.

2.1.5.4 Network analysis of human tissues and development of a work flow

While pathway analysis allows the identification of gene functional enrichment, the enrichment data does not identify the interactions between pathways, emphasise the physiological and biochemical roles of the transcriptome observations. Our network analysis assesses the gene changes observed in the pathway analyses, in the context of an interactome. An interactome is a group of all related protein:protein and protein:genetic interactions associated with a particular function (Sun and Zhao, 2010). Within a network, these interactions are grouped together by gene expression changes observed as "seed" genes. Thus an interactome model of all inferred interactions is created around germinal gene points. Such interactome models are generated using databases of base-level biological interactions, in this study we used BioGRID (http://thebiogrid.org/). BioGRID is an actively curated database of all basic primary gene-gene, protein-protein and complex gene-protein interactions. The BioGRID database contains over 70 000 interactions and the most recent updates include post- translational modifications (Chatr-Aryamontri et al. 2013; Chatr-Aryamontri et al. 2015). This study used the human BioGRID database.

The interactome map was created using Cytoscape 2.8.3, an academic open access freeware used for network visualisation (Shannon et al. 2003). The main network parameter used to analyse the function of these interactions was "connectivity", i.e. the number of connections a node has within the network, and the resulting "clusters" of genes (nodes) that arise (Yu et al., 2007). In a biological system, genes are rarely expressed in isolation and these features allow genes to be prioritised within the interactome. The Cytoscape plug-in ModuLand was used to determine these functional clusters using a process known as "community network modelling"; this uses an algorithm that allows the comprehensive hierarchical analysis of overlapping network modules (Szalay-Beko et al. 2012). The extensive overlaps forms meta-nodes, where each meta-node represents nodes of the lower layers, and so enabling the functional prioritisation of network elements.

2.1.5.5 Development of a network analysis workflow and identifying master regulators

We identified a hierarchy in the transcript data using network analyses, and to ensure confidence in the predicted high hierarchal interactions, two different algorithms were utilised. Those ranked highest in the hierarchy, were then analysed for their downstream effects in the network, using causal analysis (Chapter 3.2 Figure 1). In brief, the work flow was as follows:

1. Statistical restriction of microarray data to PPLR $1x10^{-5}$ and 0.997 (choriodecidua) or $p<0.02$ (myometrium).

2. Mathemathical modelling using software Cytoscape 2.8.3 (Shannon et al. 2003; Cline et al. 2007; Smoot et al. 2011; Saito et al. 2012) and the BioGRID database. This study used the Cytoscape plugin ModuLand to mathemathically analyse the basic interactions and primary interactors from the BioGRID database of the differentially expressed genes. Gene clusters are determined algorithmically by centrality over the whole network topology using an overlapping community approach, thus forming a hierarchy where nodes (clusters of genes) form the core of the next upper level, designated by a meta-node (cluster of nodes) (Szalay-Beko et al. 2012). As the nodes and meta-nodes are clusters identified by the top most central gene, these were cross referenced back to the microarray to identify the position of the most interconnected genes.

3. Secondary mathemathical modelling to cluster major functional cores, but include basic interaction of protein complexes, gene-protein, protein-protein interactions ClusterONE (Nepusz et al. 2012). To prioritise the volume of hierarchially-ranked data, the two mathematical models were cross referenced for common genes. Only those clusters with a $p<0.05$ were included for causal analysis.

4. Causal analysis within Ingenuity pathway analysis (IPA) on selected targets to verify labour related significance. Causal analysis analyses array transcripts for master regulators relationships, using literature-based analysis of all known gene interactions. Analysing our candidates of interest with causal analysis supports the *in silico* predicted central relationships within our data set, and identifies specific downstream effects. Targets were further refined to only those with a high number of downstream genes associated with labour. The statistical significance is related to the connectivity of the causal network (p-value) and whether the activity of the gene, when placed as an upstream component affects the direction of the causal network (activation z-score).

The two mathematical algorthims in combination with causal analysis, provide detailed hierarchical network structure of the myometrium and choriodecidua to investigate the following: (1) determine the meta-nodes or the core of labour-associated genes; (2) define key network nodes that govern large numbers of downstream genes and, (3) identify individual genes that possess a downstream profile that includes a number of labour-associated genes, i.e. Identify potential key, high level regulator gene candidates.

2.1.6 Immunohistochemistry of human tissues

Immunohistochemistry was carried to determine the protein expression of the master regulator genes using colorimetric detection. After dehydration in ascending series of ethanol and xylene, and embedding in lambswax, (Thermo Scientific, UK) tissues were sectioned at 5µm and transferred onto poly-l-lysine (Sigma, UK) coated glass slides. The tissue sections were deparaffinised in Histoclear (Thermo scientific, UK) and rehydrated in descending series of alcohol and dH_2O. Antigen retrieval consisted of 10 minutes of microwave boiling in sodium citrate buffer (0.01M, pH 6.0) and incubation in the hot buffer at room temperature for a further 20 minutes. Slides were thoroughly washed in dH_2O before treatment for 10 minutes in 3% (v/v) hydrogen peroxide, to deplete endogenous peroxide activity. Slides were washed in high salt (0.05M) Tris

buffered saline (TBS) before blocking non-specific antibody binding with 2% (v/v) normal goat serum, 2% human serum (v/v) in 0.1% TBS/Tween20. The primary antibody was diluted in TBS, incubated overnight at 4°C (Table 4) and washed in TBS/ Tween 20 (0.1% v/v) for 1 X 5 min and in TBS for 2 X 5 min. The biotinylated goat anti-mouse secondary antibodies were diluted in TBS and applied for 30 min at room temperature. Slides were washed as above in TBS/Tween and TBS, before avidin peroxidase was applied (5µg/ml in TBS; Sigma Aldrich, UK) for 20 min at room temperature. Slides were thoroughly washed in TBS and colour developed with 3,3 Diaminobenzidine (DAB; 0.7mg/ml; Sigma Aldrich, UK) for 2-6mins. Washing with dH_2O halted further DAB development. Nuclei were counter-stained with Harris's haematoxylin. Slides were mounted in DPX (Sigma Aldrich, UK) after dehydration in alcohol and clearing in Histoclear. The negative controls for all antibodies were the appropriate rabbit or mouse non-immune IgG, substituting the primary antibody at the same concentrations. Images were taken using a Leitz Dialux 22 Microscope (Ernst Leitz Wetzlar GMBH, Germany) and Qicam Fast 1394 camera (Qimaging, Surrey, Canada) and Image Pro Plus 6.0 software (Media Cybernetics UK, Marlow, UK).

Table 4. Summary of antibodies used for human tissues

Primary antibody	**Company**	C**oncentration**
TLR4 mouse monoclonal (ab22048)	Abcam, UK	0.63 µg/ml
CXCR4 mouse monoclonal (SC53534)	Santa Cruz, DEU	1 µg/ml
CD44 mouse monoclonal (M7082)	DAKO, DNK	1.6 µg/mL
Vimentin mouse monoclonal (M0760)	DAKO	0.364 µg/mL
Secondary antibody		
Goat anti mouse	DAKO	3.86 µg/mL

2.1.7 Choriodecidual explant culture

An ex vivo human choriodecidual explant culture model was used to investigate the effect of inhibiting the function of putative decidual master regulator proteins. Human choriodecidual explants were randomly sampled following amnion removal, as detailed above. After thoroughly washing in cold sterile PBS to remove excess red blood cells, the choriodecidual explants were washed in serum-free RPMI culture media containing penicillin streptomycin (0.5mg/ml) and amphotericin (1.25µg/ml) (Sigma-Aldrich, UK). Three explants of approximately equal size (2mm x 2mm) were cultured per well, and in duplicate per treatment on netwells for 24 hours at 37°C, 21% O_2 and 5% CO_2. All media culture products were purchased from GIBCO, Life Technologies, UK, unless otherwise stated.

To inhibit VIM, this study used the PKC inhibitor Gö 6983 at 1µM (G1918; Sigma Aldrich, UK). This specific antagonist has been shown to reduce cellular VIM protein expression (Mor-Vaknin et al. 2003), decrease focal attachments between adherent cells and also reduce cellular motility

(Peterman et al. 2004). This study used another antagonist to inhibit VIM, cytochalasin D at 100nM (C2618; Sigma Aldrich, UK). This mycotoxin has been shown to inhibit actin polymerisation (Tsuruta & Jones 2003) and decrease VIM protein and mRNA expression (Wu et al. 1999; Krucker et al. 2000; Jung et al. 2013). For the specific inhibition of TLR4 this study used the cyclohexene inhibitor TAK-242 at 1μM (tlrl-cli95; Invivogen, France) (Zhang et al. 2014). After 24 hours, the explants were treated with RNAlater for 24 hours at 4oC, prior to RNA isolation. The vehicle control used was at the highest concentration of DMSO used in the inhibitor treatments (0.002% v/v).

2.1.8 Human myometrial cell culture

An *in vitro* cell culture model was utilised to investigate the outcome of inhibiting the function of putative myometrial master regulator proteins. An immortalised human myometrial cell line Hret-C3-Sy, a kind gift from Dr Sylvie Girard (CHU Sainte-Justine, University of Montreal, Canada), was seeded in a 6-well plate at $5x10^4$ per well. The cells were cultured with or without inhibitors for 24h in DMEM/F12 (1:1) media supplemented with L-glutamine (1.5 mg/ml), penicillin and streptomycin (0.5mg/ml), gentomycin (100 μg/ml) and 10% (v/v) fetal bovine calf serum, in a humidified incubator at 37ºC, with 21% O_2 and 5% CO_2. After 24 hours, the cells were incubated in RNAlater for 24 hours at 4ºC prior to RNA isolation. All media culture products were purchased from GIBCO, Life Technologies, UK, unless otherwise stated.

The PKC inhibitor GÖ6983 (Sigma Aldrich, UK), was used to inhibit metallothionein 2 (MT2), as PKC inhibition has been shown to inhibit MT2 mRNA expression at 15μM (Yu et al. 1997). For the specific inhibition of TLR2, this study used a neutralising antibody Mab hTLR2, Clone TL2.1 at 100ng/ml (Invivogen, France) (Schindler & Baichwal 1994). The vehicle control used was at the highest concentration of DMSO used in the inhibitor treatments (0.002% v/v).

2.1.9 Isolation of primary human choriodecidual cells

This part of the project did not reach completion and data was not generated but, primary human choriodecidual cells were isolated for future work. In summary, a protocol to isolate of primary human choriodecidual cells was compiled after consulting a number of previously published protocols (Fleming et al. 1980; Starkey et al. 1988; Vince et al. 1990; Tabanelli et al. 1992). The final enzymatic digestion and blood separation steps were optimised in our laboratory by Ms Cyntia Duval (University of Montreal, Canada).

After fetal membranes were collected and amnion removed as described above, they were washed thoroughly in PBS containing penicillin streptomycin (0.5mg/ml) and amphotericin (1.25μg/ml) (Sigma-Aldrich, UK). The membranes were weighed and 30g was macerated using scalpel blades. Enzymatic digestion of the tissue was carried out in RPMI containing penicillin streptomycin (0.5mg/ml), 1.0mg/mL collagenase (Sigma-Aldrich, UK) and 20mg/ml DNAase (Sigma-Aldrich, UK) at 37ºC with mechanical stirring up to 60 minutes. At 15 minute intervals, the digestion was examined under the microscope for the degree of cell separation. To stop the digestion process, fetal bovine serum (FBS) (Gibco, UK) at 10% of total volume was added. The suspension was then strained through a sterile metal sieve, followed by a 40μm filter to remove undigested tissue. The

cellular supernatant was centrifuged at 2500rpm for 5 minutes and washed with PBS twice. After the third spin, the cells were resuspended in 3ml of DMEM/F12 and layered onto an equal volume of HistoPaque (Sigma-Aldrich, UK). The gradient was centrifuged at 400g for 30 min at room temperature. The upper layer containing red blood cells were discarded and the opaque layer of cells was centrifuged and washed three times in sterile PBS. At the fourth spin, the choriodecidual cells were resuspended in complete DMEM/F-12 culture media (Gibco, UK) containing L-glutamine (1.5 mg/ml), penicillin and streptomycin (0.5mg/ml) (Sigma-Aldrich, UK), and 10% FBS (v/v) (Gibco, UK). Cells were cultured at $5x10^5$ per ml on plastic. To maintain the decidual phenotype, the cells were cultured in complete media with β-estradiol at 10^{-8}M and progesterone at $5x10^{-7}$M. Both reagents were from Sigma-Aldrich, UK.

The isolated choriodecidual cells were characterised by IHC staining using mouse monoclonal antibodies IGFBP-1 (21.2µg/ml) for decidual cells, CD45 (1.25µg/ml) (DAKO, UK) for leukocytes, vimentin (2.5µg/ml) (DAKO, UK) for decidual fibroblast cells, cytokeratin 7 (0.4µg/ml) (DAKO, UK) for epithelial cells and a negative non-immune mouse IgG (Sigma-Aldrich, UK) substituting for the primary antibody. The secondary was a biotinylated goat anti-mouse (DAKO, UK). The resulting IHC indicated there were decidual, fibroblastic and some epithelial cells with few leukocytes (Figure 7).

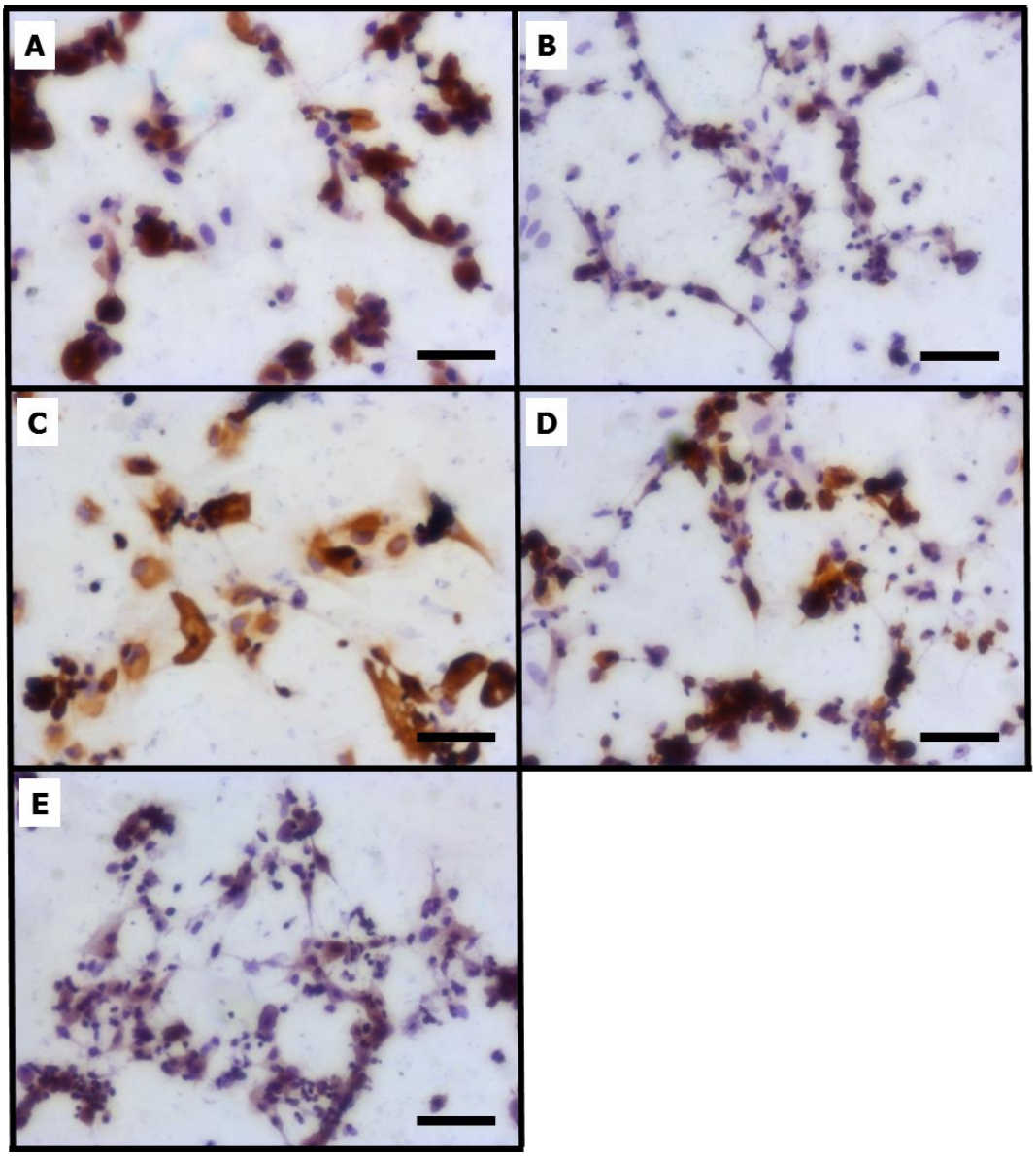


Figure 7. Immunohistochemistry staining of isolated human choriodecidual cells.
(A) IGFBP-1, (B) CD45, (C) vimentin, (D) cytokeratin 7 and (E) negative IgG control. Scale bar = 50μM.

2.2 Mouse tissue collection and sampling

Animals were kept in accordance with the UK Animals Scientific Procedures Act 1986. Pregnant female C57BL/6 mice were between 8 to 12 weeks old. Embryonic day 0.5 (E0.5) was used to designate the vaginal plug as the first day of gestation, day of parturition was E19. Animals were housed under a 12h light/dark cycle at 21–23 °C with food (Beekay Mouse Diet, Bantin & Kingman, Hull, UK) and water *ad libitum*.

To examine changes in gene expression leading up to active labour, uterine tissues (N=6 dams) were collected at different stages of gestation (E16.5, E17.5, E18.5) and during active labour (E19) (Figure 8). Pups started to litter on the evening of E18.5 (i.e. E19). Active labour was defined as the delivery of a pup with umbilical cord still attached; mice were sacrificed immediately following delivery of the first pup. After cervical dislocation, uterine horns were rapidly harvested; pups, fetal membranes and placental tissue were removed (fetal membranes and placentas stored separately), and the uteri were randomly selected for either fixation for 24h in 4% neutral buffered formalin (in house) or incubated for 48h at 4°C in RNAlater (Sigma, UK) and storage at -80°C. This ensured that initial tissue handling was minimal, allowing isolation of high quality, stable RNA, suitable for microarrays. For the microarray and PCR validation, at least two uterine sections immediately surrounding the pups were randomly selected from the uterine horns (Figure 9). The immediate uterine section surrounding the pup will be referred to as the implantation site. RNA was extracted individually for each implantation site sampled. Each extraction was assessed for RNA integrity before pooling the two samples per dam. The samples for the microarray hybridisation were pooled and hybridised in duplicate.

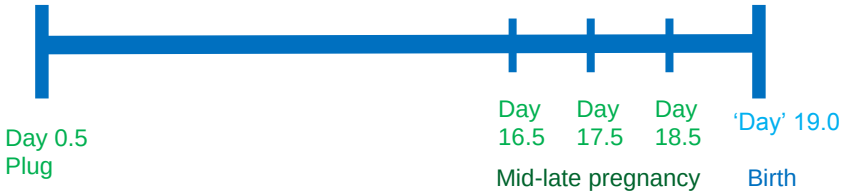


Figure 8. Timeline of tissue collection points. Non-labouring and labouring tissues. Detection of vaginal plug is denoted as embryonic day 0.5.

2.2.1 Dissection of mouse myometrium and decidua

The harvested uterine tissues were separated into myometrium and decidua. Per dam, the uterine horns contained 2-6 pups each (Figure 9A). For each of the implantation sites, a dissecting microscope was used to separate the distinct layers of decidua parietalis, decidua basalis and myometrium (Figure 9B). Fetal membranes were also collected and stored. To assess consistency and accuracy of uterine layer separation, several implantation sites were fixed in 4% NBF for immunohistochemistry (IHC); these were also separated into the individual uterine layers following fixation. IHC was carried out to confirm the identity of the distinct uterine layers separated.

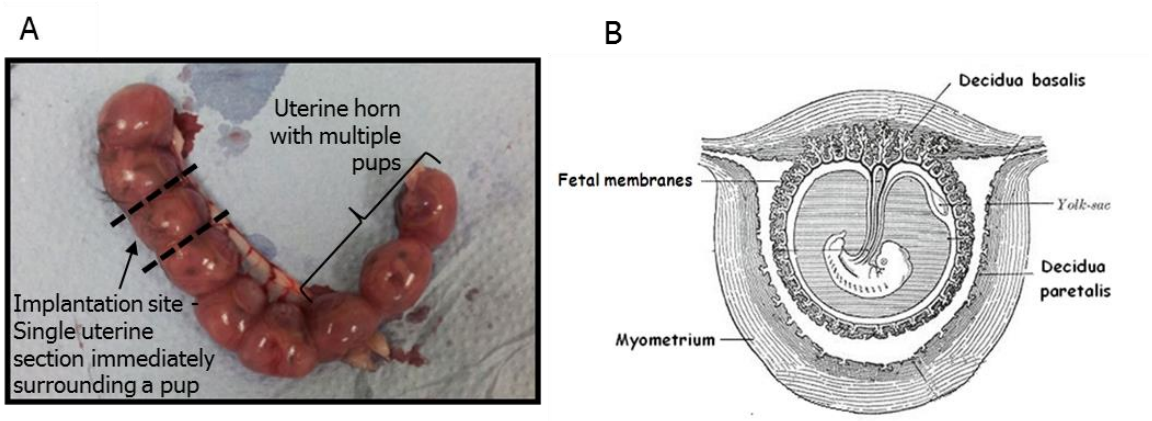


Figure 9. Diagram demonstrating the anatomy of the mouse uterus.

A Illustrating the two uterine horns and the distinct implantation sites of a pregnant mouse. B Image demonstrating the different uterine tissue layers surrounding a single fetus (Image modified from Gray's anatomy text)

2.2.1.1 Analysis of the intact mouse decidua and myometrium using immunohistochemistry

To ensure the decidual layer remained intact after fetal tissue removal, immunolocalisation was performed on the intact uteri. After dehydration in ascending series of ethanol and xylene, and embedding in lambswax (Thermo Scientific, UK) uterine tissues (N=3) were sectioned, and processed as described earlier for human tissue IHC. The Vector M.O.M. (Mouse on mouse) Immunodetection Kit (Vector Labs, UK) was used to localise the mouse raised primary antibodies from Table 5. The M.O.M kit follows the same protocol as conventional immunohistochemistry but incorporates additional blocking steps with a mouse Ig blocking agent, a non-immune protein diluent and a biotinylated anti-mouse Ig. These extra steps reduce non-specific binding of the secondary antibody (Figure 10). The IHC indicated that after removal of the fetal tissue, intact myometrium and decidua paretalis remained. In brief, contraction-associated actin calponin was strongly expressed in the decidual epithelium and in the myometrium (Figure 10A); the muscle-associated intermediate filament desmin was positive in decidual stroma and in the underlying myometrium (Figure 10B); The smooth muscle actin- α (SMA-α) was highly abundant in the myometrium and in the walls of blood vessels (Figure 10C); and decidua-associated Thy1 (Searle & Matthews 1988) was strongly positive in the decidua (Figure 10D).

Table 5. Summary of mouse antibodies used to assess separated decidua and myometrium.

Primary antibody	Company	Concentration
Calponin mouse monoclonal	DAKO	0.86 µg/mL
Desmin rabbit polyclonal	Abcam	5 µg/mL
Smooth muscle α mouse monoclonal	DAKO	0.71 µg/mL
Thy1 (CD90) mouse monoclonal	Abcam	4 µg/mL
Vimentin mouse monoclonal	DAKO	0.25 µg/mL
Secondary antibody		
Goat anti mouse	DAKO	3.86 µg/mL
Swine anti rabbit	DAKO	2 µg/mL

2.2.1.2 Structural analysis of the separated mouse decidua and myometrium using haematoxylin and eosin

For structural analysis, 5µm slides were stained with Harris' Haematoxylin and Eosin (Figure 11). Wax embedding and IHC rehydration was performed as previously described above. Uterine tissue sections (N=3) were incubated with filtered Harris' haematoxylin for 2 minutes to stain the nuclei. An acid/ alcohol rinse (1% HCl acid with 30% alcohol and 69% dH_20) for 2-3 seconds removed excess cytoplasmic staining, before sections were incubated for 5 minutes in warm water to 'blue' the haematoxylin. Eosin was similarly filtered before use. Haematoxylin stained sections were incubated in eosin for 5 minutes before washing in water (3x5 minutes) to remove excess eosin. Sections were then dehydrated and mounted as described in the earlier methods section.

After confirming that separation of the decidua and myometrium had been successful, the remaining uterine tissues were manually separated and processed for molecular analysis.

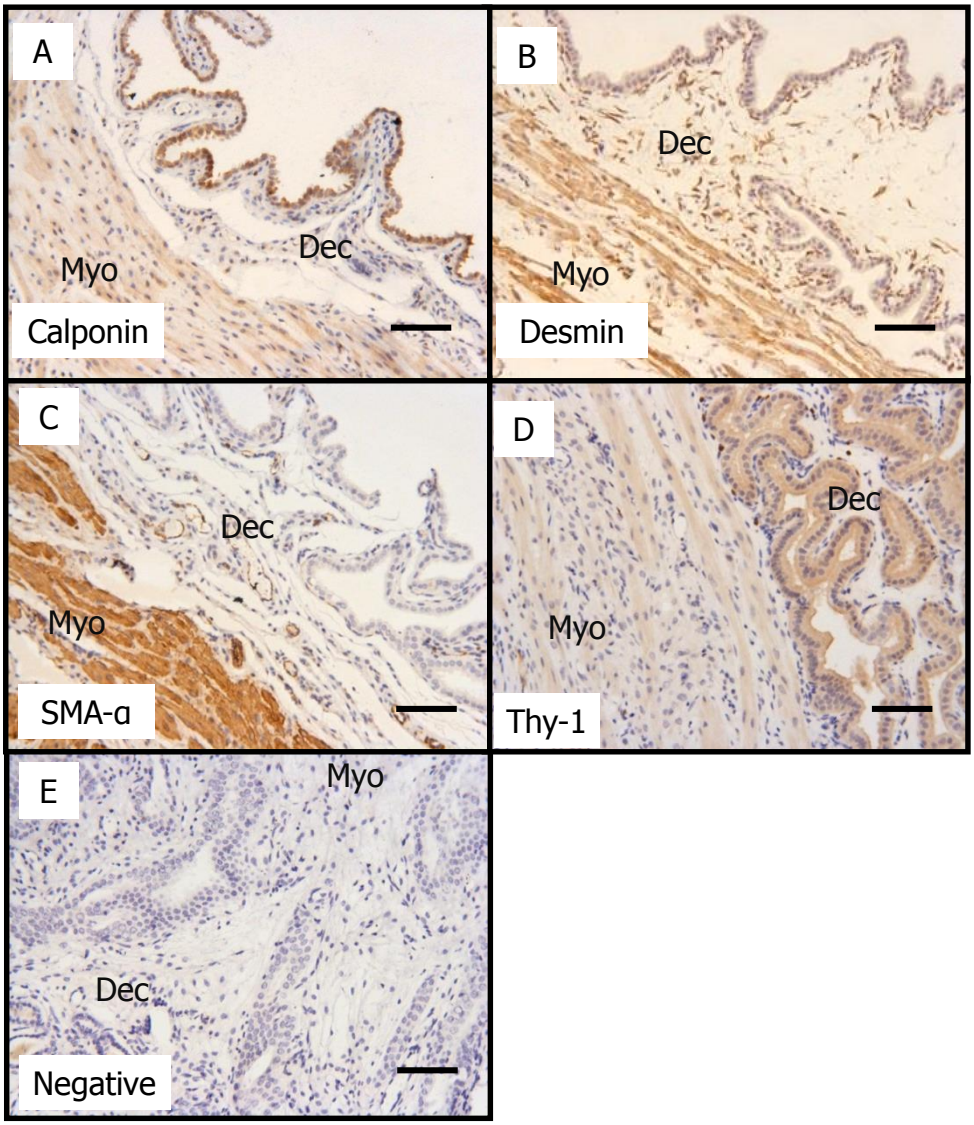


Figure 10. Immunohistochemistry of intact uterine tissues to demonstrate the distinct uterine compartments.

(A) Calponin, (B) Desmin, (C) SMA-α, (D) Thy1 and (E) Mouse IgG (negative control). Myo = myometrium, Dec = decidua paretalis. Scale bars = 55 μm.

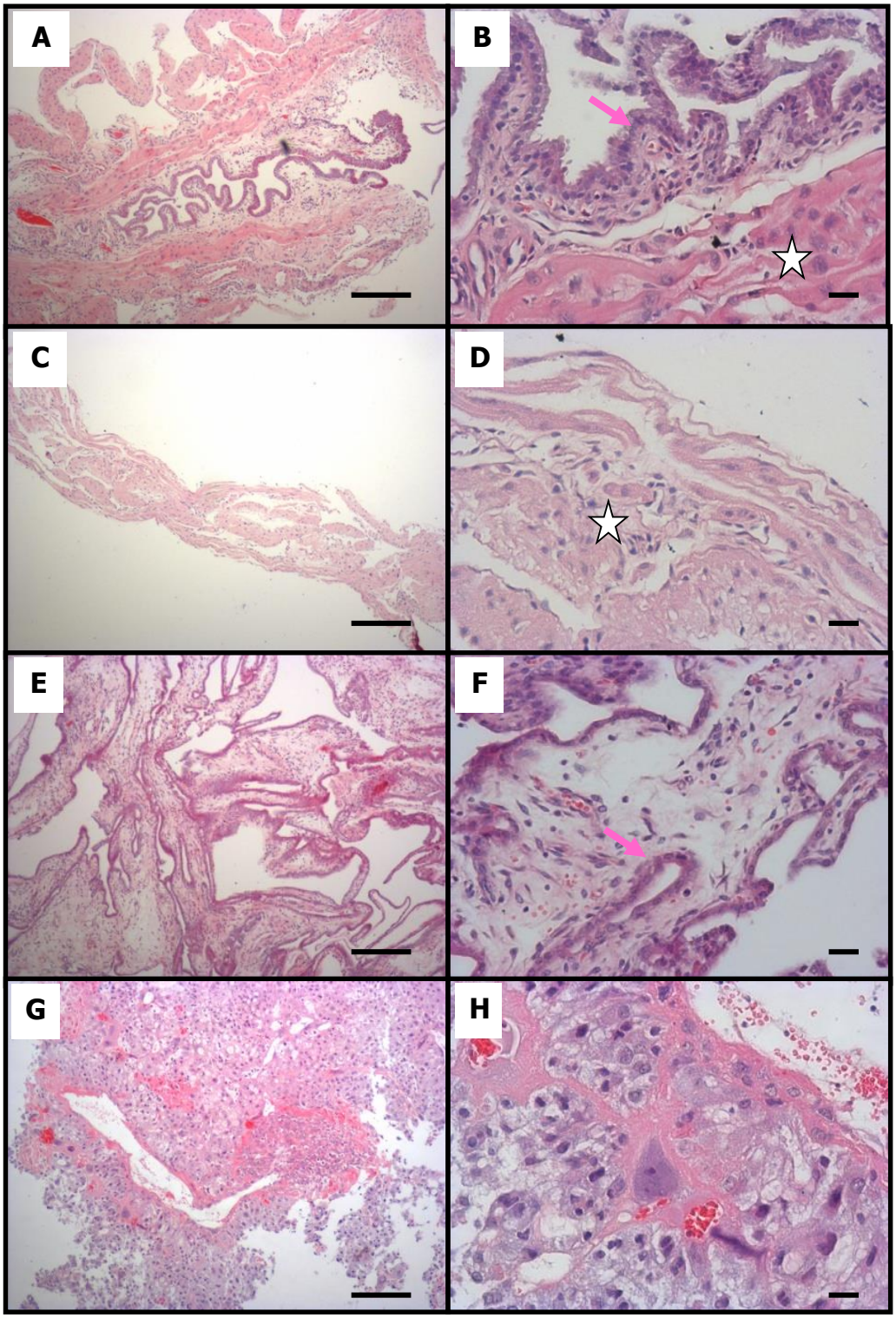


Figure 11. Haematoxylin and eosin staining of separated mouse uterine tissue.
Left column is at low magnification at x10, whereas the right column is higher magnification at x25. (A -B) Whole uterus with the myometrium and decidua intact, (arrow) indicating the decidual layer and (star) indicating the smooth muscle of the myometrium. Panels (C-H) show tissue after separation. (C-D) Myometrium only, demonstrating smooth muscle. (E-F) Decidua parietalis only, where only the epithelium and underlying tissue is present. (G-H) Decidua basalis only with trophoblast giant cells visible. Scale bars on the left column (A, C, E, G) = 55 µm; on the right column (B, D, F, H) = 25µm.

2.2.2 Inhibition of master regulators

After analysing the network relationships, using the network analysis workflow developed for the human data, we identified potential master regulators in the mouse uterine tissues. Using an aminobenzimidazole, this study aimed to inhibit IRAK 1 and 4 (Powers et al. 2006; Bansal & Silakari 2012). IRAK 1 is involved in TRAF6/ NF-κB signalling (Rhyasen et al. 2013) and IRAK 4 is essential in toll-like and interleukin signalling (Isnardi et al. 2008). The immune-modulatory properties of protein kinases imparts them with therapeutic potential; they are targets in diseases such as chronic inflammation (Cohen 2009). The toll and interleukin-1 receptor adaptor protein (TIRAP) functions as part of the TLR4 pathway and contributes to the cellular differences observed in microbial response. A TIRAP peptide inhibitor was developed with an antennapedia sequence at the end of amino acid: TIRAP 138–151 (RQIKIWFQNRRMKWKK positioned at the NH_2-terminal end of LQLRDAAPGGAIVS; mouse TIRAP aa 138–151) (Horng et al. 2001).

For the inhibition of TLR4, this study used the cyclohexene inhibitor, TAK-242 (tlrl-cli95; Invivogen, France) (Zhang et al. 2014). Wnt5a has been identified as a non-canonical β-catenin independent WNT-signalling molecule involved in cancer cell motility, adhesion and extravasation (Katoh & Katoh 2009; Bergenfelz et al. 2013; Li et al. 2013), and in nerve inflammation and injury (Wang et al. 2015). To inhibit WNT5a signalling, we used a peptide analogue of WNT5a with a t-boc substitution of the N-terminal group transforming it into an antagonist, N-butyloxycarbonyl Hexapeptide (BOX5; peptide: t-boc-Met-Asp-Gly-Cys-Glu-Leu) (Jenei et al. 2009). The vehicle control used was at the highest concentration of DMSO used in the inhibitor treatments at 0.002% v/v.

2.2.2.1 Mouse uterine tissue explants

Pregnant mice C57BL/6 at E18.5 were used. The uterine explants were approximately 2-3mm^2 and cultured with inhibitors or vehicle control for 24 hours, with drug treatment every 12 hours, in DMEM/F12 with L-glutamine (1.5 mg/ml), penicillin and streptomycin (0.5mg/ml), and 10% (v/v) fetal bovine calf serum. All media and supplements were purchased from GIBCO, Life Technologies, UK, unless otherwise stated. After treatment, the explants were incubated in RNA later for 24h and processed for PCR analysis.

2.2.2.2 Immortalised smooth muscle cells

Immortalised mouse aortic smooth muscle cells, MOVAS (ATCC® CRL-2797™), were used for *in vitro* investigation. The cells were cultured for at least two passages before experiments were undertaken. MOVAS cells were seeded in a 6-well plate at $5x10^4$ per well, with two wells per treatment. The cells were cultured with or without inhibitors for 24h in DMEM media containing L-glutamine (1.5 mg/ml), geneticin (200µg/ml) and 10% (v/v) fetal bovine calf serum. Cells were cultured in a humidified incubator at 37°C, with 21% O_2 and 5% CO_2. After 24 or 48 hours, the cells were incubated with RNAlater for 24 hours at 4°C prior to RNA isolation and qRT-PCR analysis. All media culture products were purchased from GIBCO, Life Technologies, UK, unless otherwise stated.

2.2.3 RNA isolation of mouse decidua and myometrium

RNA was isolated from mouse decidual and myometrial tissues using the MirVana miRNA Isolation Kit, following manufacturer's instructions (Life Technologies, UK). RNA was assessed using a NanoDrop 2000 UV-Vis Spectrophotometer, measuring absorbance at A260/280 and A260/230. After extraction, genomic DNA was removed as described for the human samples using, the same DNA removal kit (Ambion, UK). For microarray analysis, the RIN^e was calculated for the pooled samples using the Agilent 2200 TapeStation system as described for the human microarray data. The mouse RNA samples used for the microarray had a RIN^e of 8.3 to 9.1.

2.2.4 Mouse microarray hybridisation

The samples were then hybridised in duplicate onto a GeneChip Mouse Genome 430 2.0 Array according to manufacturer's instructions. The chip can detect over 39,000 transcripts; differentially expressed transcripts were normalised as for the human microarray with RMA. Statistical analysis was performed with PUMA and the PPLR was generated as described for the human microarray.

2.2.5 Mouse qRT-PCR

2.2.5.1 Reverse transcription

The initial cDNA was transcribed from RNA in duplicate. The experimental duplicates were then amplified in duplicate for technical accuracy, using a reference gene YWHAZ. If the expression was similar between duplicates, that is, the coefficient of variation of the $2^{(-Ct\ value)}$ was <15%, the experimental duplicates were combined for later experiments to analyse of genes of interest.

The first strand of cDNA was transcribed using the same Affinity Script Multiple Temperature cDNA Synthesis Kit (Agilent, UK) according to supplied instructions. Samples were assayed in duplicate using 250ng total RNA per sample, with the same heating cycles as used for the human cDNA transcription. The cDNA was then stored immediately at -20°C until needed.

2.2.5.2 QRT-PCR

A panel of housekeeping genes were assessed for stable expression. For PCR validation of the microarray, GAPDH was used for analysis for the validations, and TBP was the most stable in the inhibition treatments (Table 6A). Candidate genes of interest were selected based on differential expression from the microarray and the network predicted downstream genes (Table 6B). The master regulators were determined by network analysis, the work flow developed as previously described. The genes were purchased from Eurofins (DEU), except for GAPDH, ARG1 and IL-1β (Qiagen, UK).

Table 6. Summary of mouse primers used. A) Housekeeping genes used and B) Genes of interest.

A

ID	Forward 5'	Reverse 3'	Accession number
TBP	CACAGGAGCCAAGAGTGAAGA	CACAAGGCCTTCCAGCCTTA	NM_013684.3
YWHAZ	TGAGCTGTCGAATGAGGAGAG	CCTCCACGATGACCTACGG	NM_011740.3
GAPDH	Not provided by supplier		
MBP	TCACAGCGATCCAAGTACCTG	CCCCTGTCACCGCTAAAGAA	NM_001025251

B

ID	Forward 5'	Reverse 3'	Accession number
AQP5	TCTTGTGGGGATCTACTTCACC	TGAGAGGGGCTGAACCGAT	NM_009701
ARG1	Not provided by supplier		NM_007482
CASP3	GGGGAGCTTGGAACGCTAAG	GAGTCCACTGACTTGCTCCC	NM_009810.3
CCL6	AGGCTGGCCTCATACAAGAAAT	ACATGGGATCTGTGTGGCAT	NM_009139
CCL9	TCACACATGCAACAGAGACA	TGTAGGTCCGTGGTTGTGAG	NM_011338
CTNNB1	CCCAGTCCTTCACGCAAGAG	CATCTAGCGTCTCAGGGAACA	NM_007614
CXCL12	AGATTGTTGCACGGCTGAAGA	CCTTTGGGCTGTTGTGCTTAC	NM_021704
CXCL16	AACTCTGCAGGTTTGCAGCTC	TCACTGATGGAGACGAGCCT	NM_023158
CXCL17	TGCAAAGATTGGTTCCTGCAA	TCCTGTGGTGCTTTTGGTGT	NM_153576
CXCR4	GACTGGCATAGTCGGCAATG	AGAAGGGGAGTGTGATGACAAA	NM_009911
DUSP10	TGGGGATCAATGAAGCTGAGTG	TGAGATCCTGAGGTCGGACA	NM_022019.5
EGR1	TATGAGCACCTGACCACAGAG	GCTGGGATAACTCGTCTCCA	NM_007913
FOS	CCTGGGACAGAAGACCACTC	TGAGATCTCCCGGACATGGT	NM_010216.1
GAB1	GAAGTTGAAGCGTTATGCGTG	TCCAGGACATCCGGGTCTC	NM_021356
GJA1	CAGGTCTGAGAGCCCGAACT	TCTGGGCACCTCTCTTTCACTTA	NM_010288
GNA14	AGCGATCTGAACGACGGAAA	TCCTCCATGCGGTTCTCATTG	NM_008137
IKBKB	TTTCCAGCAAGCTTGAGTCTAC	TGTGAGCATCTCTTCGCTAGT	NM_026166.2
IL-1β	Not provided by supplier		
IRAK4	CATACGCAACCTTAATGTGGGG	GGAACTGATTGTATCTGTCGTCG	NM_029926
IRF8	AGACCATGTTCCGTATCCCCT	CACAGCGTAACCTCGTCTTCC	NM_008320
MMP11	CCGGAGAGTCACCGTCATC	GCAGGACTAGGGACCCAATG	NM_008606
OXTR	GATCACGCTCGCCGTCTAC	CCGTCTTGAGTCGCAGATTC	NM_001081147
PTGS2	GCTCAGCCAGGCAGCAAATC	ATCCAGTCCGGGTACAGTCA	NM_011198
SLIT2	GGCAGACACTGTCCCTATCG	GTGTTGCGGGGGATATTCCT	NM_178804
TLR4	ATGGCATGGCTTACACCACC	GAGGCCAATTTTGTCTCCACA	NM_021297

2.2.6 Pathway and network analysis of mouse tissues at active labour

Analyses of the microarray data followed the same protocol as for the human data. In brief, principle components analysis was used to explore the relationships of the initial data. Ingenuity knowledge database in IPA was used to analyse the gene functions, and canonical pathways to characterise the activated genes. Network analysis was carried out on tissues isolated during active labour (E19.0) and compared to non-labouring tissues obtained at mid-late (E16.5) gestation (E16.5), to predict the master regulators of active labour.

2.2.7 Mouse choriodecidual and myometrial absolute expression and network analysis of genes in the stages leading up to and including active labour

This study sought to analyse the true globally expressed genes at each stage of pregnancy from E16.5, through to active labour E19. For the mouse tissues, the gene data sets were also analysed using frozen RMA (fRMA), an approach that uses all known data on the 430 mouse chip to calculate presence or absence of gene expression to a high level of confidence using Gene Barcoding (McCall et al. 2010; Langfelder et al. 2011). The gene barcoding method removes the need for relative expression between samples, so each gene in every microarray (i.e gestational stages) can be analysed individually without fold change thresholds and a high level of certainty (McCall et al. 2011; McCall et al. 2014). The presence of expression of each probe signal in our data was mapped to an estimated z-score, a calculation of the average distribution of all the multiple probe signals of each gene within all cell types of the tissue. The distribution Z-score cut-off was >2.5 (Zilliox & Irizarry 2007; McCall et al. 2010; McCall et al. 2014). The presence of these genes will be referred to as the absolutely expressed genes, and these were compared across gestational stages in the lead up to labour for similarities and differences using the Venn diagram software from Bioinformatics and Evolutionary Genomics (http://bioinformatics.psb.ugent.be/software). The resulting categorically expressed genes for each time point were then analyse using IPA software and Cytoscape 2.8.3 as described above for the relative expression data.

3.0 Results chapter

Transcriptomic profiling of human choriodecidua during term labour: inflammation as a key driver of labour

[1,4] G.L. Stephen, MBChB, MRCOG*, [2,4] S. Lui, BSc*, [1,4] S.A. Hamilton MBChB, MD, MRCOG, [1,2,4] Clare L. Tower, MBChB, PhD, MRCOG, [2,4] L.K. Harris, PhD, [3,4]A. Stevens, PhD, [2,4] R.L. Jones, PhD

*Both authors contributed equally to this research.

Affiliations:

[1]St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, [2] Maternal and Fetal Health Research Centre, University of Manchester, [3] Paediatrics and Child Health Centre,

Abstract

Problem: Inflammation is a driver of labour in myometrium and cervix; however the involvement of decidua is poorly defined. We have reported decidual leukocyte infiltration prior to and during labour; the regulators of these inflammatory processes are unknown.

Method of study: Choriodecidua RNA obtained after term labour or elective caesarean delivery was applied to Affymetrix GeneChips. Pathway analysis and gene validation was performed.

Results: Extensive inflammatory activation was identified in choriodecidua following labour, predominantly upregulation of genes regulating leukocyte trafficking and cytokine signalling. Genes governing cell fate, tissue remodelling and translation were also altered. Upregulation of candidate genes (*ICAM1*, *CXCR4*, *CD44*, *TLR4*, *SOCS3*, *BCL2A, IDO)* was confirmed. NFκB, STAT1&3, HMGB1 and miRNAs-21,-146, -141, -200 were predicted upstream regulators.

Conclusions: This study confirms inflammatory processes are major players in labour events in choriodecidua, as in other gestational tissues. Suppressing uterine inflammation is likely to be critical for arresting premature labour.

Keywords: Choriodecidua, pathway analysis, chemokines, transcription factors, miRNAs

3.1 Introduction

Prematurity remains the leading cause of neonatal morbidity and mortality in developed countries, despite continued advances in neonatal medicine (www.ons.gov.uk, 2015). Globally, 15 million babies are born prematurely every year (World Health Organisation 2015) and there has been little change in the rates of preterm birth in developed countries over the past decade, with current rates of 11.5% and 7.2% in USA and UK respectively (World Health Organisation 2015; Centre for diesase control and prevention 2012). Current therapies to stop preterm labour are largely ineffective, likely due to the targeting of a single aspect of the labour process, namely arresting myometrial contractions (Alfirevic 2012; Dodd et al. 2012; Haas et al. 2012a; Roos et al. 2013; Jørgensen et al. 2014; Vogel et al. 2014). However, labour involves many other events, including cervical ripening and dilation and fetal membrane rupture, regulated by the complex integration of endocrine and paracrine signalling, and thus blockade of a single element is unlikely to halt the labour cascade. Improving tocolytic therapies therefore requires better understanding of the exact mechanisms driving labour and the concurrent processes in the distinct gestational tissues.

In addition to studies focusing on the involvement of individual factors in the labour process, there have been a number of investigations employing genome-wide analyses to comprehensively characterise the whole transcriptome of human uterine tissues following labour. At the time of reporting, the majority of these microarray studies focused on the myometrium (Chan et al. 2002; Marvin et al. 2002; Bethin et al. 2003; Charpigny et al. 2003; Esplin et al. 2005; Havelock et al. 2005; Bukowski et al. 2006; Bollapragada et al. 2009; Mittal et al. 2010; Weiner et al. 2010; Chan et al. 2014; Chandran et al. 2014), with fewer reports of gene expression changes in the cervix (Bukowski et al. 2006; Hassan et al. 2006; Bollapragada et al. 2009) and fetal membranes (Haddad et al. 2006; Bollapragada et al. 2009; Shankar et al. 2010). Importantly, all studies highlight a major involvement of inflammatory processes in labour. In the human myometrium the most prominent biological processes activated with labour were inflammatory response, immune/defence response and chemotaxis, tying in with the major molecular functions of cytokine, chemokine and growth factor activity (Bethin et al. 2003; Charpigny et al. 2003; Rehman et al. 2003; Mittal et al. 2010; Weiner et al. 2010). This was reinforced by a study by Weiner et al 2010, who identified putative initiator and effector gene sets for term and preterm labour, in which chemotaxis, inflammation and muscle contraction processes featured highly. Similar core inflammatory processes were reported in a comparison of gene expression changes between the myometrium and cervix (Havelock et al. 2005; Bukowski et al. 2006; Bollapragada et al. 2009). An "acute inflammation gene expression signature" was identified in intact fetal membranes with term labour (Haddad et al. 2006; Han et al. 2008). The genomic changes altered with labour in the global analysis studies support other studies demonstrating elevated cytokine and chemokine expression in uterine tissues, e.g. interleukin (IL)-1, IL-6, IL-8, tumour necrosis factor (TNF)-α, and CCL2 (Hamilton et al. 2013). The genomic studies also identified new candidate inflammatory mediators not previously associated with labour in the myometrium: e.g. heparin-binding EGF-like growth factor (HBEGF), suppressor of cytokine signalling (SOCS3), prokineticin 2, IL-13RA, IL-9R and CXCL-3, -5, and -6 (Mittal et al. 2010; Weiner et al. 2010); in the cervix: e.g. vascular

endothelial growth factor (VEGF) and Toll-like receptors (TLR2,-3,-4,-5) (Hassan et al. 2006); and in the amniochorion: e.g. pre-B cell colony-enhancing factor (PBEF), TLR2, and superoxide dismutase (SOD) 2 (Haddad et al. 2006).

However, there have been no genome wide gene expression studies of labour-associated changes in the human decidua, despite its hormonal actions, anatomical location at the maternal-placento-fetal interface and its major immunomodulatory actions in coordinating successful pregnancy. Recently we demonstrated infiltration of macrophages, coincident with a specific elevated chemokine expression profile, in the human decidua following term and preterm labour (Hamilton et al. 2013). In the rat, the leukocyte infiltration occurs prior to the onset of labour, and before inflammatory events in the myometrium, implicating decidual inflammation as an early, and potentially a driving, process in the labour cascade. This is consistent with evidence that the decidua is a major source of prostaglandins (Skinner & Challis 1985; Mitchell et al. 1995; Keelan et al. 2003b; Makino et al. 2007; Blanks & Brosens 2012), which are key mediators of labour through actions on the myometrium and cervix. However, the inflammatory profile and activation of other decidual cellular and molecular pathways remain uncharacterised; this knowledge is essential to fully understand the concurrent events and signalling pathways in the gestational tissues during normal term labour.

The aim of the current study was to identify and profile changes in gene expression in human choriodecidua during term labour, utilising genome wide arrays coupled with pathway analysis to identify key canonical pathways and processes associated with labour. We hypothesized that there would be widespread changes in gene expression in the choriodecidua during term labour, relating to inflammatory processes and extensive tissue remodelling.

3.2 Methods and Materials

3.2.1 Tissue collection

To investigate changes in choriodecidual gene expression during normal term labour, tissue samples were obtained with written informed consent from women who delivered at St. Mary's hospital, Manchester. Ethical approval was granted by the North West Local Research Ethics Committee (Reference Number: 08/H1010/55). Two study groups at term (37-42 weeks), were analysed in this study; term not in labour (TNL: n=12) from elective caesarean section at term without labour and normal term labour (TL: n=11) from vaginal deliveries without complications (participant demographics shown in Table 1). Strict exclusion criteria were applied to exclude any pathological pregnancies, signs of infection or maternal comorbidity as previously described (Hamilton et al. 2013). Placentae, with associated fetal membranes, were collected within 30 minutes of delivery. Choriodecidua was isolated from amnion by manual separation and incubated in RNAlater (Ambion, Warrington, UK) for 24h at 4^{o}C prior to RNA extraction.

3.2.2 RNA extraction & purification

RNA was extracted from choriodecidua using TRIZOL (Life Technologies, Paisley, UK) and purified using RNAeasy mini kit according to manufacturer's instructions (Qiagen, Crawley, UK). RNA quality and quantity was assessed by UV spectrophotometry and Quant-iT RiboGreen RNA Assay Kit (Life Technologies). DNA contamination was removed using the DNAse kit (Ambion, Life technologies) following the manufacturer's instructions.

3.2.3 Human Affymetrix genearray: choriodecidual transcriptome associated with term labour

Samples from the two groups were pooled (n=6, each) and quality-screened for integrity using the RNA 6000 Nano Assay, measured on an Agilent 2100 Bioanalyser. The two pooled groups were then hybridised onto Human Genome U133 Plus 2.0 Affymetrix GeneChip according to manufacturer's instructions. Technical quality control and outlier analysis was performed with dChip (V2005) (Li & Wong 2001) using the default settings. Normalisation and expression analysis were performed using multi-mgMos (Liu et al. 2005). Gene specific differential expression between the two groups was assessed with the Bayesian method which includes probe-level measurement error when assessing statistical significance (Liu et al. 2006). Analysis was performed with the PUMA package (Gentleman et al. 2004) to generate a probability score, probability of positive log ratio (PPLR). Probesets were considered differentially expressed between the two conditions when they had a PPLR value approaching 0 or 1. The PPLR allows data to be ranked by probability of low certainty, indicating the likelihood that there are differences in gene expression between labour and non-labour samples (Pearson et al. 2009).

3.2.4 Determination of biological functions and pathways associated with the choriodecidual transcriptome

The biological pathways and functions either enriched or decreased in the choriodecidual transcriptome during labour were assessed using Ingenuity Pathways Analysis (IPA, Redwood City, CA, USA) using a right-sided Fisher's exact test. For pathway analysis, the array list of genomic change was set with PPLR threshold hold cut-off of inclusion of ≥ 0.997 or $\leq 1x10^{-5}$.

3.2.5 Potential upstream regulators of the choriodecidual transcriptome

Upstream regulator analysis of differentially expressed gene expression was performed within IPA. This method determines expected causal effects between upstream regulators and targets; these relationships are derived from published literature and multiple database platforms curated in the Ingenuity Knowledge Base. A prediction of the activation state for each transcription factor, based on the direction of change, was calculated (z-score) using the gene expression patterns of the transcription factor and its downstream genes. An absolute z-score of $\geq |2|$ was considered significant. A p-value was also calculated by Fisher's Exact Test indicating the statistical significance of genes in the dataset that are downstream of the transcription factor or miRNA.

3.2.6 Gene networks in the choriodecidual transcriptome

To increase confidence in the observations of differentially expressed genes by correlation with biological pathways, we examined the interaction of differentially expressed genes within the

context of a model of all known interactions related to the genomic function using Ingenuity Knowledge base. Clusters of interactions were determined using the differentially expressed genes as "seeds" for generating networks that maximize their specific connectivity. Networks generated were scored based on the number of eligible molecules and their size, as well as the total number of eligible molecules analysed and the total number of molecules in the Ingenuity Knowledge Base that could potentially be included in networks. The score is the –log of the Fisher's exact test result and is not an indication of the quality or biological relevance of the network; it calculates the approximate "fit" between each network and the differentially expressed genes (Calvano et al. 2005).

3.2.7 Validation of genomic data using candidate genes

Additional choriodecidual samples for each study group were included for PCR validation studies (total TNL: n=12 and TL: n=11). Candidate genes were selected based on significance of change from the array data and their functional significance. For each individual sample, 200ng of extracted RNA was reverse transcribed using the AffinityScript multiple temperature cDNA synthesis kit (Agilent; Berkshire, UK). QPCR was carried out using Brilliant SYBRIII Green QPCR Master Mix (Stratagene, USA) with 5-carboxy-x-rhodamine as the reference dye and measured using the Stratagene MX3000P real-time PCR machine. Specific primers used are summarised in Table 2. Data were normalised for expression of the housekeeping gene and presented as $2^{-\Delta CT}$ and statistically analysed by Mann-Whitney Test.

3.2.8 Immunohistochemistry: analysis of candidate protein expression TLR4, CXCR4 and CD44

Intact fetal membranes from the same patients were formalin fixed and used to confirm expression of three of the candidate genes in the decidua (TNL: N=12 and TL: 11). Immunohistochemistry was carried as previously described (Hayward et al. 2011), using colorimetric detection and primary anti-human mouse monoclonal antibodies raised against: Toll-like receptor-4 (TLR4, ab22048, Abcam, UK, 0.63µg/ml), CXCR4 (SC53534, Santa Cruz, Germany, 1µg/ml), and CD44 (M7082, Dako, Denmark, 1.6µg/ml). Negative controls substituted with non-immunised mouse IgG at equal concentrations, for each primary antibody used. Human tonsil tissue was used as a positive control. Images were taken using Leitz Dialux 22 Microscope (Ernst Leitz Wetzlar GMBH, Germany) and Qicam Fast 1394 camera (Qimaging, Surrey, Canada) and Image Pro Plus 6.0 software (Media Cybernetics UK, Marlow, UK).

3.3 Results

3.3.1 Pathway analysis of choriodecidual transcriptomic changes with labour

Microarray analysis indicated that term labour was associated with extensive alterations in choriodecidual gene expression. 796 choriodecidual genes (396 upregulated, 400 downregulated) were significantly altered in labour (PPLR value of ≥ 0.997 or $\leq 1x10^{-5}$) using a 1.3-fold threshold to exclude false positives or changes of minimal biological significance (Full list of altered genes shown in Supplemental Table 1). The top biological functions altered by labour were predominantly inflammatory or immunological in nature. The heat map, in which each individual box represents a group of related genes altered within the indicated function ranked by z-score, demonstrates a striking upregulation of genes with term labour (orange = increased, blue = decreased) (heat map, Figure 1A). The top enriched functions were haematological system and development, cellular movement, immune cell trafficking, inflammatory response, and cell to cell signalling and interaction (ranked by z-score, $p<2.0x10^{-4}$). The majority of down-regulated genes were in cell death and survival and cancer pathways (ranked by z-score, $p<2.0x10^{-4}$).

There were a considerable number of genes with a very high magnitude of change with labour, the majority of which had inflammatory/immune characteristics. To enable analysis of inflammatory pathways, whilst also unmasking non-inflammatory pathways activated during labour, an arbitrary threshold of 3-fold change in gene expression was applied to the dataset. The biological pathways of genes with a fold change >3-fold with labour are shown in Figure 1B. Within each of the inflammatory categories, the genes were subgrouped into those governing immune cell chemotaxis and activation. Even within the apparently non-inflammatory biological functional categories (e.g. tissue morphology, cell-cell signalling, cell death and cell proliferation), each individual group consisted of genes predominantly immunological in nature (e.g. adhesion and accumulation of immune cells or proliferation, cell death and differentiation of immune cells). There was considerable overlap in the individual genes regulated in each biological function; a gene list (excluding replicates) is shown in Table 3. These include chemokines (e.g. CCL2, CCL4, CXCL1, CXCL8), chemokine receptors (CXCR4, CCLR2), cytokines (e.g. IL-1α, IL-6, IL-1RN), matrix metalloproteinases (MMP -1, -10, -12), toll-like receptors (TLR -1, -2, -4), calcium binding proteins (e.g.S100A8, -9, -12) and adhesion molecules (intercellular adhesion molecule 1 (ICAM1), integrin α2, integrin β6, junctional adhesion molecule 2). Prostaglandin G/H synthase 2 (PTGS2 / cyclooxygenase (COX-2)), whose role in labour has been well established (Challis et al. 2002), featured highly in the choriodecidual gene lists.

The gene list was also analysed according to classically activated cellular pathways (canonical pathways) to further understand their biological roles (top 15 pathways are shown in Figure 1C). These closely reflect the biological functions, i.e. predominantly inflammation and inflammatory-associated signalling ($p<7.7x10^{-6}$) (Figure 1C). Of note the majority of genes in each pathway were upregulated (red bars), with few down-regulated genes (green bars). All genes altered within each canonical pathway are represented in the list of biological functions affected by labour (highlighted with an asterisk in Table 3).

3.3.2 Unmasking non-inflammatory choriodecidual gene changes associated with labour

Separate analysis of genes with a modest, but still highly significant, change in expression (<3-fold change with labour) unmasked other biological processes affected by labour. An enrichment of genes in distinct and primarily non-inflammatory biological pathways was revealed (622 probe sets: 262 upregulated and 358 down-regulated). The top ten biological functions included: cell death and survival, cellular growth and proliferation, free radical scavenging and small molecule biochemistry (Figure 2A). Unlike the highly expressed genes, the canonical pathways of genes with modest expression included genes that were both up and down-regulated (Figure 2B). Strongly down-regulated pathways were: protein translation (comprising multiple eukaryotic initiation factors (EIF) and 16 ribosomal subunit proteins) and mitochondrial dysfunction (comprising genes encoding subunits of cytochrome c oxidase, ubiquinol-cytochrome c reductase and NADH dehydrogenase) (both pathways $p<8.4x10^{-7}$). Within the canonical pathways: histamine degradation, fatty acid oxidation and eicosanoid signalling, gene expression were predominantly upregulated ($p<0.009$). Several specific genes had multiple roles across different pathways including: amine oxidase, aldehyde dehydrogenase isoforms, COX-2, prostaglandin receptor EP4, thromboxane A2 receptor, arachidonate 5-lipoxygenase-activating protein and multiple phospholipases (details not shown).

3.3.3 Predicted upstream regulators of the choriodecidual labour transcriptome

Potential upstream regulators of the labour-associated changes in inflammatory gene expression were determined. Known regulators of the labour process featured in the top ten transcription factors of the data set (Table 4), including NFκB subunits and NR3C1 (glucocorticoid receptor) (target genes shown in Figure 3). Other predicted transcription factors included signal transducer and activator of transcription (STAT) -1 and -3, the danger associated molecular pattern (DAMP) high mobility group box 1 (HMGB1) (target genes shown in Figure 3) and mesenchyme homeobox 2 (MEOX2) (gene targets not shown). These predicted transcription factors demonstrated regulation of multiple genes involved in "inflammatory response" following labour ($p<2.3x10^{-29}$) (Figure 3). Estrogen (ER) and progesterone receptors (PGR) were predicted to regulate the labour process, but with lower activation scores and p-values (ER: $p=1.91x10^{-11}$; PR: $p=2.66x10^{-03}$). A number of miRNAs were also identified as putative regulators of choriodecidual inflammation (Table 5), including mir-21, mir-155-5p, mir-146 and -146a-5p). The target molecules for these miRNAs map onto the key biological functions and canonical pathways and also known mediators of the labour process (including PTGS2/COX-2, interleukins, chemokines and TLRs).

Interestingly, the predicted upstream regulators for the non-inflammatory changes in the <3-fold altered gene set were distinct, with a much stronger activation score and p value for PGR ($p=1.82^{-10}$), together with v-myc avian myelocytomatosis viral oncogene homolog (MYC), and master regulator of cell survival, TP53 (Table 6, Figure 4). These predicted transcription factors demonstrated regulation of multiple genes involved in "proliferation of cells" following labour ($p<5.6x10^{-13}$)(Figure 4).The predicted regulatory miRNAs of lower expressed gene targets were

largely those associated with cell survival and tissue remodelling (ECM and cytoskeletal components) (Table 7).

3.3.4 Inflammatory gene network during term labour

To understand the functional interactions and directionality of the relationships between all the inflammatory genes in the choriodecidua in labour, a network analysis was performed. The highest ranked network cluster ($p<1x10^{-51}$) features a central role for chemokine signalling and integrates the actions of the calcium binding proteins, IL-17 and other pro-inflammatory cytokines (Figure 5). This cluster of highly connected genes that govern immune cell function and migration reinforces the important interconnected actions of the genes in regulating choriodecidual inflammation.

3.3.5 Microarray validation of gene expression changes with labour

The genes selected for qPCR quantification were highly changed with labour in individual patient samples and had functional significance in the labour process. 18S rRNA was used for normalisation purposes due to its constitutive expression between the study groups following detailed examination of potential housekeeping genes (TBP, YWHAZ, β-actin and 18SrRNA). QPCR data confirmed that chemokine receptor CXCR4 (p=0.013), TLR4 (p=0.005), ICAM1 (p=0.016), SOCS3 (p=0.039), IL-1β (p=0.023), indoleamine 2, 3-dioxygenase (IDO1; p=0.007), B-cell CLL (BCL2a) (p=0.006) and CD44 (p=0.028) were significantly upregulated in the choriodecidua following labour (Figure 6). In addition, a panel of chemokines highly altered with labour (CCL2, CCL4, CCL5, CXCL8, CXCL9 and CXCL10) in the current microarray have previously been validated by real time qPCR on the same sample set, due to their prior detection by a focussed chemokine pathway gene array[13.] The changes detected in versican, folate receptor (FOLR1) and oncostatin M (OSM) mRNA expression did not reach statistical significance.

3.3.6 Immunohistochemical localisation of selected candidate genes

A selection of validated genes (TLR4, CXCR4 and CD44) were immunolocalised in fetal membranes to confirm their expression by decidual cells. All were expressed by decidual cells, with a contribution by infiltrating immune cells, but it is noteworthy that the chorion trophoblast cells also expressed the proteins examined (Figure 7). Changes in staining intensity with labour were not formally assessed due to the insensitivity of this technique.

3.4 Discussion

The current study provides a descriptive analysis of the phenotypic changes in the choriodecidua during labour at term, thus filling an important gap in our understanding of the transcriptomic alterations regulating labour in gestational tissues. Our key finding is the extensive inflammatory activation, consistent with reported inflammatory processes in other uterine tissues (Bethin et al. 2003; Haddad et al. 2006; Hassan et al. 2006; Mittal et al. 2010; Weiner et al. 2010; Lim et al. 2012), supporting the hypothesis that decidual inflammation is integral to the labour process. The demonstration that a large number of genes are altered in the choriodecidua highlights both the complexity of labour and the important contribution of these tissue layers to the labour process, in contrast to the majority of previous studies which focus solely on myometrial activation, characterised by a limited number of altered genes (e.g. COX-2, oxytocin receptor, connexin 43).

Pathway analysis was performed to identify key functional pathways altered with labour. The highly altered pathways were almost exclusively inflammatory in nature, comprising genes governing leukocyte trafficking and activation, prostaglandin synthesis and immunomodulation. We have previously reported elevated leukocyte infiltration of the decidua during term labour (Hamilton et al. 2012), hence the elevation in chemokines (CCL2, 4, 8 and CXCL1, 2, 3, 9, 10 and 11) and other regulators of immune cell trafficking (ICAM-1, integrins, CD44) (Zöller et al. 2007; Long 2011), together with leukocyte products/surface receptors (IDO, SLPI, TLRs, MMP12, interleukins CXCR4, Lymphocyte cytosolic protein 2) is as anticipated. The strong overlap between the key chemokines identified in the current study with our previously identified profile (using a pathway specific array)(Hamilton et al. 2013), together with their central involvement in the leading gene network of highly connected genes, provides compelling evidence for their involvement in the labour process. Likewise, identification of genes with established roles in labour (e.g. COX2, IL-1β, IL-6, SOCS3, CXCL6) provides reassurance of the accuracy of the gene array studies (Elliott et al. 2001; Young et al. 2002; Keelan et al. 2003; Peltier 2003; Challis et al. 2009; Khanjani et al. 2011; Gomez-Lopez et al. 2014b; Sykes et al. 2014; Kim et al. 2015; Wahid et al. 2015). We have also revealed other genes, previously not associated with labour, which have recognised actions during inflammatory processes, including: S100A8, -9 and -12 (leukocyte-derived calcium binding proteins with pro-inflammatory actions), fibroblast growth factor (FGF) 7 and its cognate receptor (FGFR2) (growth factor with immunomodulatory actions) (Ishino et al. 2013) and pentraxin 3 (cytokine-induced protein closely related to c-reactive protein) (Azzurri et al. 2005). Inflammatory processes are tightly controlled and therefore the coincident upregulation of a suite of protease inhibitors, particularly those targeting leukocyte-derived proteases: SLPI, SerpinA1 and SerpinB6, and modulators of cytokine action: SOCS3 and IL-1RA, demonstrate the innate regulatory processes to maintain tissue integrity during physiological inflammation. Furthermore, upregulation of SOCS3 and IL-1RA provide evidence of prior STAT3 and IL-1 receptor activation respectively (Arend & Guthridge 2000; Gabay et al. 2010), supporting functional consequences of the detected changes in mRNA expression.

We identified both known and novel upstream transcriptional regulators as potential triggers of the choriodecidual inflammatory responses. Activation of NFκB has the potential to induce the choriodecidual inflammatory profile observed, consistent with reported actions in driving inflammatory processes in amnion (Lim et al. 2012) and myometrium (Lindstrom & Bennett 2005). Activation of its endogenous inhibitor (NFκB1A/IκB) was also predicted to regulate choriodecidual inflammation; transcription and translation of this gene are stimulated in response to NFκB activation (Brown et al. 1993), adding evidence for functionality of this pathway in labour. Fetally-derived cortisol, acting via the glucocorticoid receptor (NR3C1), has important roles in driving the labour process in sheep and probably also in human pregnancies (McDonald et al. 2006; Whittle et al. 2006). Glucocorticoid receptors are strongly expressed by decidual cells (McDonald et al. 2006), supporting its identification as a putative upstream regulator of decidual chemokine, cytokine, protease and protease inhibitors. Novel regulators of term labour included STAT1 and STAT3, whose activation strongly mapped to the labour inflammatory profile (including pro-apoptotic genes (PHLDA1, Caspase 4), lipid metabolism genes (phospholipase A2, COX2), chemokines, interleukins and TLR4). The upstream ligands leading to STAT translocation to the nucleus are unidentified, however, a number of highly upregulated cytokines/growth factors (including IL-6, bone morphogenetic protein (BMP2), OSM) signal via STAT3, whilst interferons and FGFRs signal via STAT1.

An interesting observation in the context of physiological inflammatory processes during term labour is the predicted role for HMGB-1, a leading DAMP (danger associated molecular pattern), in regulating choriodecidual inflammation. DAMPs are endogenous stimulators of sterile inflammation released from cells in response to injury or stress. HMGB-1 is also released by activated macrophages and can interact with TLR2 and 4, activating NFκB, AP1 and IRF5, thus amplifying inflammatory reactions and immune cell trafficking (Park et al. 2006; Piccinini & Midwood 2010). The fact that TLRs (-1, -2 and -4), classical receptors for PAMPs (pathogens associated molecular patterns) feature highly in the significantly altered genes and canonical pathways activated during term labour suggests that other mediators of sterile inflammation may also participate in choriodecidual activation. Indeed many DAMPs, or their co-receptors required for TLR signalling, are upregulated in the decidua in labour (S100A8/9, versican, hylaronic acid fragments/CD44) or have established roles in labour (surfactant proteins A and D) (Condon et al. 2004; Foell et al. 2007). Whether DAMP-ligation of TLRs induces identical downstream signalling pathways to those reported in fetal membranes in response to PAMP activation (Hoang et al. 2014) has not been established.

When the most highly altered (>3 fold change) genes were excluded from the pathway analysis, we were able to delineate a number of other key biological functions and predicted pathways involved in labour, including cell death/survival, tissue remodelling and protein translation. The canonical pathways additionally highlighted mitochondrial dysfunction, ribosomal/translation suppression and fatty acid metabolism. These pathways map onto the events during parturition: decidual activation (elevated prostaglandin output), fetal membrane / placental detachment and shedding of the terminally differentiated decidual cell layer. Our tissues were sampled distant to

the rupture site; this was important as detailed analysis identified differential expression of 677 genes (including IGF family members, chemokines, cytokines and proteases) between rupture and non-rupture sites (Nhan-Chang et al. 2010). Distinct upstream regulators were predicted to drive these gene expression and phenotypic changes; predominantly regulators of cell fate, e.g. oncogenes MYC, MYCN and FOS, and the tumour suppressor gene TP53 (P53). The latter was predicted to regulate 68 downstream genes that were significantly altered in the choriodecidua during labour, including TGFβ superfamily members (inhibin βA, TGFβ2, GDF15), ECM components (collagen 3A1 and 1A1, versican), inflammatory genes (COX2, CD44) and transcription/translation factors (EIF4E2, NFκB). A role for P53 in dictating the timing of labour has previously been demonstrated in mice; conditional uterine knockout P53 in mice resulted in preterm delivery, which was reversed by pharmacological inhibition of COX-2 (Hirota et al. 2010). Our studies support a similar regulatory pathway may exist in human decidua, but also highlight broader transcriptional effects that may account for the additional phenotypes observed in the mouse model (e.g. decidual senescence, prolonged labour).

Given the established hypothesis of a switch from progesterone dominance to estrogen action being instrumental in triggering labour, it was perhaps surprising that ER and PR were not highly ranked predictive upstream regulators of choriodecidual inflammatory gene expression changes. A caveat to any conclusions drawn regarding their importance is that this type of analysis is dependent on gene interactions reported in the literature, a minority of which will be from studies of reproductive tissues. Hence, ER and PR actions may be underrepresented. Notwithstanding this issue, the stronger association of PR with primarily non-inflammatory downstream genes (such as paired related homeobox 1, H19, WNT4 and versican) suggests a more influential role in regulating labour-associated cell survival, migration and remodelling. In line with previous observations, PR can regulate prostaglandin synthesis through modulation of COX-2 and PDGH, and also other lipid metabolism enzymes (aldehyde dehydrogenase 1A3, acyl-CoA synthetase long-chain 1, carnitine palmitoyltransferase 1B), and along with the ability to alter NFκB responses via upregulation of inhibitor NFκB1A (IκB), suggests modulation of choriodecidual inflammatory responses through PR signalling.

We also investigated the theoretical regulation of the choriodecidual transcriptome by miRNAs. Studies using mouse models revealed a key role for miRNA200 as a hormonally sensitive modulator of contraction associated proteins in the myometrium during term and preterm labour, acting via regulation of the homeobox genes ZEB1/2 (Renthal et al. 2010). Our analyses found a similar interaction may exist in the choriodecidua, with both miR200b-3p and co-transcribed miR141-3p as predicted regulators of ZEB2. Other predicted miRNA regulators include miR101 consistent with previous reports of endometrial regulation of COX-2 during establishment of pregnancy (Chakrabarty et al. 2007). Altered miRNA expression was also strongly predicted to regulate choriodecidual chemokines and related pro-inflammatory mediators. Amongst the top ten potential miRNAs regulators are those with strong associations with inflammatory conditions, including miR155 (classic pro-inflammatory miRNA) and miR21 and 146, which act to resolve inflammatory responses by the suppression of TLR and cytokine (O'Connell et al. 2012) expression. These

miRNAs were not found to be altered by labour in a study of global fetal membrane miRNA expression, although significant changes in DICER expression (miRNA generating enzyme) with term labour supports their involvement in regulating labour events (Montenegro et al. 2009). Interestingly a recent study described upregulation of uterine miR200b by transgenerational maternal stress in rats; this was associated with earlier delivery and downregulation of ZEB1/2 and Stat5 (Yao et al. 2014). Whether miRs are subject to epigenetic regulation in human uterine tissues remains to be established.

A limitation of the current study is the use of choriodecidual samples rather than purified decidua. Decidua can be mechanical or enzymatic separated from the adherent chorion, but this has the potential to alter gene expression profiles – particularly inflammatory factors – and results in variable purity of the decidua. Isolation of decidual stromal cells alone introduces further confounders by loss of resident immune cells which have important paracrine actions (Castillo-Castrejon et al. 2014). We therefore performed immunohistochemistry on selected gene products to demonstrated that they were expressed by decidual cells (with some contribution by immune cells) and not exclusively by the chorion layer. In addition, our previous studies (and those of others) demonstrated that the inflammatory events were more extensive in the decidua than the chorion, at least in terms of leukocyte infiltration (Hamilton et al. 2012), thus from an inflammatory perspective at least, we believe we can assess decidual events. Previous analyses of intact fetal membranes (Haddad et al. 2006) would have included the decidual layer in the samples analysed, but there are extensive transcriptomic changes occurring in the amnion during term labour (Marvin et al. 2002; Li et al. 2011; Lim et al. 2012). Despite some overlap in the inflammatory pathways in isolated amnion cells and intact fetal membranes, there was significant divergence in the specific gene expression profiles reflecting distinct regulatory processes occurring in the different tissue layers highlighting the need to study all gestational tissues.

Our ability to validate 80% of altered genes by PCR provides confidence in the changes identified by the array. The pathway and network approaches that we have used are resistant to random error which provides further confidence in our interpretation (Albert et al. 2000). The failure to reproduce all the changes at the qPCR level is likely due to the considerable heterogeneity between clinical samples. This variability may reflect localised differences in cellular composition of the choriodecidua or the extent to which preparatory events for labour have commenced in the pregnancies delivered by caesarean section at term prior to labour. This was elegantly illustrated in the amnion in a recent study by Lim et al (Lim et al. 2012), demonstrating NFκB activation (accompanied by inflammatory changes) in a proportion of samples from women at 39-40 weeks gestation. It would be unsurprising for similar preparatory events to take place in the choriodecidua, potentially regulated by amnion. This issue could be circumvented by the use of choriodecidual samples from women who delivered preterm without undergoing labour as the control non-labour group. However, the indication for preterm delivery (either maternal or fetal pathology, e.g. pre-eclampsia, fetal growth restriction) is likely to have a significant influence on gene expression in uterine tissues and hence have a stronger confounding influence. Other potential sources of heterogeneity in the current study include maternal ethnicity. Although there is

an influence of ethnicity on susceptibility to preterm labour (MacDorman 2011), to our knowledge there is no evidence that the processes governing term labour differ between women of different ethnic origin. Furthermore, inclusion of samples from women with heterogenous ethnic backgrounds enabled identification of the common pathways activated in the choriodecidua during labour, which is important for future identification of targets for therapeutic intervention.

Summary

In an observational study using clinical samples we are unable to verify causation and determine the temporal events leading to labour, but the analysis of potential regulators and an initial foray into network analyses provides some understanding of the putative interactions between the altered genes. In depth network analyses would provide greater knowledge about interactions between the genes governing the distinct biological processes and also identify key regulatory genes affecting extensive networks of genes. These may ultimately serve as targets for therapeutic intervention which may curtail inappropriate activation of labour processes preterm. In summary, this paper confirms inflammatory processes are major players in labour events in the choriodecidua, as in other gestational tissues. We have identified additional alterations in genes governing cell death and survival, tissue remodelling and protein translation, a pattern consistent with the imminent loss of this tissue layer following delivery of the infant. Separating the inflammatory from the cell survival processes enabled identification of putative regulators of the distinct processes, illustrating the complexity of labour and the challenges involved in attempting to suppress inappropriate activation of the labour cascade in preterm deliveries.

Acknowledgements:

We thank the Genomic Technologies and Bioinformatics Core Facilities in the Faculty of Life Sciences at the University of Manchester for providing technical support and analysis with regard to Affymetrix arrays. We extend our thanks to the midwives in St Mary's Hospital for their assistance with patient recruitment, and to the women for donating tissue samples to research. This study was funded by the James Tudor Foundation, Tommy's the Baby Charity and Ferring. LKH is a BBSRC David Phillips Research Fellow. The Maternal and Fetal Health Research Centre is supported by funding from Tommy's the Baby Charity, and Action Medical Research Endowment, the Manchester Biomedical Research Centre and the Greater Manchester Comprehensive Research Network.

3.5 References

Albert, R, H Jeong, and AL Barabasi. 2000. "Error and Attack Tolerance of Complex Networks." Nature 406 (6794): 378–82. doi:10.1038/35019019.

Alfirevic, Zarko. 2012. "Tocolytics : Do They Actually Work ?" BMJ 345 (October): e6531. doi:10.1136/bmj.e6531.

Arend, W P, and C J Guthridge. 2000. "Biological Role of Interleukin 1 Receptor Antagonist Isoforms." Annals of the Rheumatic Diseases 59 Suppl 1 (November): i60–64.

Azzurri, Annalisa, Oumou Y Sow, Amedeo Amedei, Boubacar Bah, Sadio Diallo, Giuseppe Peri, Marisa Benagiano, Mario M D'Elios, Alberto Mantovani, and Gianfranco Del Prete. 2005. "IFN-Gamma-Inducible Protein 10 and Pentraxin 3 Plasma Levels Are Tools for Monitoring Inflammation and Disease Activity in Mycobacterium Tuberculosis Infection." Microbes and Infection / Institut Pasteur 7 (1): 1–8. doi:10.1016/j.micinf.2004.09.004.

Bethin, K E, Y Nagai, R Sladek, M Asada, Y Sadovsky, T J Hudson, and L J Muglia. 2003. "Microarray Analysis of Uterine Gene Expression in Mouse and Human Pregnancy." Mol Endocrinol 17 (8): 1454–69. doi:10.1210/me.2003-0007.

Blanks, A M, and J J Brosens. 2012. "Progesterone Action in the Myometrium and Decidua in Preterm Birth." Facts, Views & Vision in ObGyn 4 (3): 33–43.

Bollapragada, Shrikant, Refaat Youssef, Fiona Jordan, Ian Greer, Jane Norman, and Scott Nelson. 2009. "Term Labor Is Associated with a Core Inflammatory Response in Human Fetal Membranes, Myometrium, and Cervix." American Journal of Obstetrics and Gynecology 200 (1). Mosby, Inc.: 104.e1–11. doi:10.1016/j.ajog.2008.08.032.

Brown, K, S Park, T Kanno, G Franzoso, and U Siebenlist. 1993. "Mutual Regulation of the Transcriptional Activator NF-Kappa B and Its Inhibitor, I Kappa B-Alpha." Proceedings of the National Academy of Sciences of the United States of America 90 (6): 2532–36.

Bukowski, R, G D Hankins, G R Saade, G D Anderson, and S Thornton. 2006. "Labor-Associated Gene Expression in the Human Uterine Fundus, Lower Segment, and Cervix." PLoS Med 3 (6): e169. doi:05-PLME-RA-0327R2 [pii]10.1371/journal.pmed.0030169.

Calvano, Steve E., Wenzhong Xiao, Daniel R. Richards, Ramon M. Felciano, Henry V. Baker, Raymond J. Cho, Richard O. Chen, et al. 2005. "A Network-Based Analysis of Systemic Inflammation in Humans." Nature 437 (7061): 1032–37. doi:10.1038/nature03985.

Castillo-Castrejon, Marisol, Noemí Meraz-Cruz, Nardhy Gomez-Lopez, Arturo Flores-Pliego, Jorge Beltrán-Montoya, Martín Viveros-Alcaráz, and Felipe Vadillo-Ortega. 2014. "Choriodecidual Cells from Term Human Pregnancies Show Distinctive Functional Properties Related to the Induction of Labor." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 71 (1): 86–93. doi:10.1111/aji.12179.

Centre for disease control and prevention. 2012. "Births and Natality." http://wwwcdcgov/nchs/fastats/birthshtm. Updated July, 2014. Accessed August, 2014.

Chakrabarty, Anindita, Susanne Tranguch, Takiko Daikoku, Kevin Jensen, Henry Furneaux, and Sudhansu K Dey. 2007. "MicroRNA Regulation of Cyclooxygenase-2 during Embryo Implantation." Proceedings of the National Academy of Sciences of the United States of America 104 (38): 15144–49. doi:10.1073/pnas.0705917104.

Challis, John R G, Deborah M Sloboda, Nadia Alfaidy, Steven J Lye, William Gibb, Fal A Patel, Wendy L Whittle, and John P Newnham. 2002. "Prostaglandins and Mechanisms of Preterm Birth." Reproduction (Cambridge, England) 124 (1): 1–17.

Challis, John R, Charles J Lockwood, Leslie Myatt, Jane E Norman, J F Strauss 3rd, Felice Petraglia, and Jerome F Strauss. 2009. "Inflammation and Pregnancy." Reprod Sci 16 (2): 206–15. doi:16/2/206 [pii]10.1177/1933719108329095.

Chan, E Cheng, S Fraser, S Yin, G Yeo, K Kwek, R J Fairclough, and R Smith. 2002. "Human Myometrial Genes Are Differentially Expressed in Labor: A Suppression Subtractive Hybridization Study." The Journal of Clinical Endocrinology and Metabolism 87 (6): 2435–41. doi:10.1210/jcem.87.6.8439.

Chan, Yi-Wah, Hugo A van den Berg, Jonathan D Moore, Siobhan Quenby, and Andrew M Blanks. 2014. "Assessment of Myometrial Transcriptome Changes Associated with Spontaneous Human Labour by High-Throughput RNA-Seq." Experimental Physiology 99 (3): 510–24. doi:10.1113/expphysiol.2013.072868.

Chandran, Sreenath, Michael T. Cairns, Margaret O'Brien, and Terry J. Smith. 2014. "Transcriptomic Effects of Estradiol Treatment on Cultured Human Uterine Smooth Muscle Cells." Molecular and Cellular Endocrinology, June. doi:10.1016/j.mce.2014.05.020.

Charpigny, G, J Leroy, M Breuiller-Fouché, Z Tanfin, S Mhaouty-Kodja, Ph Robin, D Leiber, et al. 2003. "A Functional Genomic Study to Identify Differential Gene Expression in the Preterm and Term Human Myometrium." Biol Reprod 68 (6): 2289–96. doi:10.1095/biolreprod.102.013763 biolreprod.102.013763 [pii].

Condon, Jennifer C, Pancharatnam Jeyasuria, Julie M Faust, and Carole R Mendelson. 2004. "Surfactant Protein Secreted by the Maturing Mouse Fetal Lung Acts as a Hormone That Signals the Initiation of Parturition." Proceedings of the National Academy of Sciences of the United States of America 101 (14): 4978–83. doi:10.1073/pnas.0401124101.

Dodd, J M, C A Crowther, and P Middleton. 2012. "Oral Betamimetics for Maintenance Therapy after Threatened Preterm Labour." Cochrane Database Syst Rev 12: CD003927. doi:10.1002/14651858.CD003927.pub3.

Elliott, C L, J A Loudon, N Brown, D M Slater, P R Bennett, and M H Sullivan. 2001. "IL-1beta and IL-8 in Human Fetal Membranes: Changes with Gestational Age, Labor, and Culture Conditions." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 46 (4): 260–67.

Esplin, M Sean, M Bardett Fausett, Morgan R Peltier, Steven Hamblin, Robert M Silver, D Ware Branch, Eli Y Adashi, and David Whiting. 2005. "The Use of cDNA Microarray to Identify Differentially Expressed Labor-Associated Genes within the Human Myometrium during Labor." American Journal of Obstetrics and Gynecology 193 (2): 404–13. doi:10.1016/j.ajog.2004.12.021.

Foell, Dirk, Helmut Wittkowski, Thomas Vogl, and Johannes Roth. 2007. "S100 Proteins Expressed in Phagocytes: A Novel Group of Damage-Associated Molecular Pattern Molecules." Journal of Leukocyte Biology 81 (1): 28–37. doi:10.1189/jlb.0306170.

Gabay, Cem, Céline Lamacchia, and Gaby Palmer. 2010. "IL-1 Pathways in Inflammation and Human Diseases." Nature Reviews. Rheumatology 6 (4): 232–41. doi:10.1038/nrrheum.2010.4.

Gentleman, Robert C, Vincent J Carey, Douglas M Bates, Ben Bolstad, Marcel Dettling, Sandrine Dudoit, Byron Ellis, et al. 2004. "Bioconductor: Open Software Development for Computational Biology and Bioinformatics." Genome Biology 5 (10): R80. doi:10.1186/gb-2004-5-10-r80.

Gomez-Lopez, Nardhy, Derek StLouis, Marcus A Lehr, Elly N Sanchez-Rodriguez, and Marcia Arenas-Hernandez. 2014. "Immune Cells in Term and Preterm Labor." Cellular and Molecular Immunology 11 (6): 571–81. doi:10.1038/cmi.2014.46.

Haas, DM, DM Caldwell, P Kirkpatrick, J J McIntosh, and N J Welton. 2012. "Tocolytic Therapy for Preterm Delivery: Systematic Review and Network Meta-Analysis." BMJ 345: e6226.

Haddad, Ramsi, Gerard Tromp, Helena Kuivaniemi, Tinnakorn Chaiworapongsa, Yeon Mee Kim, Moshe Mazor, and Roberto Romero. 2006. "Human Spontaneous Labor without Histologic Chorioamnionitis Is Characterized by an Acute Inflammation Gene Expression Signature." American Journal of Obstetrics and Gynecology 195 (2): 394.e1–24. doi:10.1016/j.ajog.2005.08.057.

Hamilton, Sarah A, Y Oomomian, G Stephen, O Shynlova, C L Tower, A Garrod, S J Lye, and R L Jones. 2012. "Macrophages Infiltrate the Human and Rat Decidua during Term and Preterm Labor: Evidence That Decidual Inflammation Precedes Labor." Biol Reprod 86 (2): 39. doi:biolreprod.111.095505 [pii] 10.1095/biolreprod.111.095505.

Hamilton, Sarah A, Clare L Tower, and Rebecca L Jones. 2013. "Identification of Chemokines Associated with the Recruitment of Decidual Leukocytes in Human Labour: Potential Novel Targets for Preterm Labour." PLoS One 8 (2): e56946. doi:10.1371/journal.pone.0056946.

Han, Yu Mi, Roberto Romero, Jung-Sun Kim, Adi L Tarca, Sun Kwon Kim, Sorin Draghici, Juan Pedro Kusanovic, et al. 2008. "Region-Specific Gene Expression Profiling: Novel Evidence for Biological Heterogeneity of the Human Amnion." Biology of Reproduction 79 (5): 954–61. doi:10.1095/biolreprod.108.069260.

Hassan, Sonia S, Roberto Romero, Ramsi Haddad, Israel Hendler, Nahla Khalek, Gerard Tromp, Michael P Diamond, Yoram Sorokin, and John Malone. 2006. "The Transcriptome of the Uterine Cervix before and after Spontaneous Term Parturition." American Journal of Obstetrics and Gynecology 195 (3): 778–86. doi:10.1016/j.ajog.2006.06.021.

Havelock, Jon C, Patrick Keller, Ndaya Muleba, Bobbie A Mayhew, Brian M Casey, William E Rainey, and R Ann Word. 2005. "Human Myometrial Gene Expression before and during Parturition." Biology of Reproduction 72 (3): 707–19. doi:10.1095/biolreprod.104.032979.

Hayward, C E, S L Greenwood, C P Sibley, P N Baker, and R L Jones. 2011. "Effect of Young Maternal Age and Skeletal Growth on Placental Growth and Development." Placenta 32 (12): 990–98. doi:10.1016/j.placenta.2011.09.016.

Hirota, Yasushi, Takiko Daikoku, Susanne Tranguch, Huirong Xie, Heather B Bradshaw, and Sudhansu K Dey. 2010. "Uterine-Specific p53 Deficiency Confers Premature Uterine Senescence and Promotes Preterm Birth in Mice." The Journal of Clinical Investigation 120 (3): 803–15. doi:10.1172/JCI40051.

Hoang, Mai, Julie A Potter, Stefan M Gysler, Christina S Han, Seth Guller, Errol R Norwitz, and Vikki M Abrahams. 2014. "Human Fetal Membranes Generate Distinct Cytokine Profiles in Response to Bacterial Toll-like Receptor and Nod-like Receptor Agonists." Biology of Reproduction 90 (2): 39. doi:10.1095/biolreprod.113.115428.

Ishino, Ruri, Kaori Minami, Satowa Tanaka, Mami Nagai, Keiji Matsui, Natsumi Hasegawa, Robert G Roeder, Shigetaka Asano, and Mitsuhiro Ito. 2013. "FGF7 Supports Hematopoietic Stem and Progenitor Cells and Niche-Dependent Myeloblastoma Cells via Autocrine Action on Bone Marrow Stromal Cells *in vitro*." Biochemical and Biophysical Research Communications 440 (1): 125–31. doi:10.1016/j.bbrc.2013.09.044.

Jørgensen, Jan Stener, Louise Katrine Kjær Weile, and Ronald Francis Lamont. 2014. "Preterm Labor: Current Tocolytic Options for the Treatment of Preterm Labor." Expert Opinion on Pharmacotherapy 15 (5): 585–88. doi:10.1517/14656566.2014.880110.

Keelan, J A, M Blumenstein, R J A Helliwell, T A Sato, K W Marvin, and M D Mitchell. 2003. "Cytokines, Prostaglandins and Parturition--a Review." Placenta 24 Suppl A (April): S33–46.

Khanjani, Shirin, Mandeep K Kandola, Tamsin M Lindstrom, Suren R Sooranna, Manuela Melchionda, Yun S Lee, Vasso Terzidou, Mark R Johnson, and Phillip R Bennett. 2011. "NF-κB Regulates a Cassette of Immune/inflammatory Genes in Human Pregnant Myometrium at Term." Journal of Cellular and Molecular Medicine 15 (4): 809–24. doi:10.1111/j.1582-4934.2010.01069.x.

Kim, Sung Hye, David A MacIntyre, Maria Firmino Da Silva, Andrew M Blanks, Yun S Lee, Steven Thornton, Phillip R Bennett, and Vasso Terzidou. 2015. "Oxytocin Activates NF-κB-Mediated Inflammatory Pathways in Human Gestational Tissues." Molecular and Cellular Endocrinology 403 (March): 64–77. doi:10.1016/j.mce.2014.11.008.

Li, C, and W H Wong. 2001. "Model-Based Analysis of Oligonucleotide Arrays: Expression Index Computation and Outlier Detection." Proceedings of the National Academy of Sciences of the United States of America 98 (1): 31–36. doi:10.1073/pnas.011404098.

Li, Ruth, William E Ackerman, Taryn L Summerfield, Lianbo Yu, Parul Gulati, Jie Zhang, Kun Huang, Roberto Romero, and Douglas A Kniss. 2011. "Inflammatory Gene Regulatory Networks in Amnion Cells Following Cytokine Stimulation: Translational Systems Approach to Modeling Human Parturition." PloS One 6 (6): e20560. doi:10.1371/journal.pone.0020560.

Lim, Sheri, David A MacIntyre, Yun S Lee, Shirin Khanjani, Vasso Terzidou, T G Teoh, and Phillip R Bennett. 2012. "Nuclear Factor Kappa B Activation Occurs in the Amnion prior to Labour Onset and Modulates the Expression of Numerous Labour Associated Genes." PloS One 7 (4): e34707. doi:10.1371/journal.pone.0034707.

Lindstrom, T M, and P R Bennett. 2005. "The Role of Nuclear Factor Kappa B in Human Labour." Reproduction 130 (5): 569–81. doi:130/5/569 [pii]10.1530/rep.1.00197.

Liu, Xuejun, Marta Milo, Neil D Lawrence, and Magnus Rattray. 2005. "A Tractable Probabilistic Model for Affymetrix Probe-Level Analysis across Multiple Chips." Bioinformatics (Oxford, England) 21 (18): 3637–44. doi:10.1093/bioinformatics/bti583.

Liu, Xuejun, Marta Milo, Neil D Lawrence, and Magnus Rattray. 2006. "Probe-Level Measurement Error Improves Accuracy in Detecting Differential Gene Expression." Bioinformatics (Oxford, England) 22 (17): 2107–13. doi:10.1093/bioinformatics/btl361.

Long, Eric O. 2011. "ICAM-1: Getting a Grip on Leukocyte Adhesion." Journal of Immunology (Baltimore, Md. : 1950) 186 (9): 5021–23. doi:10.4049/jimmunol.1100646.

MacDorman, Marian F. 2011. "Race and Ethnic Disparities in Fetal Mortality, Preterm Birth, and Infant Mortality in the United States: An Overview." Seminars in Perinatology 35 (4): 200–208. doi:10.1053/j.semperi.2011.02.017.

Makino, S, D B Zaragoza, B F Mitchell, S Robertson, and D M Olson. 2007. "Prostaglandin F2alpha and Its Receptor as Activators of Human Decidua." Semin Reprod Med 25 (1): 60–68. doi:10.1055/s-2006-956776.

Marvin, K W, J A Keelan, R L Eykholt, T A Sato, and M D Mitchell. 2002. "Use of cDNA Arrays to Generate Differential Expression Profiles for Inflammatory Genes in Human Gestational Membranes Delivered at Term and Preterm." Mol Hum Reprod 8 (4): 399–408.

McDonald, Sarah E, Teresa A Henderson, Celso E Gomez-Sanchez, Hilary O D Critchley, and J Ian Mason. 2006. "11Beta-Hydroxysteroid Dehydrogenases in Human Endometrium." Molecular and Cellular Endocrinology 248 (1-2): 72–78. doi:10.1016/j.mce.2005.12.010.

Mitchell, M D, R J Romero, S S Edwin, and M S Trautman. 1995. "Prostaglandins and Parturition." Reprod Fertil Dev 7 (3): 623–32.

Mittal, Pooja, Roberto Romero, Adi L Tarca, Juan Gonzalez, Sorin Draghici, Yi Xu, Zhong Dong, et al. 2010. "Characterization of the Myometrial Transcriptome and Biological Pathways of Spontaneous Human Labor at Term." J Perinat Med 38 (6): 617–43. doi:10.1515/JPM.2010.097 [doi].

Montenegro, D, R Romero, S S Kim, A L Tarca, S Draghici, J P Kusanovic, J S Kim, et al. 2009. "Expression Patterns of microRNAs in the Chorioamniotic Membranes: A Role for microRNAs in Human Pregnancy and Parturition." J Pathol 217 (1): 113–21. doi:10.1002/path.2463.

Nhan-Chang, Chia-Ling, Roberto Romero, Adi L Tarca, Pooja Mittal, Juan Pedro Kusanovic, Offer Erez, Shali Mazaki-Tovi, et al. 2010. "Characterization of the Transcriptome of Chorioamniotic Membranes at the Site of Rupture in Spontaneous Labor at Term." American Journal of Obstetrics and Gynecology 202 (5): 462.e1–41. doi:10.1016/j.ajog.2010.02.045.

O'Connell, Ryan M, Dinesh S Rao, and David Baltimore. 2012. "microRNA Regulation of Inflammatory Responses." Annual Review of Immunology 30 (January): 295–312. doi:10.1146/annurev-immunol-020711-075013.

Office for National Statistics, Gestation-Specific Infant Mortality in England and Wales 2015. *http://www.ons.gov.uk/ons/taxonomy/index.html?nscl=Infant+Mortality.*

Park, Jong Sung, Fabia Gamboni-Robertson, Qianbin He, Daiva Svetkauskaite, Jae-Yeol Kim, Derek Strassheim, Jang-Won Sohn, et al. 2006. "High Mobility Group Box 1 Protein Interacts with Multiple Toll-like Receptors." American Journal of Physiology. Cell Physiology 290 (3): C917–24. doi:10.1152/ajpcell.00401.2005.

Pearson, Richard D, Xuejun Liu, Guido Sanguinetti, Marta Milo, Neil D Lawrence, and Magnus Rattray. 2009. "Puma: A Bioconductor Package for Propagating Uncertainty in Microarray Analysis." BMC Bioinformatics 10 (January): 211. doi:10.1186/1471-2105-10-211.

Peltier, Morgan R. 2003. "Immunology of Term and Preterm Labor." Reproductive Biology and Endocrinology : RB&E 1 (December): 122. doi:10.1186/1477-7827-1-122.

Piccinini, A M, and K S Midwood. 2010. "DAMPening Inflammation by Modulating TLR Signalling." Mediators of Inflammation 2010 (January). doi:10.1155/2010/672395.

Rehman, K S, S Yin, B A Mayhew, R A Word, and W E Rainey. 2003. "Human Myometrial Adaptation to Pregnancy: cDNA Microarray Gene Expression Profiling of Myometrium from Non-Pregnant and Pregnant Women." Mol Hum Reprod 9 (11): 681–700.

Renthal, N E, C C Chen, K C Williams, R D Gerard, J Prange-Kiel, and C R Mendelson. 2010. "miR-200 Family and Targets, ZEB1 and ZEB2, Modulate Uterine Quiescence and Contractility during Pregnancy and Labor." Proceedings of the National Academy of Sciences 107 (48): 20828–33. doi:10.1073/pnas.1008301107.

Roos, C, E Borowiack, M Kowalska, A Zapalska, Bw Mol, L Mignini, C Meads, J Walczak, and Ks Khan. 2013. "What Do We Know about Tocolytic Effectiveness and How Do We Use This Information in Guidelines? A Comparison of Evidence Grading." BJOG : An International Journal of Obstetrics and Gynaecology 120 (13): 1588–96; discussion 1597–98. doi:10.1111/1471-0528.12388.

Shankar, R, M P Johnson, N A Williamson, F Cullinane, A W Purcell, E K Moses, and S P Brennecke. 2010. "Molecular Markers of Preterm Labor in the Choriodecidua." Reprod Sci 17 (3): 297–310. doi:1933719109353454 [pii]10.1177/1933719109353454.

Skinner, K A, and J R Challis. 1985. "Changes in the Synthesis and Metabolism of Prostaglandins by Human Fetal Membranes and Decidua at Labor." Am J Obstet Gynecol 151 (4): 519–23. doi:0002-9378(85)90281-9 [pii].

Sykes, Lynne, David A MacIntyre, Tiong Ghee Teoh, and Phillip R Bennett. 2014. "Anti-Inflammatory Prostaglandins for the Prevention of Preterm Labour." Reproduction (Cambridge, England) 148 (2): R29–40. doi:10.1530/REP-13-0587.

Vogel, Joshua P, Juan Manuel Nardin, Therese Dowswell, Helen M West, and Olufemi T Oladapo. 2014. "Combination of Tocolytic Agents for Inhibiting Preterm Labour." The Cochrane Database of Systematic Reviews 7 (January): CD006169. doi:10.1002/14651858.CD006169.pub2.

Wahid, Hanan H., Camilla L. Dorian, Peck Yin Chin, Mark R. Hutchinson, Kenner C. Rice, David M. Olson, Lachlan M. Moldenhauer, and Sarah A. Robertson. 2015. "Toll-Like Receptor 4 Is an Essential Upstream Regulator of On-Time Parturition and Perinatal Viability in Mice." Endocrinology 156 (10): 3828–41. doi:10.1210/en.2015-1089.

Weiner, C P, C W Mason, Y Dong, I A Buhimschi, P W Swaan, and C S Buhimschi. 2010. "Human Effector/initiator Gene Sets That Regulate Myometrial Contractility during Term and Preterm Labor." Am J Obstet Gynecol 202 (5): 474 e1–20. doi:S0002-9378(10)00247-4 [pii]10.1016/j.ajog.2010.02.034.

Whittle, W L, A C Holloway, S Lye, J R G Challis, and W Gibb. 2006. "The Pattern of Glucocorticoid and Estrogen Receptors May Explain Differences in Steroid Dependency of Intrauterine Prostaglandin Production at Parturition in Sheep." Journal of the Society for Gynecologic Investigation 13 (7): 506–11. doi:10.1016/j.jsgi.2006.07.003.

World Health Organisation. 2015. "Preterm Birth Fact Sheet No. 363." http://www.who.int/mediacentre/factsheets/fs363/en/. Accessed February, 2014.

Yao, Youli, Alexandra M Robinson, Fabiola C R Zucchi, Jerrah C Robbins, Olena Babenko, Olga Kovalchuk, Igor Kovalchuk, David M Olson, and Gerlinde A S Metz. 2014. "Ancestral Exposure to Stress Epigenetically Programs Preterm Birth Risk and Adverse Maternal and Newborn Outcomes." BMC Medicine 12 (January): 121. doi:10.1186/s12916-014-0121-6.

Young, Anne, Andrew J Thomson, MarieAnne Ledingham, Fiona Jordan, Ian A Greer, and Jane E Norman. 2002. "Immunolocalization of Proinflammatory Cytokines in Myometrium, Cervix, and Fetal Membranes during Human Parturition at Term." Biol Reprod 66 (2): 445–49.

Zöller, Margot, Pooja Gupta, Rachid Marhaba, Mario Vitacolonna, and Pia Freyschmidt-Paul. 2007. "Anti-CD44-Mediated Blockade of Leukocyte Migration in Skin-Associated Immune Diseases." Journal of Leukocyte Biology 82 (1): 57–71. doi:10.1189/jlb.0107063.

Figures and tables

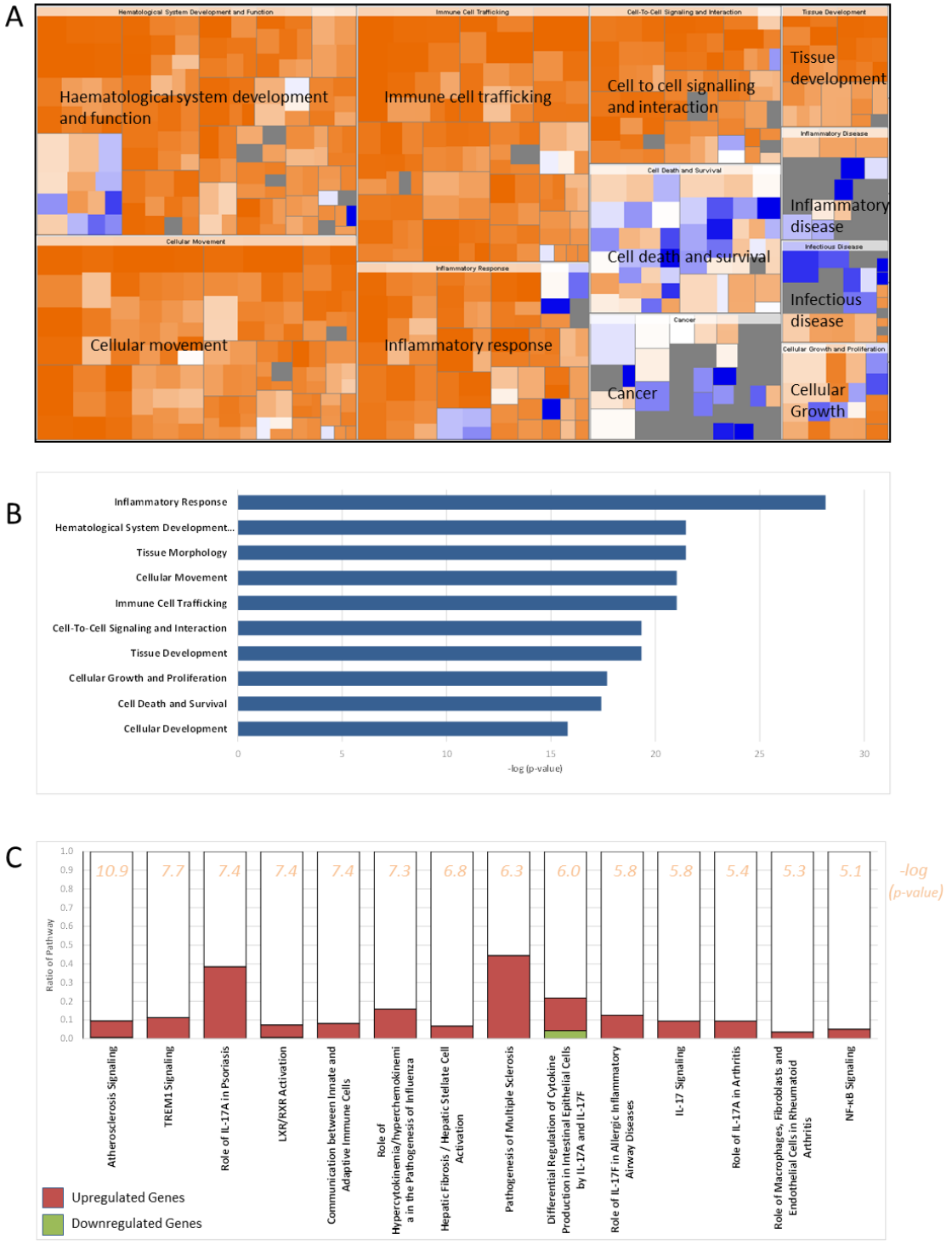


Figure 1: Pathways and functions associated with choriodecidual genes altered by term labour.

A) Heat map of function activity predicted in association with altered gene expression (z-score and p-value). B) Biological functions of genes altered >3-fold with labour, ranked by p-value of Fisher's exact test. C) Canonical pathways of genes altered >3-fold with labour ranked by p-value of Fisher's exact test (orange line, right hand vertical axis), percentage of pathway shown upregulated (red) and downregulated (green), bold number = number of genes in pathway.

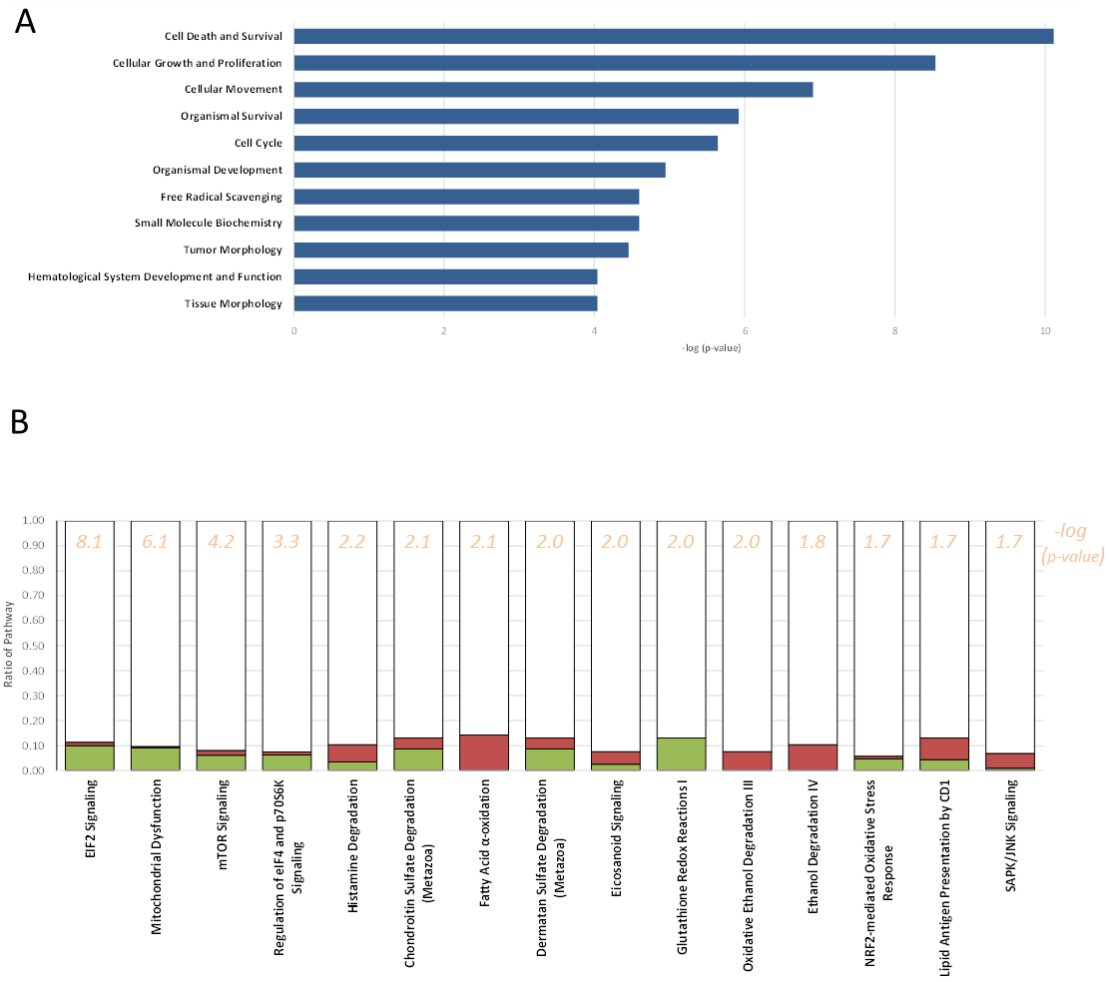


Figure 2: Pathways and functions associated with choriodecidual genes altered <3-fold by term labour.

A) Biological functions ranked by p-value of Fisher's exact test. B) Canonical pathways ranked by p-value of Fisher's exact test (orange line, right hand vertical axis), percentage of pathway shown upregulated (red) and downregulated (green), bold number = number of genes in pathway.

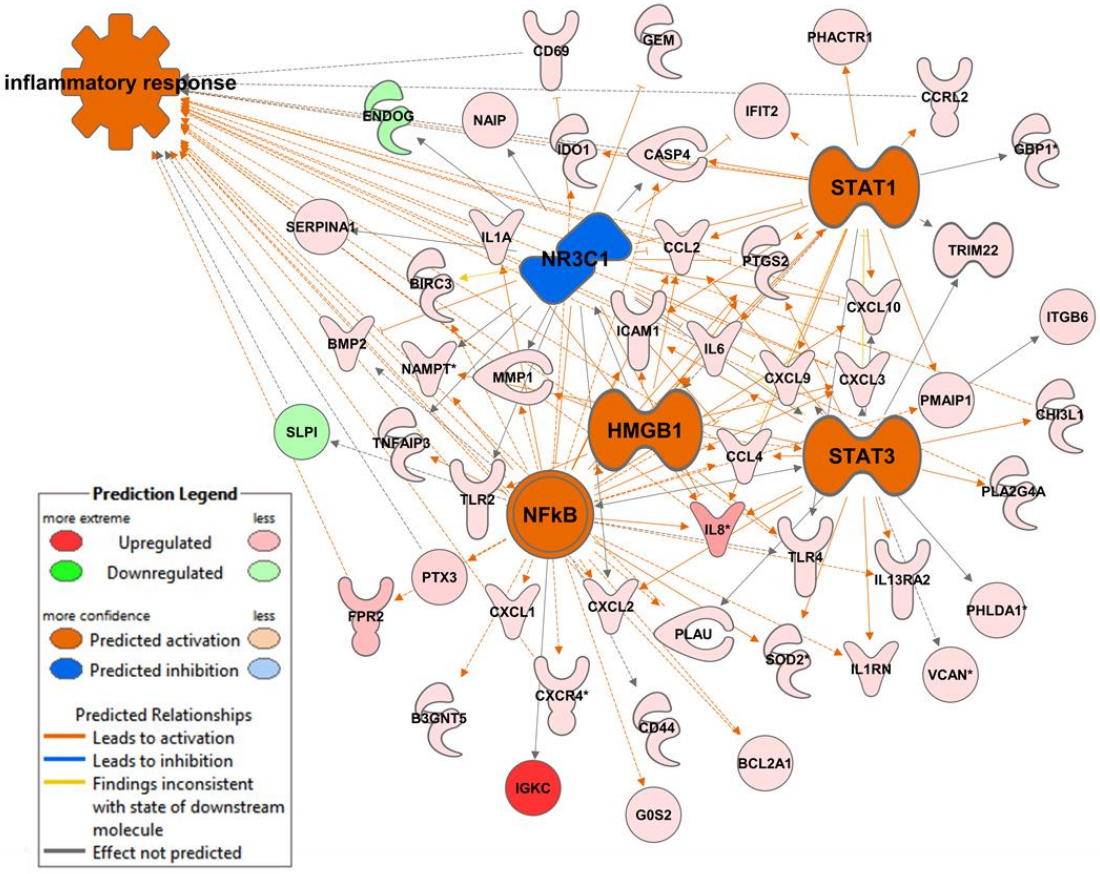


Figure 3: Upstream regulators of inflammatory pathways in choriodecidua during term labour.

Predicted activity of the upstream regulators NFκB, HMGB1, NR3C1 (glucocorticoid receptor), STAT1 and STAT3 (colour coded by predicted activation determined by z-score – see legend) and shown in relation to their altered downstream targets (see legend). Predicted relationship to the biological function of "inflammatory response" shown.

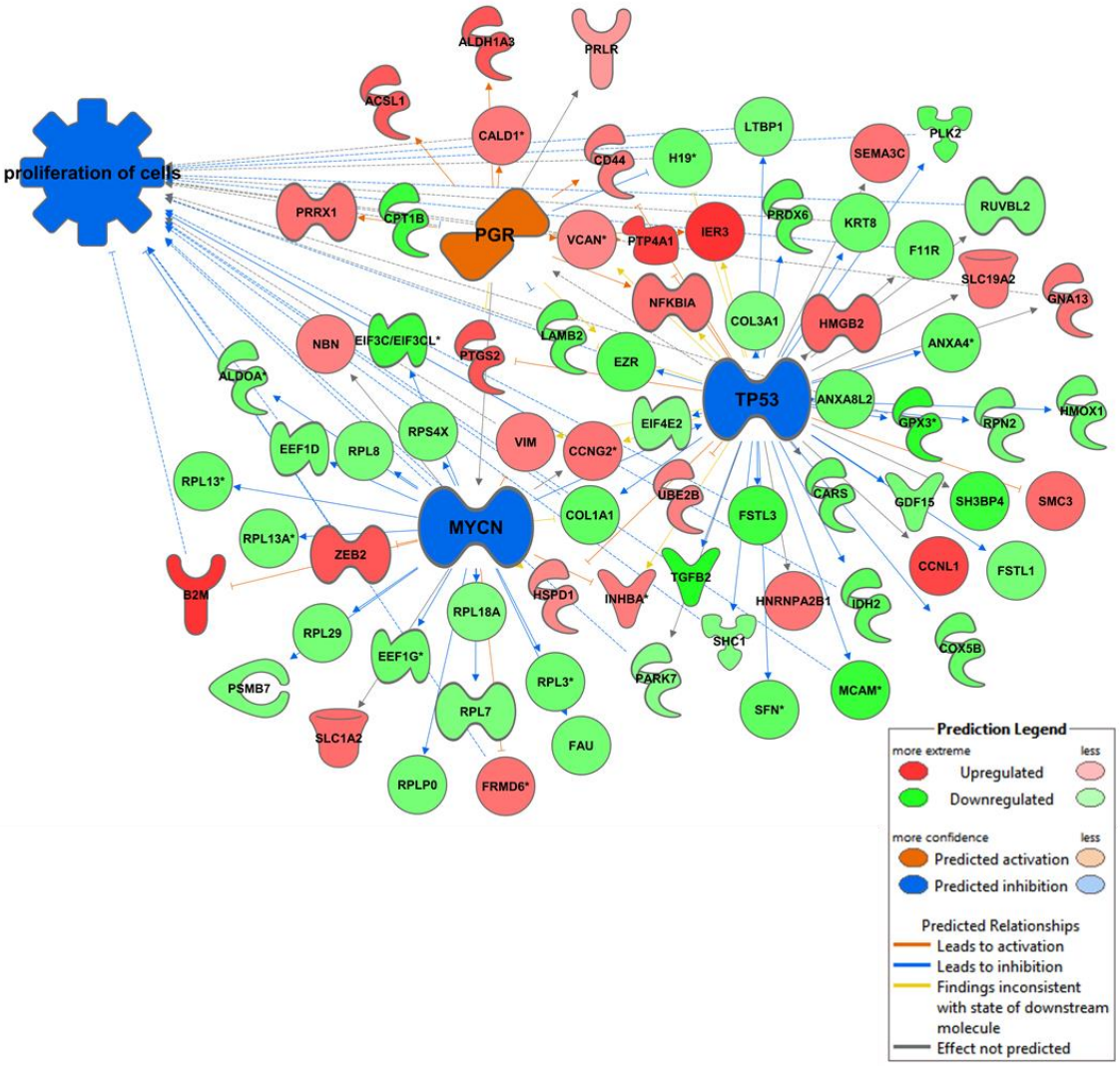


Figure 4: Potential upstream regulators of choriodecidual genes altered <3-fold by term labour.

Predicted activity of the upstream regulators MYCN, PGR, TP53 (colour coded by predicted activation determined by z-score – see legend) and shown in relation to their altered downstream targets (see legend). Predicted relationship to the biological function of "proliferation of cells" shown.

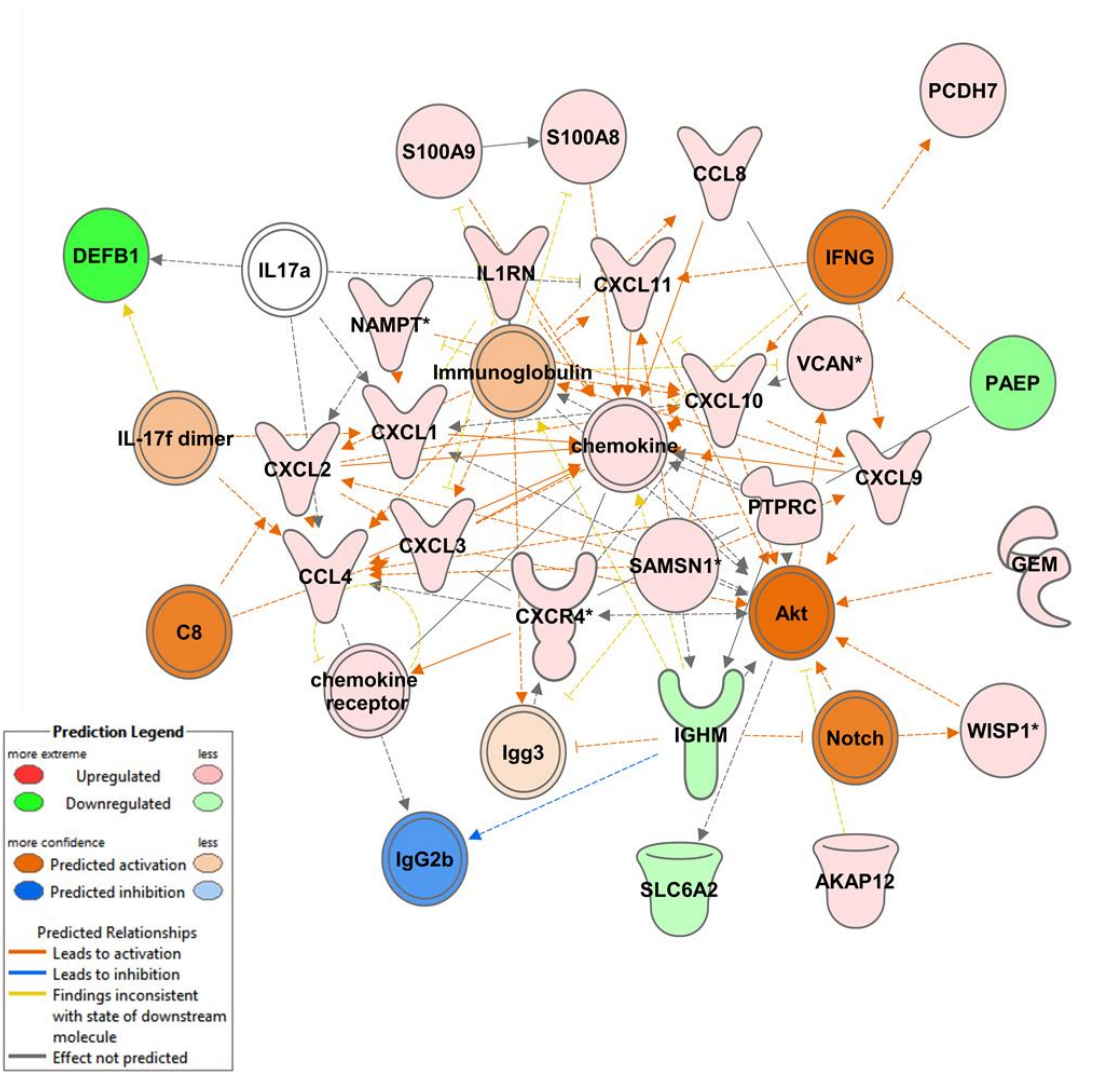


Figure 5: Network analysis of inflammatory genes in choriodecidua altered by term labour.

Altered gene expression was used to generate a network model within the context of all known interactions related to the genomic function using Ingenuity Knowledge Base (IKB). Clusters of interactions were determined using the genes with altered expression as "seeds" for generating networks that maximize their specific connectivity. Activity prediction and gene expression colour coded (see legend).

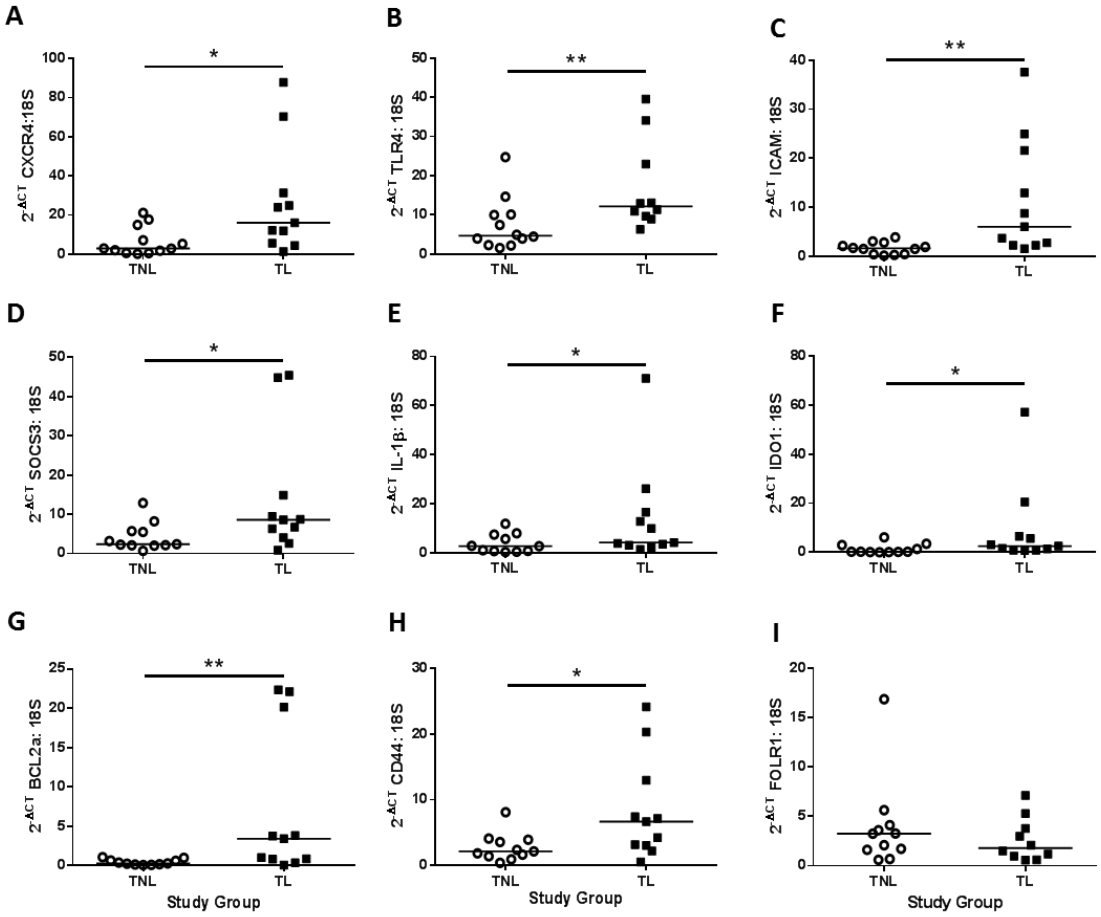


Figure 6: Q-PCR validation of candidate genes altered by term labour in choriodecidua. Data were normalised to 18S rRNA expression levels. TNL (term not labour, n=12), TL (term labour, n=11). *p<0.05, **p<0.01, Mann-Whitney Test.

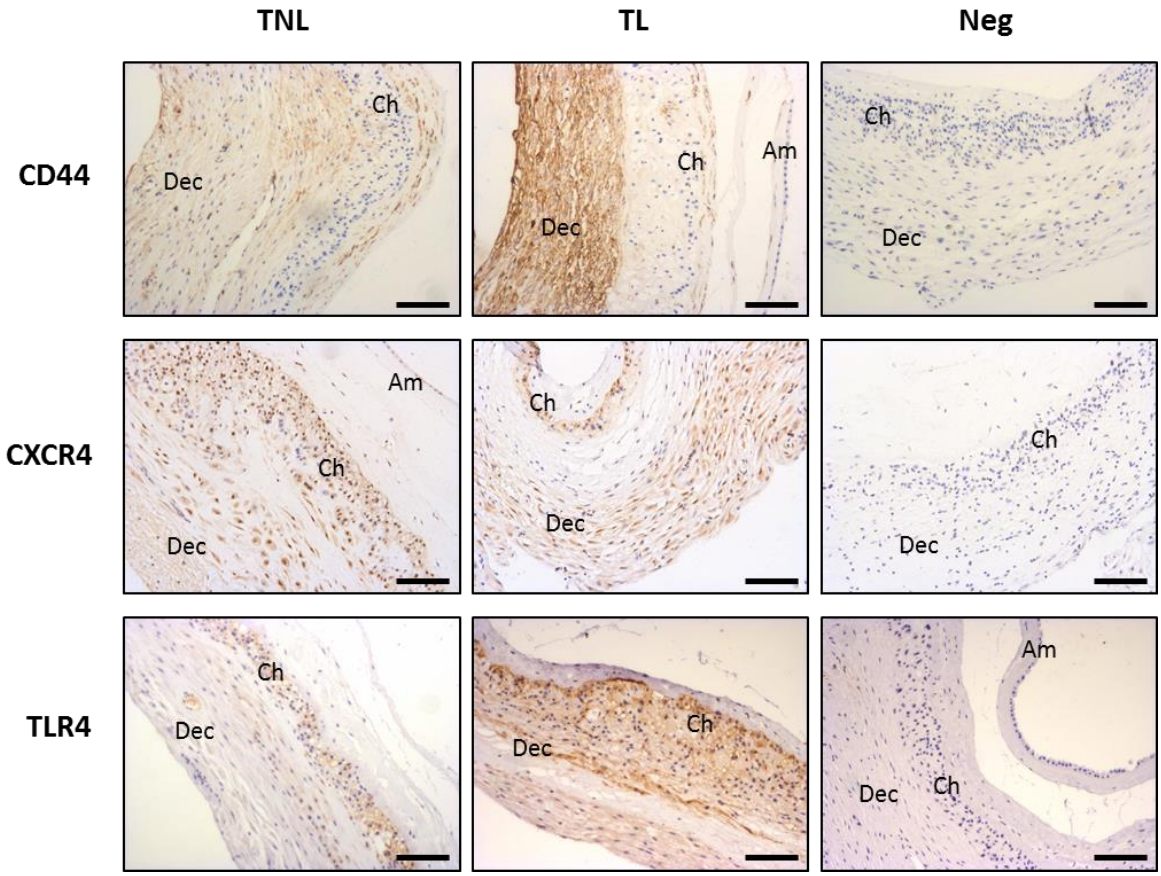


Figure 7: Immunolocalisation of candidate genes (CXCR4, CD44, TLR4) altered by term labour in fetal membranes.

Representative images from TNL (term not labour, n=12), TL (term labour, n=11), Neg (negative IgG control). Original magnification x10. Scale bar = 100μm. Dec=decidua, Ch=chorion, Am = amnion.

Table 1. Demographic and biophysical characteristics of the clinical study groups.

The numbers are median and range unless otherwise indicated. TNL (Term not in labour), TL (Term labour), BMI (body mass index at booking to antenatal care). * $p<0.05$ indicates TL compared to TNL (Mann-Whitney test); † statistics performed with Fisher's Exact Test.

	TNL (n=12)	***TL (n=11)***	***P value***
Age (years)	31 (21-38)	25 (20-33)	NS
Gravidity	2 (1-2.5)	1(1-3)	NS
Parity	1 (0-3)	0 (0-6)*	0.04
Ethnicity: n	9 Caucasian 3 Afro – Caribbean 1 Asian	10 Caucasian 1 Afro-Caribbean	NS†
BMI (kg/m^2)	26 (17-34)	26 (22-43)	NS
Smoking: n (%)	2 (16%)	1 (9%)	NS†
Gestational age at delivery (weeks)	39 (37-41)	40 (38-42)	NS

Table 2. Summary of primer sequences used for qPCR validation.

Gene	Symbol	Forward 5'
B-cell CLL or Lymphoma 2	BCL2a	5′-TACAGGCTGGCTCAGGACTAT-3′ 5′-CGCAACATTTTGTAGCACTCTG-3′
Chemokine (C-X-C motif) receptor 4	CXCR4	5′-ACTACACCGAGGAAATGGGCT-3′ 5′-CCCACAATGCCAGTTAAGAAGA-3′
Cluster of differentiation 44	CD44	5′-CTGCCGCTTTGCAGGTGTA-3′ 5′- CATTGTGGGCAAGGTGCTATT-3′
Indoleamine-pyrrole 2,3-dioxygenase	IDO1	5′-GCCAGCTTCGAGAAAGAGTTG-3′ 5′-ATCCCAGAACTAGACGTGCAA-3′
Intercellular adhesion molecule 1	ICAM1	5′-ATGCCCAGACATCTGTGTCC-3′ 5′-GGGGTCTCTATGCCCAACAA-3′
Toll-like receptor 4	TLR4	5′-AGACCTGTCCCTGAACCCTAT-3′ 5′-CGATGGACTTCTAAACCAGCCA-3′
Interleukin-1β	IL-1 β	5′-CTCGCCAGTGAAATGATGGCT-3′ 5′-GTCGGAGATTCGTAGCTGGAT-3′
Suppressor of cytokine signalling 3	SOCS3	5′-CCTGCGCCTCAAGACCTTC-3′ 5′-GTCACTGCGCTCCAGTAGAA-3′
Folate Receptor 1	FOLR1	5′-GCTCAGCGGATGACAACACA-3′ 5′-CCTGGCCCATGCAATCCTT-3′
Versican	VCAN	5′-GTAACCCATGCGCTACATAAAGT-3′ 5′-GGCAAAGTAGGCATCGTTGAAA-3′
Oncostatin M	OSM	5′-ATGGCTCTATTTGCAGTCTTTCA-3′ 5′-CACCCAGATGACATTGGATGTT-3′
18S ribosomal RNA	18S rRNA	5′-GCTGGAATTACCGCGGCT-3′ 5′-CGGCTACCACATCCAAGGAA-3′

Table 3. Choriodecidual genes altered by labour in the top 10 biological functions

Genes highlighted in blue were selected for validation studies; those in red were previously validated (Hamilton et al. 2013). † indicates the gene was also detected in the top canonical pathways altered by labour.

Symbol	Entrez Gene Name	Entrez Gene ID for Human	Type(s)	Fold Change
AKAP12	A kinase (PRKA) anchor protein 12	9590	transporter	3.1
APOBEC3A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A	200315	enzyme	4.4
†APOD	apolipoprotein D	347	transporter	-3.3
B3GNT5	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5	84002	enzyme	3.9
BCL2A1	BCL2-related protein A1	597	other	15.7
BIRC3	baculoviral IAP repeat containing 3	330	enzyme	20.5
†BMP2	bone morphogenetic protein 2	650	growth factor	3.5
CASP4	caspase 4	837	peptidase	3.9
†CCL2	chemokine (C-C motif) ligand 2	6347	cytokine	3.4
†CCL4	chemokine (C-C motif) ligand 4	6351	cytokine	3.1
CCL8	chemokine (C-C motif) ligand 8	6355	cytokine	3.8
CCRL2	chemokine (C-C motif) receptor-like 2	9034	G-protein coupled receptor	5.0
CD44	CD44 molecule	960	enzyme	5.6
CD69	CD69 molecule	969	transmembrane receptor	13.1
CHI3L1	chitinase 3-like 1 (cartilage glycoprotein-39)	1116	enzyme	11.0
†CXCL1	chemokine (C-X-C motif) ligand 1	2919	cytokine	10.6
CXCL2	chemokine (C-X-C motif) ligand 2	2920	cytokine	5.7
†CXCL3	chemokine (C-X-C motif) ligand 3	2921	cytokine	9.8
CXCL9	chemokine (C-X-C motif) ligand 9	4283	cytokine	8.5
†CXCL10	chemokine (C-X-C motif) ligand 10	3627	cytokine	3.7
†CXCL11	chemokine (C-X-C motif) ligand 11	6373	cytokine	13.7
†CXCR4	chemokine (C-X-C motif) receptor 4	7852	G-protein coupled receptor	14.2
CYTIP	cytohesin 1 interacting protein	9595	other	4.6
†DEFB1	defensin, beta 1	1672	other	-9.6
EBI3	Epstein-Barr virus induced 3	10148	cytokine	-3.7
ENDOG	endonuclease G	2021	enzyme	-4.0
EREG	epiregulin	2069	growth factor	17.3
FAT1	FAT atypical cadherin 1	2195	other	
FCGR3B	Fc fragment of IgG, low affinity IIIb, receptor (CD16b)	2215	transmembrane receptor	11.9
FGF7	fibroblast growth factor 7	2252	growth factor	5.9
†FGFR2	fibroblast growth factor receptor 2	2263	kinase	53.3
FOLR1	folate receptor 1 (adult)	2348	transporter	-4.3
FPR2	formyl peptide receptor 2	2358	G-protein coupled receptor	53.3
G0S2	G0/G1switch 2	50486	other	6.2
GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1	2550	G-protein coupled receptor	3.3
GBP1	guanylate binding protein 1, interferon-inducible	2633	enzyme	4.7
GEM	GTP binding protein overexpressed in skeletal muscle	2669	enzyme	8.7
†ICAM1	intercellular adhesion molecule 1	3383	transmembrane	8.7

			receptor	
IDO1	indoleamine 2,3-dioxygenase 1	3620	enzyme	6.6
IFIT2	interferon-induced protein with tetratricopeptide repeats 2	3433	other	4.5
IGHM	immunoglobulin heavy constant mu	3507	transmembrane receptor	-3.4
IGKC	immunoglobulin kappa constant	3514	other	165.1
†IL6	interleukin 6 (interferon, beta 2)	3569	cytokine	19.6
†IL8	interleukin 8	3576	cytokine	78.5
IL-13Rα2	interleukin 13 receptor, alpha 2	3598	transmembrane receptor	5.5
†IL-1α	interleukin 1, alpha	3552	cytokine	18.7
†IL-1RN	interleukin 1 receptor antagonist	3557	cytokine	28.4
IRAK3	interleukin-1 receptor-associated kinase 3	11213	kinase	3.1
ITGA2	integrin, alpha 2	3673	transmembrane receptor	5.8
ITGB6	integrin, beta 6	3694	other	28.2
JAM2	junctional adhesion molecule 2	58494	other	-3.2
LCP2	lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)	3937	other	5.8
LMNB1	lamin B1	4001	other	11.7
†LYZ	lysozyme	4069	enzyme	3.5
MBD3	methyl-CpG binding domain protein 3	53615	other	-3.1
†MMP1	matrix metallopeptidase 1	4312	peptidase	4.5
MMP10	matrix metallopeptidase 10	4319	peptidase	6.2
MMP12	matrix metallopeptidase 12	4321	peptidase	3.1
MNDA	myeloid cell nuclear differentiation antigen	4332	transcription regulator	4.0
NAIP	NLR family, apoptosis inhibitory protein	4671	other	3.6
NAMPT	nicotinamide phosphoribosyltransferase	10135	cytokine	19.7
NPW	neuropeptide W	283869	other	-3.5
†OSM	oncostatin M	5008	cytokine	31.4
PAEP	progestagen-associated endometrial protein	5047	glycoprotein	-5.5
PAG1	phosphoprotein associated with glycosphingolipid microdomains 1	55824	other	3.3
PCDH7	protocadherin 7	5099	other	
PELI1	pellino E3 ubiquitin protein ligase 1	57162	enzyme	4.8
PHLDA1	pleckstrin homology-like domain, family A, member 1	22822	other	8.2
†PLA2G4A	phospholipase A2, group IVA	5321	enzyme	3.0
PLAU	plasminogen activator, urokinase	5328	peptidase	5.3
PLEK	pleckstrin	5341	other	4.3
PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1	5366	other	5.2
†PTGS2	prostaglandin-endoperoxide synthase 2 (cyclooxygenase)	5743	enzyme	3.0
PTPRC	protein tyrosine phosphatase, receptor type, C	5788	phosphatase	3.5
PTX3	pentraxin 3, long	5806	other	34.5
RGS1	regulator of G-protein signaling 1	5996	other	4.1
RHOD	ras homolog family member D	29984	enzyme	-3.3
RHOH	ras homolog family member H	399	enzyme	38.9

†**S100A8**	S100 calcium binding protein A8	6279	other	5.1
†**S100A9**	S100 calcium binding protein A9	6280	other	3.2
†**S100A12**	S100 calcium binding protein A12	6283	other	7.9
SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1	64092	other	6.1
SCAF11	SR-related CTD-associated factor 11	9169	other	4.7
SELL	selectin L	6402	transmembrane receptor	6.9
†**SERPINA1**	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	5265	other	7.0
SERPINB6	serpin peptidase inhibitor, clade B (ovalbumin), member 6	5269	other	93.0
SKAP2	src kinase associated phosphoprotein 2	8935	other	3.4
SLC20A1	solute carrier family 20 (phosphate transporter), member 1	6574	transporter	4.6
SLC6A2	solute carrier family 6 (neurotransmitter transporter), member 2	6530	transporter	-4.1
SLC7A1	solute carrier family 7 (cationic amino acid transporter, y+ system), member 1	6541	transporter	4.8
SLPI	secretory leukocyte peptidase inhibitor	6590	other	-3.8
SOD2	superoxide dismutase 2, mitochondrial	6648	enzyme	22.6
SRGN	serglycin	5552	other	4.0
†**TLR1**	Toll-like receptor 1	7096	transmembrane receptor	3.0
†**TLR2**	Toll-like receptor 2	7097	transmembrane receptor	5.1
†**TLR4**	Toll-like receptor 4	7099	transmembrane receptor	3.7
TMEFF1	transmembrane protein with EGF-like and two follistatin-like domains 1	8577	other	7.3
TNFAIP3	tumor necrosis factor, alpha-induced protein 3	7128	enzyme	3.5
†**TNFAIP6**	tumor necrosis factor, alpha-induced protein 6	7130	enzyme	3.5
TRIM22	tripartite motif containing 22	10346	transcription regulator	3.1
UCP2	uncoupling protein 2 (mitochondrial, proton carrier)	7351	transporter	-4.2
VCAN	versican	1462	other	3.6
WISP1	WNT1 inducible signaling pathway protein 1	8840	other	4.7

Table 4. Potential upstream transcription factors regulating choriodecidual gene expression changes with term labour.

The top 10 transcription factors are presented, as ranked by highest significance (lowest p value).

Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap	No. of target molecules
NFκB (complex)	Activated	5.422	1.60×10^{-29}	39
RELA	Activated	4.289	9.18×10^{-24}	28
NFκBIA	Activated	1.080	1.15×10^{-22}	30
NFκB1	Activated	2.555	1.70×10^{-21}	23
STAT1	Activated	3.182	1.57×10^{-16}	20
MEOX2	Inhibited	-3.286	7.37×10^{-16}	11
STAT3	Activated	2.670	4.35×10^{-15}	22
NR3C1	Inhibited	-3.147	1.48×10^{-14}	26
HMGB1	Activated	3.266	3.28×10^{-13}	11
JUN	Activated	2.277	3.93×10^{-13}	20

Table 5. Potential upstream miRNAs regulating high choriodecidual gene expression changes with term labour.

The top predicted miRNA regulators are presented, as ranked by highest significance (lowest p value).

Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap	Target molecules in dataset
mir-21	Inhibited	-2.137	$4.90x10^{-11}$	CASP4, CXCL1, CXCL10, CXCL9, ICAM1, IDO1, IGHM, SLPI, SOD2, TLR1, TLR2, TLR4
mir-146	Inhibited	-2.186	$2.22\ x10^{-07}$	CCL2, CXCL10, IL6, IL8, PTGS2
miR-155-5p	Inhibited	-2.941	$6.72\ x10^{-07}$	CCL4, CXCL1, CXCL2, CXCL3, FGF7, IL1A, IL6, IL8, PELI1
miR-146a-5p	Inhibited	-2.607	$9.37x10^{-07}$	CCL2, CCL8, CXCR4, IL8, S100A12, TLR1, TLR4
mir-155		-1.446	$1.69\ x10^{-06}$	CCL2, CD69, CXCL1, CXCL11, IL6, PTGS2
miR-16-5p		-0.342	$2.01x10^{-04}$	FGF7, ITGA2, PSAT1, PTGS2, SKAP2, SLC7A1, UCP2

Table 6. Potential upstream transcription factors regulating choriodecidual gene expression changes (<3-fold and >1.3 fold) with term labour

The top 10 transcription factors are presented, as ranked by highest significance (lowest p value).

Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap
MYCN	Inhibited	-2.412	$4.72x10^{-16}$
MYC		-1.002	$3.21x10^{-14}$
PGR		0.666	$1.82x10^{-10}$
TP53		0.072	$1.25x10^{-09}$
FOS		-0.671	$5.93x10^{-09}$
PDX1		1.807	$2.24x10^{-08}$
CEBPB		0.984	$1.05x10^{-06}$
PPARG		-0.622	$1.05x10^{-06}$
CEBPA		1.091	$4.09x10^{-06}$
NFκBIA		0.892	$2.11x10^{-05}$

Table 7. Potential upstream miRNA regulating choriodecidual gene expression with modest changes (<3-fold and >1.3 fold) with term labour.

The top predicted miRNA regulators are presented, as ranked by highest significance (lowest p value).

Upstream Regulator	Activation z-score	p-value of overlap	Target molecules in dataset
miR-141-3p	-0.627	$6.26x10^{-04}$	CTBP2, ERBB2IP, TGFB2, VIM, ZEB2
miR-200b-3p	-1.980	$3.90x10^{-03}$	ERBB2IP, MARCKS, VIM, ZEB2
miR-29b-3p	0.579	$4.91x10^{-03}$	COL1A1, COL3A1, GAS7, HMGN3, MCL1, SRSF10
miR-21-5p	-0.374	$5.83x10^{-03}$	FAS, MARCKS, PELI1, PRRG4, TPM1

Supplemental table 1. Microarray analysis indicated that term labour was associated with extensive alterations in choriodecidual gene expression.

796 choriodecidual genes were significantly altered in labour (PPLR value of ≥0.997 or ≤1x10^{-5}) using a 1.3-fold threshold. Analysis was performed with the PUMA package using probability of positive log ratio (PPLR). Probesets were considered differentially expressed between the two conditions when they had a PPLR value approaching 0 or 1.

Fold Change	PPLR	ID	Symbol	Entrez Gene Name
-2.208	0	53071_s_at	OGFOD3	2-oxoglutarate and iron-dependent oxygenase domain containing 3
2.013	1	206638_at	HTR2B	5-hydroxytryptamine (serotonin) receptor 2B, G protein-coupled
-2.281	0	218387_s_at	PGLS	6-phosphogluconolactonase
-2.198	0	218388_at	PGLS	6-phosphogluconolactonase
3.099	1	227529_s_at	AKAP12	A kinase (PRKA) anchor protein 12
-2.355	0	49452_at	ACACB	acetyl-CoA carboxylase beta
-2.263	0	204638_at	ACP5	acid phosphatase 5, tartrate resistant
1.57	1	221505_at	ANP32E	acidic (leucine-rich) nuclear phosphoprotein 32 family, member E
-1.848	0	207988_s_at	ARPC2	actin related protein 2/3 complex, subunit 2, 34kDa
1.932	1	212815_at	ASCC3	activating signal cointegrator 1 complex subunit 3
1.551	0.999	202672_s_at	ATF3	activating transcription factor 3
2.362	1	201963_at	ACSL1	acyl-CoA synthetase long-chain family member 1
-2.039	0	208002_s_at	ACOT7	acyl-CoA thioesterase 7
-2.81	0	209765_at	ADAM19	ADAM metallopeptidase domain 19
2.827	0.998	208268_at	ADAM28	ADAM metallopeptidase domain 28
-2.738	0	229004_at	ADAMTS15	ADAM metallopeptidase with thrombospondin type 1 motif, 15
1.98	0.999	219023_at	AP1AR	adaptor-related protein complex 1 associated regulatory protein
-1.921	0	213892_s_at	APRT	adenine phosphoribosyltransferase
-1.969	0	225016_at	APCDD1	adenomatosis polyposis coli down-regulated 1
-1.852	0	200903_s_at	AHCY	adenosylhomocysteinase
-1.84	0	201196_s_at	AMD1	adenosylmethionine decarboxylase 1
-2.423	0	203586_s_at	ARL4D	ADP-ribosylation factor-like 4D
1.793	0.999	232865_at	AFF4	AF4/FMR2 family, member 4
2.289	1	212224_at	ALDH1A1	aldehyde dehydrogenase 1 family, member A1
2.412	0.998	203180_at	ALDH1A3	aldehyde dehydrogenase 1 family, member A3
-1.545	0	202139_at	AKR7A2	aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)
-1.934	0	200966_x_at	ALDOA	aldolase A, fructose-bisphosphate
-1.778	0	214687_x_at	ALDOA	aldolase A, fructose-bisphosphate
2.151	0.997	205584_at	ALG13	ALG13, UDP-N-acetylglucosaminyltransferase subunit
-1.445	0	218203_at	ALG5	ALG5, dolichyl-phosphate beta-glucosyltransferase
-2.5	0	223075_s_at	AIF1L	allograft inflammatory factor 1-like
-1.903	0	219329_s_at	ATRAID	all-trans retinoic acid-induced differentiation factor
-2.342	0	203559_s_at	AOC1	amine oxidase, copper containing 1
2.037	1	222608_s_at	ANLN	anillin, actin binding protein
1.938	1	212286_at	ANKRD12	ankyrin repeat domain 12
-2.399	0	224810_s_at	ANKRD13A	ankyrin repeat domain 13A
2.854	0.999	1561079_at	ANKRD28	ankyrin repeat domain 28
-2.023	0	204671_s_at	ANKRD6	ankyrin repeat domain 6
-1.983	0	201302_at	ANXA4	annexin A4
-1.858	0	201301_s_at	ANXA4	annexin A4
-1.924	0	203074_at	ANXA8/ ANXA8L1	annexin A8-like 1
4.425	0.998	210873_x_at	APOBEC3A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A
-3.282	0	201525_at	APOD	apolipoprotein D
-2.863	0	39248_at	AQP3	aquaporin 3 (Gill blood group)
2.773	1	204174_at	ALOX5AP	arachidonate 5-lipoxygenase-activating protein
-2.388	0	214749_s_at	ARMCX6	armadillo repeat containing, X-linked 6
1.686	0.999	224797_at	ARRDC3	arrestin domain containing 3

-1.803	0	222912_at	ARRB1	arrestin, beta 1
-1.958	0	38703_at	DNPEP	aspartyl aminopeptidase
1.53	0.999	201624_at	DARS	aspartyl-tRNA synthetase
-2.233	0	213041_s_at	ATP5D	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit
-2.027	0	201172_x_at	ATP6V0E1	ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e1
-2.039	0	201242_s_at	ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide
20.484	1	210538_s_at	BIRC3	baculoviral IAP repeat containing 3
-2.277	0	217809_at	BZW2	basic leucine zipper and W2 domains 2
1.398	0.999	201084_s_at	BCLAF1	BCL2-associated transcription factor 1
1.655	0.999	201101_s_at	BCLAF1	BCL2-associated transcription factor 1
15.654	1	205681_at	BCL2A1	BCL2-related protein A1
-2.677	0	208906_at	BSCL2	Berardinelli-Seip congenital lipodystrophy 2 (seipin)
2.928	0.998	232311_at	B2M	beta-2-microglobulin
-2.528	0	213905_x_at	BGN	biglycan
2.352	0.999	235009_at	BOD1L1	biorientation of chromosomes in cell division 1-like 1
-1.804	0	201032_at	BLCAP	bladder cancer associated protein
3.497	0.999	205289_at	BMP2	bone morphogenetic protein 2
3.29	0.999	1568768_s_at	BRE-AS1	BRE antisense RNA 1
-3.502	0	230854_at	BCAR4	breast cancer anti-estrogen resistance 4 (non-protein coding)
-1.953	0	202102_s_at	BRD4	bromodomain containing 4
2.029	0.999	204194_at	BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor 1
2.301	0.998	38241_at	BTN3A3	butyrophilin, subfamily 3, member A3
-2.059	0	218309_at	CAMK2N1	calcium/calmodulin-dependent protein kinase II inhibitor 1
1.678	0.999	201617_x_at	CALD1	caldesmon 1
1.906	0.997	201616_s_at	CALD1	caldesmon 1
-2.164	0	225693_s_at	CAMTA1	calmodulin binding transcription activator 1
-1.808	0	214315_x_at	CALR	calreticulin
-2.016	0	202857_at	CNPY2	canopy FGF signaling regulator 2
-1.863	0	209796_s_at	CNPY2	canopy FGF signaling regulator 2
1.697	1	227364_at	CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1
-2.555	0	210069_at	CPT1B	carnitine palmitoyltransferase 1B (muscle)
-1.554	0	1555889_a_at	CRTAP	cartilage associated protein
1.55	0.999	1556006_s_at	CSNK1A1	casein kinase 1, alpha 1
3.92	0.997	213596_at	CASP4	caspase 4, apoptosis-related cysteine peptidase
-1.883	0	213274_s_at	CTSB	cathepsin B
1.976	1	202902_s_at	CTSS	cathepsin S
-2.275	0	219025_at	CD248	CD248 molecule, endosialin
1.922	1	212063_at	CD44	CD44 molecule (Indian blood group)
5.625	1	217523_at	CD44	CD44 molecule (Indian blood group)
1.972	0.999	203416_at	CD53	CD53 molecule
13.057	1	209795_at	CD69	CD69 molecule
-1.937	0	201897_s_at	CKS1B	CDC28 protein kinase regulatory subunit 1B
2.028	1	214683_s_at	CLK1	CDC-like kinase 1
-2.681	0	212540_at	CDC34	cell division cycle 34
8.095	0.998	212942_s_at	CEMIP	cell migration inducing protein, hyaluronan binding
2.534	0.998	206003_at	CEP135	centrosomal protein 135kDa
3.432	1	216598_s_at	CCL2	chemokine (C-C motif) ligand 2
3.075	1	204103_at	CCL4	chemokine (C-C motif) ligand 4
3.799	1	214038_at	CCL8	chemokine (C-C motif) ligand 8
4.98	1	211434_s_at	CCRL2	chemokine (C-C motif) receptor-like 2
10.644	1	204470_at	CXCL1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
3.664	1	204533_at	CXCL10	chemokine (C-X-C motif) ligand 10
13.707	0.999	211122_s_at	CXCL11	chemokine (C-X-C motif) ligand 11
5.734	1	209774_x_at	CXCL2	chemokine (C-X-C motif) ligand 2
9.764	0.999	207850_at	CXCL3	chemokine (C-X-C motif) ligand 3
10.109	1	202859_x_at	CXCL8	chemokine (C-X-C motif) ligand 8
78.495	1	211506_s_at	CXCL8	chemokine (C-X-C motif) ligand 8
8.46	1	203915_at	CXCL9	chemokine (C-X-C motif) ligand 9
3.21	1	217028_at	CXCR4	chemokine (C-X-C motif) receptor 4
6.073	0.999	211919_s_at	CXCR4	chemokine (C-X-C motif) receptor 4

14.246	0.998	209201_x_at	CXCR4	chemokine (C-X-C motif) receptor 4
10.991	0.999	209395_at	CHI3L1	chitinase 3-like 1 (cartilage glycoprotein-39)
-2.351	0	223061_at	CHID1	chitinase domain containing 1
-1.731	0	219529_at	CLIC3	chloride intracellular channel 3
-3.214	0	211739_x_at	CSH1/CSH2	chorionic somatomammotropin hormone 1 (placental lactogen)
-3.109	0	208356_x_at	CSH1/CSH2	chorionic somatomammotropin hormone 1 (placental lactogen)
-3.026	0	202493_x_at	CSH1/CSH2	chorionic somatomammotropin hormone 1 (placental lactogen)
-3.007	0	208357_x_at	CSH1/CSH2	chorionic somatomammotropin hormone 1 (placental lactogen)
-2.966	0	208342_x_at	CSH1/CSH2	chorionic somatomammotropin hormone 1 (placental lactogen)
-2.952	0	208341_x_at	CSH1/CSH2	chorionic somatomammotropin hormone 1 (placental lactogen)
-3.969	0	222281_s_at	C1orf186	chromosome 1 open reading frame 186
-2.128	0	229099_at	C11orf83	chromosome 11 open reading frame 83
-1.969	0	224719_s_at	C12orf57	chromosome 12 open reading frame 57
1.521	0.999	223484_at	C15orf48	chromosome 15 open reading frame 48
-2.495	0	227378_x_at	C16orf13	chromosome 16 open reading frame 13
-1.907	0	229860_x_at	C4orf48	chromosome 4 open reading frame 48
2.54	0.999	225919_s_at	C9orf72	chromosome 9 open reading frame 72
-2.677	0	211043_s_at	CLTB	clathrin, light chain B
-2.439	0	206284_x_at	CLTB	clathrin, light chain B
-3.542	0	1554804_a_at	CLDN19	claudin 19
-3.217	0	1552535_at	CLDN19	claudin 19
-1.705	0	208792_s_at	CLU	clusterin
-2.849	0	212512_s_at	CARM1	coactivator-associated arginine methyltransferase 1
-2.063	0	224583_at	COTL1	coactosin-like F-actin binding protein 1
-2.518	0	222890_at	CCDC113	coiled-coil domain containing 113
1.69	0.999	226031_at	CCDC132	coiled-coil domain containing 132
-6.145	0	227091_at	CCDC146	coiled-coil domain containing 146
-2.029	0	1556499_s_at	COL1A1	collagen, type I, alpha 1
-1.654	0	201852_x_at	COL3A1	collagen, type III, alpha 1
-2.282	0	205159_at	CSF2RB	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)
-2.05	0	202953_at	C1QB	complement component 1, q subcomponent, B chain
1.651	0.999	203854_at	CFI	complement factor I
-2.736	0	223796_at	CNTNAP3	contactin associated protein-like 3
-2.893	0	1552301_a_at	CORO6	coronin 6
-1.702	0	223191_at	COX16	COX16 cytochrome c oxidase assembly homolog (S. cerevisiae)
-1.927	0	210835_s_at	CTBP2	C-terminal binding protein 2
1.535	0.999	209732_at	CLEC2B	C-type lectin domain family 2, member B
-2.891	0	205200_at	CLEC3B	C-type lectin domain family 3, member B
5.71	1	219947_at	CLEC4A	C-type lectin domain family 4, member A
18.243	0.999	221724_s_at	CLEC4A	C-type lectin domain family 4, member A
2.219	1	1555756_a_at	CLEC7A	C-type lectin domain family 7, member A
-2.342	0	221488_s_at	CUTA	cutA divalent cation tolerance homolog (E. coli)
1.935	0.999	211559_s_at	CCNG2	cyclin G2
1.987	0.999	202770_s_at	CCNG2	cyclin G2
2.648	1	1555411_a_at	CCNL1	cyclin L1
1.643	0.998	213743_at	CCNT2	cyclin T2
2.08	0.998	204645_at	CCNT2	cyclin T2
-1.614	0	212899_at	CDK19	cyclin-dependent kinase 19
-2.566	0	206595_at	CST6	cystatin E/M
-2.122	0	207030_s_at	CSRP2	cysteine and glycine-rich protein 2
-2.464	0	205081_at	CRIP1	cysteine-rich protein 1 (intestinal)
-2.038	0	212971_at	CARS	cysteinyl-tRNA synthetase
-2.186	0	205627_at	CDA	cytidine deaminase
-1.806	0	202263_at	CYB5R1	cytochrome b5 reductase 1
-1.725	0	218026_at	COA3	cytochrome c oxidase assembly factor 3
-1.696	0	202698_x_at	COX4I1	cytochrome c oxidase subunit IV isoform 1
-1.983	0	213735_s_at	COX5B	cytochrome c oxidase subunit Vb
-1.971	0	201441_at	COX6B1	cytochrome c oxidase subunit VIb polypeptide 1 (ubiquitous)
2.763	0.999	244546_at	CYCS	cytochrome c, somatic
-2.442	0	204309_at	CYP11A1	cytochrome P450, family 11, subfamily A, polypeptide 1
4.565	1	209606_at	CYTIP	cytohesin 1 interacting protein
-2.296	0	206315_at	CRLF1	cytokine receptor-like factor 1

1.482	1	226939_at	CPEB2	cytoplasmic polyadenylation element binding protein 2
1.951	0.998	224831_at	CPEB4	cytoplasmic polyadenylation element binding protein 4
-1.908	0	200999_s_at	CKAP4	cytoskeleton-associated protein 4
1.577	0.999	208896_at	DDX18	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18
1.817	0.999	218986_s_at	DDX60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60
1.952	1	225502_at	DOCK8	dedicator of cytokinesis 8
-9.574	0	210397_at	DEFB1	defensin, beta 1
-3.321	0	203699_s_at	DIO2	deiodinase, iodothyronine, type II
-2.159	0	212611_at	DTX4	deltex 4, E3 ubiquitin ligase
-2.134	0	213068_at	DPT	dermatopontin
1.641	0.997	226817_at	DSC2	desmocollin 2
1.961	1	206032_at	DSC3	desmocollin 3
2.859	1	206033_s_at	DSC3	desmocollin 3
2.114	1	212820_at	DMXL2	Dmx-like 2
-2.356	0	202500_at	DNAJB2	DnaJ (Hsp40) homolog, subfamily B, member 2
-1.908	0	208675_s_at	DDOST	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit (non-catalytic)
1.88	1	208892_s_at	DUSP6	dual specificity phosphatase 6
1.871	1	225885_at	EEA1	early endosome antigen 1
3.846	1	211742_s_at	EVI2B	ecotropic viral integration site 2B
-2.217	0	202942_at	ETFB	electron-transfer-flavoprotein, beta polypeptide
1.65	1	221773_at	ELK3	ELK3, ETS-domain protein (SRF accessory protein 2)
1.645	1	226099_at	ELL2	elongation factor, RNA polymerase II, 2
-4.038	0	204824_at	ENDOG	endonuclease G
-1.828	0	224576_at	ERGIC1	endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1
17.301	0.999	205767_at	EREG	epiregulin
1.846	1	227609_at	EPSTI1	epithelial stromal interaction 1 (breast)
-2.413	0	218180_s_at	EPS8L2	EPS8-like 2
-3.709	0	219424_at	EBI3	Epstein-Barr virus induced 3
1.642	1	203584_at	EMC2	ER membrane protein complex subunit 2
1.727	1	217941_s_at	ERBB2IP	erbb2 interacting protein
2.185	1	222473_s_at	ERBB2IP	erbb2 interacting protein
2.103	1	222646_s_at	ERO1L	ERO1-like (S. cerevisiae)
1.391	1	219017_at	ETNK1	ethanolamine kinase 1
-2.033	0	204034_at	ETHE1	ethylmalonic encephalopathy 1
-1.864	0	203113_s_at	EEF1D	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)
-1.893	0	211927_x_at	EEF1G	eukaryotic translation elongation factor 1 gamma
-1.891	0	211345_x_at	EEF1G	eukaryotic translation elongation factor 1 gamma
-1.824	0	200689_x_at	EEF1G	eukaryotic translation elongation factor 1 gamma
-2.5	0	215230_x_at	EIF3C	eukaryotic translation initiation factor 3, subunit C
-1.812	0	210949_s_at	EIF3C	eukaryotic translation initiation factor 3, subunit C
-2.067	0	208887_at	EIF3G	eukaryotic translation initiation factor 3, subunit G
-1.88	0	217719_at	EIF3L	eukaryotic translation initiation factor 3, subunit L
-2.57	0	221539_at	EIF4EBP1	eukaryotic translation initiation factor 4E binding protein 1
-1.602	0	213571_s_at	EIF4E2	eukaryotic translation initiation factor 4E family member 2
-1.796	0	202013_s_at	EXT2	exostosin glycosyltransferase 2
1.972	1	235927_at	XPO1	exportin 1
-2.18	0	208622_s_at	EZR	ezrin
-1.878	0	223000_s_at	F11R	F11 receptor
1.366	0.999	212979_s_at	FAM115A	family with sequence similarity 115, member A
1.516	0.999	229460_at	FAM126B	family with sequence similarity 126, member B
2.094	0.999	1554178_a_at	FAM126B	family with sequence similarity 126, member B
-1.866	0	221984_s_at	FAM134A	family with sequence similarity 134, member A
-1.728	0	203262_s_at	FAM50A	family with sequence similarity 50, member A
2.677	1	209829_at	FAM65B	family with sequence similarity 65, member B
-1.73	0	208647_at	FDFT1	farnesyl-diphosphate farnesyltransferase 1
1.913	1	204780_s_at	FAS	Fas cell surface death receptor
2.986	0.999	215719_x_at	FAS	Fas cell surface death receptor
-4.218	0	201579_at	FAT1	FAT atypical cadherin 1
1.832	1	241763_s_at	FBXO32	F-box protein 32
1.665	0.999	204232_at	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide

2.514	1	1554899_s_at	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide
5.722	0.999	204007_at	FCGR3A/ FCGR3B	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)
11.9	1	204006_s_at	FCGR3A/ FCGR3B	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)
2.079	0.998	213341_at	FEM1C	fem-1 homolog c (C. elegans)
1.731	0.998	225464_at	FRMD6	FERM domain containing 6
2.023	1	225481_at	FRMD6	FERM domain containing 6
2.244	1	223263_s_at	FGFR1OP2	FGFR1 oncogene partner 2
5.878	0.998	205782_at	FGF7	fibroblast growth factor 7
2.292	1	203638_s_at	FGFR2	fibroblast growth factor receptor 2
3.195	0.997	208228_s_at	FGFR2	fibroblast growth factor receptor 2
-2.053	0	202994_s_at	FBLN1	fibulin 1
-1.792	0	200019_s_at	FAU	Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed
-2.116	0	218034_at	FIS1	fission 1 (mitochondrial outer membrane) homolog (S. cerevisiae)
-4.336	0	204437_s_at	FOLR1	folate receptor 1 (adult)
-1.76	0	208782_at	FSTL1	follistatin-like 1
-2.47	0	203592_s_at	FSTL3	follistatin-like 3 (secreted glycoprotein)
2.41	0.999	205119_s_at	FPR1	formyl peptide receptor 1
53.282	0.999	210772_at	FPR2	formyl peptide receptor 2
-1.956	0	224252_s_at	FXYD5	FXYD domain containing ion transport regulator 5
-1.852	0	218084_x_at	FXYD5	FXYD domain containing ion transport regulator 5
6.202	1	213524_s_at	G0S2	G0/G1 switch 2
1.937	0.998	213142_x_at	GSAP	gamma-secretase activating protein
2.15	1	222150_s_at	GSAP	gamma-secretase activating protein
2.098	1	226269_at	GDAP1	ganglioside induced differentiation associated protein 1
-1.82	0	201338_x_at	GTF3A	general transcription factor IIIA
-2.298	0	1554486_a_at	GFOD1	glucose-fructose oxidoreductase domain containing 1
-2.228	0	202812_at	GAA	glucosidase, alpha; acid
-1.619	0	202605_at	GUSB	glucuronidase, beta
2.377	1	204844_at	ENPEP	glutamyl aminopeptidase (aminopeptidase A)
-2.735	0	201348_at	GPX3	glutathione peroxidase 3 (plasma)
-2.594	0	214091_s_at	GPX3	glutathione peroxidase 3 (plasma)
-2.358	0	203924_at	GSTA1	glutathione S-transferase alpha 1
2.014	1	224826_at	GPCPD1	glycerophosphocholine phosphodiesterase GDE1 homolog (S. cerevisiae)
3.042	0.998	230492_s_at	GPCPD1	glycerophosphocholine phosphodiesterase GDE1 homolog (S. cerevisiae)
-3.153	0	219722_s_at	GDPD3	glycerophosphodiester phosphodiesterase domain containing 3
-2.24	0	212737_at	GM2A	GM2 ganglioside activator
-2.141	0	35820_at	GM2A	GM2 ganglioside activator
2.841	1	203765_at	GCA	grancalcin, EF-hand calcium binding protein
2.069	1	203725_at	GADD45A	growth arrest and DNA-damage-inducible, alpha
-2.828	0	31874_at	GAS2L1	growth arrest-specific 2 like 1
-2.79	0	202191_s_at	GAS7	growth arrest-specific 7
-1.76	0	221577_x_at	GDF15	growth differentiation factor 15
3.419	1	204472_at	GEM	GTP binding protein overexpressed in skeletal muscle
1.988	0.998	227539_at	GNA13	guanine nucleotide binding protein (G protein), alpha 13
1.653	0.999	227692_at	GNAI1	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1
2.428	0.999	224964_s_at	GNG2	guanine nucleotide binding protein (G protein), gamma 2
2.373	0.998	231578_at	GBP1	guanylate binding protein 1, interferon-inducible
3.825	1	202269_x_at	GBP1	guanylate binding protein 1, interferon-inducible
4.384	1	202270_at	GBP1	guanylate binding protein 1, interferon-inducible
4.682	1	231577_s_at	GBP1	guanylate binding protein 1, interferon-inducible
2.526	1	242907_at	GBP2	guanylate binding protein 2, interferon-inducible
2.212	1	223434_at	GBP3	guanylate binding protein 3
-1.948	0	224646_x_at	H19	H19, imprinted maternally expressed transcript (non-protein coding)
-1.584	0	224997_x_at	H19	H19, imprinted maternally expressed transcript (non-protein coding)

1.681	0.999	200806_s_at	HSPD1	heat shock 60kDa protein 1 (chaperonin)
-2.583	0	217755_at	HN1	hematological and neurological expressed 1
-2.44	0	222396_at	HN1	hematological and neurological expressed 1
-1.912	0	203665_at	HMOX1	heme oxygenase (decycling) 1
-1.625	0	209116_x_at	HBB	hemoglobin, beta
-2.42	0	204848_x_at	HBG1	hemoglobin, gamma A
-2.484	0	204419_x_at	HBG2	hemoglobin, gamma G
2.791	0.999	227361_at	HS3ST3B1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1
-5.205	0	231928_at	HES2	hes family bHLH transcription factor 2
1.952	0.999	225107_at	HNRNPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1
1.629	0.999	1554678_s_at	HNRNPDL	heterogeneous nuclear ribonucleoprotein D-like
-2.324	0	201944_at	HEXB	hexosaminidase B (beta polypeptide)
2.216	1	208808_s_at	HMGB2	high mobility group box 2
-2.039	0	225601_at	HMGB3	high mobility group box 3
-1.82	0	209377_s_at	HMGN3	high mobility group nucleosomal binding domain 3
-1.621	0	209806_at	HIST1H2BK	histone cluster 1, H2bk
1.482	0.997	218280_x_at	HIST2H2AA3/ HIST2H2AA4	histone cluster 2, H2aa3
-1.755	0	217168_s_at	HERPUD1	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1
-2.614	0	201185_at	HTRA1	HtrA serine peptidase 1
1.71	1	205220_at	HCAR3	hydroxycarboxylic acid receptor 3
-2.065	0	211549_s_at	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)
-1.944	0	203914_x_at	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)
-1.872	0	211548_s_at	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)
-2.236	0	210624_s_at	ILVBL	ilvB (bacterial acetolactate synthase)-like
2.969	1	201631_s_at	IER3	immediate early response 3
-3.375	0	211430_s_at	IGHG1	immunoglobulin heavy constant gamma 1 (G1m marker)
165.093	0.997	215176_x_at	IGKC	immunoglobulin kappa constant
4.641	1	214677_x_at	IGLC1	immunoglobulin lambda constant 1 (Mcg marker)
6.649	0.999	210029_at	IDO1	indoleamine 2,3-dioxygenase 1
1.553	0.997	201362_at	IVNS1ABP	influenza virus NS1A binding protein
2.075	1	201363_s_at	IVNS1ABP	influenza virus NS1A binding protein
2.704	1	206245_s_at	IVNS1ABP	influenza virus NS1A binding protein
1.745	0.999	210511_s_at	INHBA	inhibin, beta A
1.876	0.999	227140_at	INHBA	inhibin, beta A
-1.79	0	227792_at	ITPRIPL2	inositol 1,4,5-trisphosphate receptor interacting protein-like 2
1.865	1	209566_at	INSIG2	insulin induced gene 2
-1.948	0	53968_at	INTS5	integrator complex subunit 5
5.823	1	227314_at	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
2.572	1	226535_at	ITGB6	integrin, beta 6
28.196	0.999	208083_s_at	ITGB6	integrin, beta 6
-2.74	0	219064_at	ITIH5	inter-alpha-trypsin inhibitor heavy chain family, member 5
8.718	0.998	202637_s_at	ICAM1	intercellular adhesion molecule 1
1.856	1	211676_s_at	IFNGR1	interferon gamma receptor 1
-2.165	0	223474_at	IRF2BPL	interferon regulatory factor 2 binding protein-like
2.383	1	208966_x_at	IFI16	interferon, gamma-inducible protein 16
2.391	1	206332_s_at	IFI16	interferon, gamma-inducible protein 16
1.951	1	214453_s_at	IFI44	interferon-induced protein 44
1.684	0.998	204439_at	IFI44L	interferon-induced protein 44-like
2.342	0.999	203153_at	IFIT1	interferon-induced protein with tetratricopeptide repeats 1
4.465	0.999	226757_at	IFIT2	interferon-induced protein with tetratricopeptide repeats 2
2.089	1	229450_at	IFIT3	interferon-induced protein with tetratricopeptide repeats 3
2.323	0.997	204747_at	IFIT3	interferon-induced protein with tetratricopeptide repeats 3
28.353	1	212657_s_at	IL1RN	interleukin 1 receptor antagonist
18.703	1	210118_s_at	IL1A	interleukin 1, alpha
5.491	1	206172_at	IL13RA2	interleukin 13 receptor, alpha 2
-2.516	0	205291_at	IL2RB	interleukin 2 receptor, beta
19.56	1	205207_at	IL6	interleukin 6
3.086	1	213817_at	IRAK3	interleukin-1 receptor-associated kinase 3
-2.11	0	210046_s_at	IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial
-1.905	0	223104_at	JAGN1	jagunal homolog 1 (Drosophila)

-1.782	0	200048_s_at	JTB	jumping translocation breakpoint
-1.646	0	203752_s_at	JUND	jun D proto-oncogene
-3.222	0	219213_at	JAM2	junctional adhesion molecule 2
-2.465	0	229127_at	JAM2	junctional adhesion molecule 2
-1.836	0	200700_s_at	KDELR2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2
1.412	0.999	221986_s_at	KLHL24	kelch-like family member 24
-1.776	0	201596_x_at	KRT18	keratin 18
-1.932	0	201650_at	KRT19	keratin 19
1.697	0.999	209125_at	KRT6A	keratin 6A
-1.898	0	209008_x_at	KRT8	keratin 8
-2.358	0	217118_s_at	KIAA0930	KIAA0930
1.576	1	212794_s_at	KIAA1033	KIAA1033
1.652	0.998	212795_at	KIAA1033	KIAA1033
2.847	1	215936_s_at	KIAA1033	KIAA1033
1.847	1	227152_at	KIAA1551	KIAA1551
2.062	0.999	218614_at	KIAA1551	KIAA1551
-1.903	0	226003_at	KIF21A	kinesin family member 21A
1.765	1	34031_i_at	KRIT1	KRIT1, ankyrin repeat containing
1.673	1	224606_at	KLF6	Kruppel-like factor 6
1.955	1	228937_at	LACC1	laccase (multicopper oxidoreductase) domain containing 1
11.691	0.999	203276_at	LMNB1	lamin B1
1.581	0.997	234608_at	LAMA3	laminin, alpha 3
-2.158	0	216264_s_at	LAMB2	laminin, beta 2 (laminin S)
-1.703	0	202729_s_at	LTBP1	latent transforming growth factor beta binding protein 1
-2.094	0	220158_at	LGALS14	lectin, galactoside-binding, soluble, 14
-2.407	0	213909_at	LRRC15	leucine rich repeat containing 15
1.486	1	218577_at	LRRC40	leucine rich repeat containing 40
-2.743	0	1552546_a_at	LETM2	leucine zipper-EF-hand containing transmembrane protein 2
3.271	0.999	215838_at	LILRA5	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5
3.544	0.999	242767_at	LMCD1	LIM and cysteine-rich domains 1
1.891	1	212687_at	LIMS1	LIM and senescent cell antigen-like domains 1
2.073	0.999	1570259_at	LIMS1	LIM and senescent cell antigen-like domains 1
-2.836	0	220765_s_at	LIMS2	LIM and senescent cell antigen-like domains 2
-2.552	0	209204_at	LMO4	LIM domain only 4
-1.745	0	229349_at	LIN28B	lin-28 homolog B (C. elegans)
7.34	1	1559573_at	LINC01093	long intergenic non-protein coding RNA 1093
2.608	0.999	205668_at	LY75	lymphocyte antigen 75
5.801	1	205269_at	LCP2	lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)
1.362	0.998	226100_at	KMT2E	lysine (K)-specific methyltransferase 2E
3.51	0.999	1555745_a_at	LYZ	lysozyme
-1.913	0	228763_at	MDP1	magnesium-dependent phosphatase 1
1.75	0.999	222805_at	MANEA	mannosidase, endo-alpha
-1.777	0	201126_s_at	MGAT1	mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
-2.613	0	226039_at	MGAT4A	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme A
-1.904	0	212098_at	MGAT5	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase
4.492	1	204475_at	MMP1	matrix metallopeptidase 1 (interstitial collagenase)
6.175	1	205680_at	MMP10	matrix metallopeptidase 10 (stromelysin 2)
3.083	1	204580_at	MMP12	matrix metallopeptidase 12 (macrophage elastase)
1.992	1	228846_at	MXD1	MAX dimerization protein 1
1.759	1	213761_at	MDM1	Mdm1 nuclear protein homolog (mouse)
-2.997	0	43544_at	MED16	mediator complex subunit 16
-2.524	0	1553993_s_at	MED25	mediator complex subunit 25
-2.584	0	223347_at	MUM1	melanoma associated antigen (mutated) 1
1.746	1	229160_at	MUM1L1	melanoma associated antigen (mutated) 1-like 1
-2.567	0	209087_x_at	MCAM	melanoma cell adhesion molecule
-2.271	0	210869_s_at	MCAM	melanoma cell adhesion molecule
1.442	0.999	203434_s_at	MME	membrane metallo-endopeptidase

1.902	1	226675_s_at	MALAT1	metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)
2.056	1	224568_x_at	MALAT1	metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)
2.094	0.998	223940_x_at	MALAT1	metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)
-1.897	0	218773_s_at	MSRB2	methionine sulfoxide reductase B2
1.523	0.999	209861_s_at	METAP2	methionyl aminopeptidase 2
-3.048	0	41160_at	MBD3	methyl-CpG binding domain protein 3
-2.892	0	91816_f_at	MEX3D	mex-3 RNA binding family member D
-1.959	0	55081_at	MICALL1	MICAL-like 1
-2.482	0	209758_s_at	MFAP5	microfibrillar associated protein 5
-2.268	0	201403_s_at	MGST3	microsomal glutathione S-transferase 3
-1.864	0	212566_at	MAP4	microtubule-associated protein 4
-1.698	0	218027_at	MRPL15	mitochondrial ribosomal protein L15
-1.798	0	204386_s_at	MRPL57	mitochondrial ribosomal protein L57
-2.375	0	218385_at	MRPS18A	mitochondrial ribosomal protein S18A
-2.059	0	224948_at	MRPS24	mitochondrial ribosomal protein S24
-1.707	0	224919_at	MRPS6	mitochondrial ribosomal protein S6
1.662	0.998	218311_at	MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3
-1.626	0	211026_s_at	MGLL	monoglyceride lipase
2.4	1	201151_s_at	MBNL1	muscleblind-like splicing regulator 1
1.959	1	200798_x_at	MCL1	myeloid cell leukemia 1
4.005	1	204959_at	MNDA	myeloid cell nuclear differentiation antigen
2.022	1	212364_at	MYO1B	myosin IB
1.968	1	224823_at	MYLK	myosin light chain kinase
1.541	0.998	225897_at	MARCKS	myristoylated alanine-rich protein kinase C substrate
-1.927	0	224512_s_at	NAA38	N(alpha)-acetyltransferase 38, NatC auxiliary subunit
-2.176	0	220864_s_at	NDUFA13	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13
-1.779	0	209224_s_at	NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa
-1.732	0	218200_s_at	NDUFB2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa
-1.86	0	201227_s_at	NDUFB8	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19kDa
-1.652	0	202077_at	NDUFAB1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1, 8kDa
-1.611	0	209177_at	NDUFAF3	NADH dehydrogenase (ubiquinone) complex I, assembly factor 3
-2.322	0	203190_at	NDUFS8	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)
-2.049	0	37005_at	MINOS1-NBL1/NBL1	neuroblastoma 1, DAN family BMP antagonist
2.948	1	210387_at	NCALD	neurocalcin delta
-3.451	0	243110_x_at	NPW	neuropeptide W
2.316	0.998	212298_at	NRP1	neuropilin 1
1.819	1	202907_s_at	NBN	nibrin
6.544	1	217738_at	NAMPT	nicotinamide phosphoribosyltransferase
6.648	1	243296_at	NAMPT	nicotinamide phosphoribosyltransferase
8.289	1	217739_s_at	NAMPT	nicotinamide phosphoribosyltransferase
19.727	1	1555167_s_at	NAMPT	nicotinamide phosphoribosyltransferase
-2.664	0	217950_at	NOSIP	nitric oxide synthase interacting protein
3.598	0.997	204860_s_at	NAIP	NLR family, apoptosis inhibitory protein
-2.323	0	223206_s_at	NMRAL1	NmrA-like family domain containing 1
-2.186	0	224666_at	NSMCE1	non-SMC element 1 homolog (S. cerevisiae)
-2.288	0	228649_at	NOTUM	notum pectinacetylesterase homolog (Drosophila)
-1.669	0	220248_x_at	NSFL1C	NSFL1 (p97) cofactor (p47)
-2.772	0	204589_at	NUAK1	NUAK family, SNF1-like kinase, 1
2.048	1	201502_s_at	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
1.966	1	223218_s_at	NFKBIZ	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
2.91	1	223217_s_at	NFKBIZ	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
1.761	1	225344_at	NCOA7	nuclear receptor coactivator 7
-1.652	0	202397_at	NUTF2	nuclear transport factor 2
1.944	1	228062_at	NAP1L5	nucleosome assembly protein 1-like 5

-2.373	0	213946_s_at	OBSL1	obscurin-like 1
-1.864	0	212776_s_at	OBSL1	obscurin-like 1
31.393	0.997	230170_at	OSM	oncostatin M
2.14	1	226810_at	OGFRL1	opioid growth factor receptor-like 1
1.586	1	202073_at	OPTN	optineurin
1.921	0.999	205908_s_at	OMD	osteomodulin
2.148	1	210004_at	OLR1	oxidized low density lipoprotein (lectin-like) receptor 1
1.5	1	212585_at	OSBPL8	oxysterol binding protein-like 8
1.555	1	212582_at	OSBPL8	oxysterol binding protein-like 8
2.018	0.998	226695_at	PRRX1	paired related homeobox 1
1.601	0.997	225761_at	PAPD4	PAP associated domain containing 4
-2.204	0	213332_at	PAPPA2	pappalysin 2
-1.538	0	200006_at	PARK7	parkinson protein 7
1.493	0.997	223310_x_at	PNPLA8	patatin-like phospholipase domain containing 8
2.12	1	209243_s_at	PEG3	paternally expressed 3
-2.207	0	219630_at	PDZK1IP1	PDZK1 interacting protein 1
1.777	0.998	218319_at	PELI1	pellino E3 ubiquitin protein ligase 1
4.824	0.999	232304_at	PELI1	pellino E3 ubiquitin protein ligase 1
34.497	1	206157_at	PTX3	pentraxin 3, long
12.089	1	229947_at	PI15	peptidase inhibitor 15
-1.889	0	226336_at	PPIA	peptidylprolyl isomerase A (cyclophilin A)
1.385	0.997	209122_at	PLIN2	perilipin 2
-1.69	0	208680_at	PRDX1	peroxiredoxin 1
-1.919	0	1560587_s_at	PRDX5	peroxiredoxin 5
-2.217	0	200844_s_at	PRDX6	peroxiredoxin 6
4.033	0.999	204285_s_at	PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1
27.33	0.999	213638_at	PHACTR1	phosphatase and actin regulator 1
8.056	0.999	242277_at	PHACTR2	phosphatase and actin regulator 2
1.788	1	241905_at	PIK3C2A	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha
-1.851	0	201968_s_at	PGM1	phosphoglucomutase 1
2.269	0.998	226459_at	PIK3AP1	phosphoinositide-3-kinase adaptor protein 1
3.027	1	210145_at	PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)
3.336	0.998	225626_at	PAG1	phosphoprotein membrane anchor with glycosphingolipid microdomains 1
-1.964	0	202529_at	PRPSAP1	phosphoribosyl pyrophosphate synthetase-associated protein 1
-11.201	0	223062_s_at	PSAT1	phosphoserine aminotransferase 1
-1.624	0	203335_at	PHYH	phytanoyl-CoA 2-hydroxylase
-3.662	0	221605_s_at	PIPOX	pipecolic acid oxidase
-2.64	0	219014_at	PLAC8	placenta-specific 8
5.255	0.999	205479_s_at	PLAU	plasminogen activator, urokinase
4.338	1	203471_s_at	PLEK	pleckstrin
1.493	0.998	212542_s_at	PHIP	pleckstrin homology domain interacting protein
4.944	1	217997_at	PHLDA1	pleckstrin homology-like domain, family A, member 1
6.813	1	217996_at	PHLDA1	pleckstrin homology-like domain, family A, member 1
8.184	1	225842_at	PHLDA1	pleckstrin homology-like domain, family A, member 1
-1.984	0	209466_x_at	PTN	pleiotrophin
-1.963	0	211737_x_at	PTN	pleiotrophin
-2.123	0	201939_at	PLK2	polo-like kinase 2
-1.813	0	214239_x_at	PCGF2	polycomb group ring finger 2
1.33	0.997	226508_at	PHC3	polyhomeotic homolog 3 (Drosophila)
-2.498	0	212955_s_at	POLR2I	polymerase (RNA) II (DNA directed) polypeptide I, 14.5kDa
-2.26	0	219956_at	GALNT6	polypeptide N-acetylgalactosaminyltransferase 6
-1.907	0	224617_at	PTBP3	polypyrimidine tract binding protein 3
1.831	0.998	225291_at	PNPT1	polyribonucleotide nucleotidyltransferase 1
-2.127	0	207132_x_at	PFDN5	prefoldin subunit 5
-2.004	0	210908_s_at	PFDN5	prefoldin subunit 5
-2.15	0	204830_x_at	PSG5	pregnancy specific beta-1-glycoprotein 5
-2.537	0	201982_s_at	PAPPA	pregnancy-associated plasma protein A, pappalysin 1
-1.713	0	1559400_s_at	PAPPA	pregnancy-associated plasma protein A, pappalysin 1
1.756	0.999	220553_s_at	PRPF39	pre-mRNA processing factor 39
2.531	0.998	211090_s_at	PRPF4B	pre-mRNA processing factor 4B
-5.454	0	206859_s_at	PAEP	progestagen-associated endometrial protein

-2.404	0	1569110_x_at	LOC728613	programmed cell death 6 pseudogene
-1.82	0	229124_at	PROK1	prokineticin 1
1.458	0.999	206346_at	PRLR	prolactin receptor
-2.458	0	227325_at	PRR24	proline rich 24
-2.48	0	238513_at	PRRG4	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)
-4.861	0	203860_at	PCCA	propionyl CoA carboxylase, alpha polypeptide
2.075	1	204897_at	PTGER4	prostaglandin E receptor 4 (subtype EP4)
2.43	1	1554997_a_at	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
3.013	1	204748_at	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
-2.173	0	212032_s_at	PTOV1	prostate tumor overexpressed 1
-2.444	0	202525_at	PRSS8	protease, serine, 8
-1.569	0	214288_s_at	PSMB1	proteasome (prosome, macropain) subunit, beta type, 1
-1.78	0	201400_at	PSMB3	proteasome (prosome, macropain) subunit, beta type, 3
-1.676	0	208799_at	PSMB5	proteasome (prosome, macropain) subunit, beta type, 5
-1.698	0	200786_at	PSMB7	proteasome (prosome, macropain) subunit, beta type, 7
-2.491	0	218292_s_at	PRKAG2	protein kinase, AMP-activated, gamma 2 non-catalytic subunit
1.305	0.999	228222_at	PPP1CB	protein phosphatase 1, catalytic subunit, beta isozyme
1.35	0.999	222467_s_at	PPP6R3	protein phosphatase 6, regulatory subunit 3
2.744	1	200730_s_at	PTP4A1	protein tyrosine phosphatase type IVA, member 1
2.373	1	212587_s_at	PTPRC	protein tyrosine phosphatase, receptor type, C
3.48	1	212588_at	PTPRC	protein tyrosine phosphatase, receptor type, C
-2.059	0	200635_s_at	PTPRF	protein tyrosine phosphatase, receptor type, F
-1.968	0	244050_at	PTPLAD2	protein tyrosine phosphatase-like A domain containing 2
-2.099	0	211743_s_at	PRG2	proteoglycan 2, bone marrow (natural killer cell activator, eosinophil granule major basic protein)
2.592	0.999	225975_at	PCDH18	protocadherin 18
3.111	1	228640_at	PCDH7	protocadherin 7
-2.503	0	242414_at	QPRT	quinolinate phosphoribosyltransferase
-2.129	0	203136_at	RABAC1	Rab acceptor 1 (prenylated)
-2.179	0	227698_s_at	RAB40C	RAB40C, member RAS oncogene family
2.044	1	222846_at	RAB8B	RAB8B, member RAS oncogene family
2.141	1	242625_at	RSAD2	radical S-adenosyl methionine domain containing 2
-2.814	0	227425_at	REPS2	RALBP1 associated Eps domain containing 2
-1.981	0	221827_at	RBCK1	RanBP-type and C3HC4-type zinc finger containing 1
1.717	0.999	1553186_x_at	RASEF	RAS and EF-hand domain containing
1.751	1	1553185_at	RASEF	RAS and EF-hand domain containing
-3.341	0	209885_at	RHOD	ras homolog family member D
38.921	0.998	204951_at	RHOH	ras homolog family member H
1.816	0.998	212122_at	RHOQ	ras homolog family member Q
-2.442	0	228109_at	RASGRF2	Ras protein-specific guanine nucleotide-releasing factor 2
1.839	0.998	243463_s_at	RIT1	Ras-like without CAAX 1
-1.992	0	224972_at	ROMO1	reactive oxygen species modulator 1
-2.007	0	204916_at	RAMP1	receptor (G protein-coupled) activity modifying protein 1
1.647	1	218352_at	RCBTB1	regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1
4.144	1	202988_s_at	RGS1	regulator of G-protein signaling 1
-2.467	0	34408_at	RTN2	reticulon 2
-1.804	0	203423_at	RBP1	retinol binding protein 1, cellular
1.818	0.999	212724_at	RND3	Rho family GTPase 3
-1.726	0	201785_at	RNASE1	ribonuclease, RNase A family, 1 (pancreatic)
-1.839	0	213399_x_at	RPN2	ribophorin II
-1.778	0	229563_s_at	RPL10A	ribosomal protein L10a
-1.898	0	208929_x_at	RPL13	ribosomal protein L13
-1.835	0	212734_x_at	RPL13	ribosomal protein L13
-1.752	0	212191_x_at	RPL13	ribosomal protein L13
-1.794	0	200716_x_at	RPL13A	ribosomal protein L13a
-1.78	0	212790_x_at	RPL13A	ribosomal protein L13a
-1.779	0	210646_x_at	RPL13A	ribosomal protein L13a
-1.607	0	200869_at	RPL18A	ribosomal protein L18a
-1.763	0	200823_x_at	RPL29	ribosomal protein L29
-1.843	0	201217_x_at	RPL3	ribosomal protein L3

-1.824	0	211073_x_at	RPL3	ribosomal protein L3
-1.647	0	225190_x_at	RPL35A	ribosomal protein L35a
-1.684	0	200717_x_at	RPL7	ribosomal protein L7
-1.712	0	200936_at	RPL8	ribosomal protein L8
-1.823	0	211542_x_at	RPS10	ribosomal protein S10
-1.785	0	200817_x_at	RPS10	ribosomal protein S10
-1.751	0	214003_x_at	RPS20	ribosomal protein S20
-1.669	0	200834_s_at	RPS21	ribosomal protein S21
2.11	0.999	236621_at	RPS27	ribosomal protein S27
-1.762	0	200933_x_at	RPS4X	ribosomal protein S4, X-linked
-1.845	0	213801_x_at	RPSA	ribosomal protein SA
-1.774	0	214167_s_at	RPLP0	ribosomal protein, large, P0
1.686	1	201845_s_at	RYBP	RING1 and YY1 binding protein
2.086	0.999	201846_s_at	RYBP	RING1 and YY1 binding protein
-1.669	0	1559946_s_at	RUVBL2	RuvB-like AAA ATPase 2
7.94	1	205863_at	S100A12	S100 calcium binding protein A12
-2.064	0	202598_at	S100A13	S100 calcium binding protein A13
5.062	1	202917_s_at	S100A8	S100 calcium binding protein A8
3.174	1	203535_at	S100A9	S100 calcium binding protein A9
-2.076	0	204351_at	S100P	S100 calcium binding protein P
4.866	1	220330_s_at	SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1
6.067	0.999	1555638_a_at	SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1
-2.484	0	236520_at	SDCBP2-AS1	SDCBP2 antisense RNA 1
-3.838	0	203021_at	SLPI	secretory leukocyte peptidase inhibitor
6.857	1	204563_at	SELL	selectin L
-2.033	0	226051_at	SELM	selenoprotein M
2.004	1	203789_s_at	SEMA3C	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C
2.217	0.998	201859_at	SRGN	serglycin
3.841	1	201858_s_at	SRGN	serglycin
3.95	0.997	1554676_at	SRGN	serglycin
-1.984	0	210715_s_at	SPINT2	serine peptidase inhibitor, Kunitz type, 2
1.659	0.999	204299_at	SRSF10	serine/arginine-rich splicing factor 10
-1.594	0	208804_s_at	SRSF6	serine/arginine-rich splicing factor 6
-1.474	0	208855_s_at	STK24	serine/threonine kinase 24
2.46	0.997	223746_at	STK4	serine/threonine kinase 4
7.044	1	202833_s_at	SERPINA1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
-2.346	0	202376_at	SERPINA3	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
92.99	0.998	231628_s_at	SERPINB6	serpin peptidase inhibitor, clade B (ovalbumin), member 6
-2.292	0	200802_at	SARS	seryl-tRNA synthetase
-2.515	0	222258_s_at	SH3BP4	SH3-domain binding protein 4
-1.618	0	214853_s_at	SHC1	SHC (Src homology 2 domain containing) transforming protein 1
-3.253	0	228400_at	SHROOM3	shroom family member 3
-1.932	0	201004_at	SSR4	signal sequence receptor, delta
-2.274	0	206118_at	STAT4	signal transducer and activator of transcription 4
1.829	0.999	201139_s_at	SSB	Sjogren syndrome antigen B (autoantigen La)
-1.72	0	225534_at	SMIM19	small integral membrane protein 19
-1.97	0	219097_x_at	SMIM7	small integral membrane protein 7
-2.301	0	218493_at	SNRNP25	small nuclear ribonucleoprotein 25kDa (U11/U12)
-1.805	0	200826_at	SNRPD2	small nuclear ribonucleoprotein D2 polypeptide 16.5kDa
-2.275	0	225155_at	SNHG5	small nucleolar RNA host gene 5 (non-protein coding)
2.106	1	225491_at	SLC1A2	solute carrier family 1 (glial high affinity glutamate transporter), member 2
-2.196	0	1554593_s_at	SLC1A6	solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6
-2.164	0	1554592_a_at	SLC1A6	solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6
2.652	1	230748_at	SLC16A6	solute carrier family 16, member 6
3.298	0.998	207038_at	SLC16A6	solute carrier family 16, member 6
-1.836	0	223441_at	SLC17A5	solute carrier family 17 (acidic sugar transporter), member 5
1.953	1	209681_at	SLC19A2	solute carrier family 19 (thiamine transporter), member 2

1.883	0.997	216236_s_at	SLC2A14	solute carrier family 2 (facilitated glucose transporter), member 14
1.571	0.998	201920_at	SLC20A1	solute carrier family 20 (phosphate transporter), member 1
4.62	0.998	230494_at	SLC20A1	solute carrier family 20 (phosphate transporter), member 1
1.942	0.998	222528_s_at	SLC25A37	solute carrier family 25 (mitochondrial iron transporter), member 37
-3.338	0	226728_at	SLC27A1	solute carrier family 27 (fatty acid transporter), member 1
1.33	0.997	224595_at	SLC44A1	solute carrier family 44 (choline transporter), member 1
-3.072	0	217621_at	SLC6A2	solute carrier family 6 (neurotransmitter transporter), member 2
-3.467	0	212295_s_at	SLC7A1	solute carrier family 7 (cationic amino acid transporter, y+ system), member 1
2.488	1	218404_at	SNX10	sorting nexin 10
-2.107	0	1553960_at	SNX21	sorting nexin family member 21
1.675	0.999	200795_at	SPARCL1	SPARC-like 1 (hevin)
-2.714	0	215235_at	SPTAN1	spectrin, alpha, non-erythrocytic 1
1.709	1	212470_at	SPAG9	sperm associated antigen 9
-1.875	0	221268_s_at	SGPP1	sphingosine-1-phosphate phosphatase 1
1.876	0.999	228176_at	S1PR3	sphingosine-1-phosphate receptor 3
3.387	0.998	204361_s_at	SKAP2	src kinase associated phosphoprotein 2
4.713	0.998	1570507_at	SCAF11	SR-related CTD-associated factor 11
1.461	0.999	227607_at	STAMBPL1	STAM binding protein-like 1
1.893	0.998	230746_s_at	STC1	stanniocalcin 1
1.9	0.999	225987_at	STEAP4	STEAP family member 4
1.802	1	226603_at	SAMD9L	sterile alpha motif domain containing 9-like
1.973	0.999	230036_at	SAMD9L	sterile alpha motif domain containing 9-like
-1.933	0	233049_x_at	STUB1	STIP1 homology and U-box containing protein 1, E3 ubiquitin protein ligase
1.899	1	213413_at	STON1	stonin 1
-2.049	0	33323_r_at	SFN	stratifin
-2.01	0	33322_i_at	SFN	stratifin
2.098	0.999	209257_s_at	SMC3	structural maintenance of chromosomes 3
4.58	1	215223_s_at	SOD2	superoxide dismutase 2, mitochondrial
5.22	0.999	216841_s_at	SOD2	superoxide dismutase 2, mitochondrial
22.609	1	215078_at	SOD2	superoxide dismutase 2, mitochondrial
1.762	1	227697_at	SOCS3	suppressor of cytokine signaling 3
-2.34	0	44702_at	SYDE1	synapse defective 1, Rho GTPase, homolog 1 (C. elegans)
-8.765	0	219992_at	TAC3	tachykinin 3
1.715	1	227685_at	TMF1	TATA element modulatory factor 1
4.83	1	229723_at	TAGAP	T-cell activation RhoGTPase activating protein
5.678	0.998	209813_x_at	TARP	TCR gamma alternate reading frame protein
-1.93	0	217853_at	TNS3	tensin 3
1.966	0.999	1554029_a_at	TTC37	tetratricopeptide repeat domain 37
1.713	1	225308_s_at	TANC1	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1
2.112	1	201110_s_at	THBS1	thrombospondin 1
2.87	0.998	201109_s_at	THBS1	thrombospondin 1
-2.295	0	336_at	TBXA2R	thromboxane A2 receptor
2.047	0.997	210785_s_at	THEMIS2	thymocyte selection associated family member 2
-3.398	0	229385_s_at	TINCR	tissue differentiation-inducing non-protein coding RNA
3.034	0.999	210176_at	TLR1	toll-like receptor 1
5.089	1	204924_at	TLR2	toll-like receptor 2
3.677	1	232068_s_at	TLR4	toll-like receptor 4
-1.702	0	209917_s_at	TP53TG1	TP53 target 1 (non-protein coding)
-2.794	0	226388_at	TCEA3	transcription elongation factor A (SII), 3
-2.017	0	236094_at	TCF7L2	transcription factor 7-like 2 (T-cell specific, HMG-box)
-3.238	0	222219_s_at	TLE6	transducin-like enhancer of split 6
-2.857	0	209909_s_at	TGFB2	transforming growth factor, beta 2
-2.381	0	208837_at	TMED3	transmembrane emp24 protein transport domain containing 3
-1.792	0	205812_s_at	TMED9	transmembrane emp24 protein transport domain containing 9
1.867	1	211967_at	TMEM123	transmembrane protein 123
-1.963	0	224981_at	TMEM219	transmembrane protein 219
57.288	0.997	238429_at	TMEM71	transmembrane protein 71
7.254	0.999	205122_at	TMEFF1	transmembrane protein with EGF-like and two follistatin-like

				domains 1
-2.611	0	218145_at	TRIB3	tribbles pseudokinase 3
-1.513	0	203148_s_at	TRIM14	tripartite motif containing 14
3.135	1	213293_s_at	TRIM22	tripartite motif containing 22
-1.663	0	202504_at	TRIM29	tripartite motif containing 29
-1.605	0	200742_s_at	TPP1	tripeptidyl peptidase I
-2.006	0	203476_at	TPBG	trophoblast glycoprotein
-1.908	0	210987_x_at	TPM1	tropomyosin 1 (alpha)
1.795	1	226181_at	TUBE1	tubulin, epsilon 1
2.058	0.997	223501_at	TNFSF13B	tumor necrosis factor (ligand) superfamily, member 13b
2.25	1	202644_s_at	TNFAIP3	tumor necrosis factor, alpha-induced protein 3
3.515	1	202643_s_at	TNFAIP3	tumor necrosis factor, alpha-induced protein 3
5.563	1	206026_s_at	TNFAIP6	tumor necrosis factor, alpha-induced protein 6
8.05	1	206025_s_at	TNFAIP6	tumor necrosis factor, alpha-induced protein 6
-2.434	0	227420_at	TNFAIP8L1	tumor necrosis factor, alpha-induced protein 8-like 1
1.838	0.998	201689_s_at	TPD52	tumor protein D52
-2.109	0	227388_at	TUSC1	tumor suppressor candidate 1
1.987	1	226784_at	TWISTNB	TWIST neighbor
-1.618	0	218190_s_at	UQCR10	ubiquinol-cytochrome c reductase, complex III subunit X
-1.955	0	202090_s_at	UQCR11	ubiquinol-cytochrome c reductase, complex III subunit XI
-1.494	0	208909_at	UQCRFS1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
3.309	0.997	205890_s_at	UBD	ubiquitin D
2.64	1	230083_at	USP53	ubiquitin specific peptidase 53
1.813	0.997	239163_at	UBE2B	ubiquitin-conjugating enzyme E2B
1.499	1	240383_at	UBE2D3	ubiquitin-conjugating enzyme E2D 3
3.89	1	225612_s_at	B3GNT5	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5
-2.853	0	1555963_x_at	B3GNT7	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 7
1.974	1	212633_at	UFL1	UFM1-specific ligase 1
-2.465	0	243871_at	LOC100130476	uncharacterized LOC100130476
-1.687	0	1557161_at	LOC100132735	uncharacterized LOC100132735
-2.338	0	231473_at	LOC100506530	uncharacterized LOC100506530
-4.229	0	208998_at	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
-1.837	0	221998_s_at	VRK3	vaccinia related kinase 3
-1.922	0	218679_s_at	VPS28	vacuolar protein sorting 28 homolog (S. cerevisiae)
-3.1	0	217969_at	VPS51	vacuolar protein sorting 51 homolog (S. cerevisiae)
1.399	0.997	224917_at	VMP1	vacuole membrane protein 1
-2.751	0	226213_at	ERBB3	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3
1.683	0.998	204619_s_at	VCAN	versican
1.732	0.999	204620_s_at	VCAN	versican
3.304	1	215646_s_at	VCAN	versican
3.554	1	211571_s_at	VCAN	versican
-2.734	0	209822_s_at	VLDLR	very low density lipoprotein receptor
-2.138	0	208626_s_at	VAT1	vesicle amine transport 1
-1.877	0	208091_s_at	VOPP1	vesicular, overexpressed in cancer, prosurvival protein 1
2.252	0.998	224833_at	ETS1	v-ets avian erythroblastosis virus E26 oncogene homolog 1
1.852	0.999	1555938_x_at	VIM	vimentin
-1.76	0	217949_s_at	VKORC1	vitamin K epoxide reductase complex, subunit 1
-2.481	0	233929_x_at	WASH3P	WAS protein family homolog 3 pseudogene
-2.214	0	226340_x_at	WASH3P	WAS protein family homolog 3 pseudogene
-1.931	0	224562_at	WASF2	WAS protein family, member 2
-3.065	0	227174_at	WDR72	WD repeat domain 72
-2.043	0	208606_s_at	WNT4	wingless-type MMTV integration site family, member 4
4.528	0.998	235821_at	WISP1	WNT1 inducible signaling pathway protein 1
4.709	1	229802_at	WISP1	WNT1 inducible signaling pathway protein 1
-2.071	0	202908_at	WFS1	Wolfram syndrome 1 (wolframin)
1.446	0.998	224895_at	YAP1	Yes-associated protein 1
1.77	1	224894_at	YAP1	Yes-associated protein 1
1.618	0.999	201368_at	ZFP36L2	ZFP36 ring finger protein-like 2
2.421	0.999	203603_s_at	ZEB2	zinc finger E-box binding homeobox 2

2.165	0.998	219228_at	ZNF331	zinc finger protein 331
-2.019	0	204175_at	ZNF593	zinc finger protein 593
-2.078	0	223212_at	ZDHHC16	zinc finger, DHHC-type containing 16
-13.804	0	231063_at		
-4.784	0	228977_at		
-3.432	0	242460_at		
-3.217	0	238805_at		
-2.984	0	237263_at		
-2.984	0	236180_at		
-2.944	0	203807_x_at		
-2.62	0	206548_at		
-2.518	0	213515_x_at		
-2.356	0	1557807_a_at		
-2.342	0	232935_at		
-2.184	0	232191_at		
-1.568	0	213828_x_at		
-1.56	0	212639_x_at		
1.611	0.999	242881_x_at		
1.761	0.998	222088_s_at		
1.809	0.998	1560622_at		
1.883	1	228812_at		
1.928	0.998	224549_x_at		
1.932	0.999	233020_at		
1.937	1	213872_at		
1.94	1	241916_at		
1.95	0.998	233614_at		
1.957	0.999	241773_at		
1.974	1	227368_at		
1.979	1	235456_at		
2.049	0.998	242712_x_at		
2.073	0.999	1564424_at		
2.13	0.998	1565886_at		
2.219	0.999	236685_at		
2.26	0.997	233303_at		
2.397	0.998	235959_at		
2.423	0.998	232268_at		
2.445	1	235419_at		
2.79	1	237496_at		
2.887	1	240038_at		
3.081	1	232628_at		
3.178	0.998	1558783_at		
3.391	0.998	1562260_at		
3.95	0.997	243819_at		
7.323	0.997	215806_x_at		
7.462	1	222326_at		
8.172	0.998	235438_at		
9.262	1	236495_at		
24.601	0.998	1560859_at		
48.057	0.998	234632_x_at		

4.0 Results Chapter

Identification of potential regulators of labour the human choriodecidua and myometrium, using *in silico* and *in vitro* analysis

Sylvia Lui [1], Farkhondeh Farrokhnia [1], Cyntia Duval[4,] Lynda K. Harris [1,3], Clare L Tower [1], Adam Stevens [1,2], Rebecca L. Jones [1]

Affliations:

[1]Maternal and Fetal Health Research Centre, Institute of Human Development, University Of Manchester, UK. [2]Institute of Human Development, University Of Manchester, UK. [3]School of Pharmacy, University Of Manchester, UK. [4]Sainte-Justine Hospital Research Center, Department of Obstetrics and Gynecology, University of Montreal, Canada.

Abstract

Preterm delivery is an international health issue and remains a leading cause of neonatal morbidity and mortality. The current available tocolytic therapies target one function, to impair myometrial contractions, but little evidence exists they are effective beyond 48 hours. There are a number of established processes required for labour, but identifying the regulatory pathways of normal term labour is vital to the understanding of labour pathologies and development of effective therapies in delaying spontaneous premature birth.

To identify upstream master regulators of labour that have multiple downstream labour-associated effects, this study used microarray transcriptomic data to globally analyse for functional causal networks using Cytoscape 2.8.3 plugins ModuLand and ClusterONE, as well as Ingenuity Pathway Analysis software, on the human choriodecidua and myometrium. *In vitro* experiments using chemical inhibition were assessed using qRT-PCR.

The comprehensive network analysis identified several upstream molecular components in the decidua including vimentin (VIM), TLR4 and TNFSF13B. In the myometrium, regulator candidates include metallothionein 2 (MT2), TLR2 and RelB. These master regulators have significant differential gene expression during labour, hierarchically high centrality in gene networks with interactions amongst the labour gene set, and causal relationships with numerous downstream effects. *In vitro* experiments indicate inhibition of the candidate master regulator MT2 effectively decreased several network predicted downstream genes. These initial results encourage further investigation into the efficacy of predicted master regulators in blocking multiple pathways of the labour processes and ultimately, potential as therapeutic antagonists that could effectively block the labour cascade.

4.1 Introduction

Preterm delivery remains a leading cause of infant morbidity and mortality worldwide (Blencowe et al. 2012). Idiopathic, spontaneous preterm births account for 45-50% of cases (Rudra et al. 2008; Beck et al. 2010). Current treatments to prevent labour are relatively ineffective. Tocolytics target myometrial contractions and are the only available therapy to date. However, they are associated with maternal side effects, do not improve fetal outcomes and there is poor evidence for the delay of premature labour even up to 48 hours, compared to a placebo (Haas et al. 2012). Labour involves the sensitisation of the uterus to contractile signals resulting in amplification and increased intensity of myometrial contractions. However, labour and successful parturition involves a multitude of other events: inflammatory signalling, decidual activation, fetal and maternal endocrine coordination, cervical ripening and dilation, fetal membrane dissociation and placental separation (Norman et al. 2007). Targeting multiple pathways within the labouring cascade may be more effective in preventing premature deliveries than targeting a single process. Whilst there is a wealth of studies on labouring processes, the regulatory molecules and pathways of these critical events remain to be elucidated.

Pathologies are rarely the result of singular gene dysregulations and exploring the roles of upstream regulating molecules of a suite of essential mediator genes, are vital in the understanding of the triggers of true labour. Identifying the regulators of the normal term labour condition is essential to the future development of therapies effective in delaying spontaneous premature labour and premature birth. Extensive gene lists using genomic microarray technology have identified thousands of genes whose expression changes with labour, suggesting a significant role in the parturition process. Transcriptomic changes in the normal term labouring myometrium include contractile-associated genes: prostaglandin synthase 2 (PTGS2), prostaglandin F 2α (PGF2-α) oxytocin (OXT), oxytocin receptor (OXTR) and gap junction 1 (GJA1) (Chan et al. 2002; Rehman et al. 2003; Mittal et al. 2010). Other significantly upregulated genes include those involved in cytoskeletal structure, cell metabolism, cell death, signal transduction, and inflammatory cytokines (Aguan et al. 2000; Chan et al. 2002; Rehman et al. 2003; Esplin et al. 2005; Havelock et al. 2005; Bukowski et al. 2006; Bollapragada et al. 2009; Germeyer et al. 2009; Mittal et al. 2010; Khanjani et al. 2011).

When compared to non-labouring tissues, genomic analysis of fetal membranes (decidua, chorion and amnion) obtained from normal labouring pregnancies at term identified a number of differentially expressed genes that included multiple inflammatory-related complex components (multiple chemokines and their resepective receptors; interleukins (IL); the Nuclear factor kappa B complex (NFκB); toll-like receptors (TLR), intercellular adhesion molecule 1 (ICAM1), and cell-survival related genes: bone morphogenic protein (BMP) 2 and 3; b-cell lymphoma related (BCL) 2 and 3 (Haddad et al. 2006). Our recent study of isolated choriodecidua supports these findings, where we observed a strong immune response during normal term labour. Upregulated genes included: ICAM1, CD44, TLR4, BCL2A, suppressor of cytokine signalling (SOCS) 3, along with multiple chemokines, cytokines, interleukins and also many matrix metalloproteinases (MMP)

(Stephen et al. 2014). Preliminary network analysis identified potential transcription factors that are upstream of these labour-associated molecules including: NFκB, and several micro-RNAs (miR-21, -146, -141, and -200) (Stephen et al. 2014). Further analysis is required in order to identify robust causal relationships, and regulators with the greatest number of downstream effector genes in labour-associated tissues.

This study hypothesed that undertaking a comprehensive global network analysis of the choriodecidua and myometrium would identify master regulators of the labour process, by identifying and prioritising the most significant genomic interrelationships and interactions. To explore the global list of genes whose expression changes with labour in intimately associated tissues, the choriodecidua and myometrium, this study aimed to: 1) use hierarchical network analysis to prioritise relationships and interactions between genes that are differentially expressed in labour; 2) identify potential regulatory candidate genes; and 3) to test whether chemical inhibition of the predicted master regulator candidates will alter expression of multiple downstream labour-associated molecules, and therefore have potential as therapeutic antagonists that could effectively stop the labour cascade.

4.2 Methods

4.2.1 Gene microarray data

The term labour choriodecidua transcriptome data was extracted from Stephen et. al. 2014. In brief, the choriodecidual samples were from two groups of women at term: elective caesareans with no labour (N=12; 37 to 41 weeks gestation; not in labour) and vaginal deliveries (N=11; 38 to 42 weeks gestation; normal term labour). The extracted RNA samples were hybridised to GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix, UK) according to the manufacturer's instructions. These arrays can detect over 47 000 transcripts (approximately 38 500 known genes). The choriodecidual microarray raw data was normalised using robust multiarray analysis (RMA) from the Affymetrix Microarray Suite (Irizarry et al. 2003) and statistically processed using the PUMA package (Gentleman et al. 2004). For a stringent data constraint, this study used the parameters of probability of positive log ratio (PPLR) of at least 0.997 and less than 0.00001 (gene expression change occurring by chance of 0.003% and 0.00001%).

The publicly available myometrial transcriptomic data PubMed GEO series GSE9159 was analysed (Weiner et al. 2010), after normalisation using the *R* statistical package, and with statistical restriction ($P<0.02$). In summary, the data used were from women undergoing caesarean section after labour (N=6; 39 to 41 weeks gestation; emergency section after arrest of cervical dilation), or not in labour (N=6; 38 to 41 weeks gestation; elective section). The myometrium transcript data was generated by the authors using the GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix, UK).

4.2.2 Identification of master regulators of the transcriptome of labour using global network analysis

To investigate the interactions and relationships between the differentially expressed non-labouring and labouring genes, all known interactions were referenced using the 'Biological General Repository for Interaction Datasets' (BioGRID) database (BioGRID: http://thebiogrid.org). A network analysis protocol was developed to comprehensively analyse the large-scale microarray results, as well as identify a hierarchy of the most interconnected, central regulatory candidates from the gene/protein interactions. We identified a hierarchy in the transcript data using network analyses, and to ensure confidence in the predicted high hierarchal interactions two different algorithms from the Cytoscape 2.8.3 software, ModuLand and ClusterONE were utilised. Those ranked highest in the hierarchy were then analysed for their downstream effects in the network using causal analysis (Figure 1).

In brief, the work flow was as follows:

1. Statistical restriction of microarray data to PPLR $1x10^{-5}$ and 0.997 (choriodecidua) or 0.02 (myometrium).

2. Mathemathical modelling using software Cytoscape 2.8.3 (Shannon et al. 2003; Cline et al. 2007; Smoot et al. 2011; Saito et al. 2012) and the BioGRID database. This study used the Cytoscape plugin ModuLand to mathemathically analyse the basic interactions and primary interactors, from the BioGRID database, of the differentially expressed genes. Gene clusters were determined algorithmically by centrality over the whole network topology using an overlapping community approach, thus forming a hierarchy where nodes (clusters of genes) form the core of the next upper level, designated by a meta-node (cluster of nodes) (Szalay-Beko et al. 2012). As the nodes and meta-nodes are clusters identified by the top-most central gene, these were cross referenced back to the microarray to identify the position of the most interconnected genes.

3. Secondary mathemathical modelling to cluster major functional cores, but include basic interaction of protein complexes, gene-protein, protein-protein interactions ClusterONE (Nepusz et al. 2012). To prioritise the volume of hierarchially-ranked data, the two mathematical models were cross referenced for common genes.

4. Causal analysis using Ingenuity Pathway Analysis (IPA) on selected targets to verify labour-related significance. Causal analysis maps array transcripts into master regulators relationships, using literature-based analysis of all known gene interactions. Analysing our candidates of interest using causal analysis supports the *in silico* predicted central relationships within our dataset, and identifies specific downstream effects. Targets were further refined to only include those with a high number of downstream genes associated with labour.

The two mathematical algorthims in combination with causal analysis provide a detailed hierarchical network structure of the myometrium and choriodecidua to investigate the following: (1) determine the meta-nodes or the core of labour-associated genes; (2) define key network

nodes that govern large numbers of downstream genes and, (3) identify genes that also possess a downstream profile that includes a number of labour-associated genes i.e. potential key regulator candidates.

4.2.3 Tissue sampling for *in vitro* investigation

Human choriodecidual tissue explants were used to investigate the effect of inhibiting the master regulator candidates. Written informed consent was obtained from women undergoing elective caesarean section at term (gestational range 37-42 weeks) presenting with an uncomplicated pregnancy and who were not in labour (TNL, N=6) at St Mary's Hospital, Manchester, UK. Ethical approval was granted by the North West Local Research Ethics Committee (Reference Number: 08/H1010/55 (+55)).

4.2.4 Choriodecidual explant culture and treatment

The choriodecidua samples were taken from the midzone area of the membranes, distant from the rupture side and placenta (Hamilton et al. 2012; Hamilton et al. 2013). The decidual parietalis with attached chorion were then separated from the fetal amnion membrane. After thoroughly washing in cold sterile PBS to remove excess red blood cells, the choriodecidual explants were prepared for sterile culture by thoroughly washing in serum-free RPMI culture media containing penicillin streptomycin (0.5mg/ml) and amphotericin (1.25µg/ml) (Sigma-Aldrich, UK). The explants were then cultured with or without inhibitors for 24h (3-4 explants per well, two wells per treatment or control), on netwells in DMEM/F12 (1:1) media supplemented with L-glutamine (1.5mg/ml), penicillin streptomycin (0.5mg/ml), and 10% (v/v) FBS in a humidified incubator at 37^{o}C, with 21% O_2 and 5% CO_2. At the end of the 24 hour incubation, the tissues were either treated with RNAlater (Ambion, UK) for 24 hours for RNA isolation, or fixed in 4% neutral buffered formalin (NBF) for immunohistochemistry. All media culture products were purchased from GIBCO (Life Technologies, UK) unless otherwise stated.

To inhibit VIM, this study used the PKC inhibitor Gö 6983 at 1µM (G1918; Sigma Aldrich, UK). This antagonist has been shown to reduce cellular VIM protein expression (Mor-Vaknin et al. 2003), decrease focal attachments between adherent cells and also reduce cellular motility (Peterman et al. 2004). This study used another antagonist to inhibit VIM, cytochalasin D at 100nM (C2618; Sigma Aldrich, UK). This mycotoxin has been shown to inhibit actin polymerisation (Tsuruta & Jones 2003) and decrease VIM protein and mRNA expression (Wu et al. 1999; Krucker et al. 2000; Jung et al. 2013). For the specific inhibition of TLR4, this study used the cyclohexene inhibitor TAK-242 at 1µM (tlrl-cli95; Invivogen, France) (Zhang et al. 2014). The vehicle control used was at the highest concentration of DMSO used in the inhibitor treatments (0.002% v/v).

4.2.5 Myometrial cell culture and treatment

An *in vitro* cell culture model was utilised to investigate the potential of inhibiting the function of putative myometrial master regulator proteins. An immortalised human myometrial cell line, Hret-C3-Sy, a kind gift from Dr Sylvie Girard (CHU Sainte-Justine, University of Montreal, Canada) was seeded in a 6-well plate at $5x10^4$ per well. The cells were cultured with or without inhibitors for

24h in DMEM/F12 (1:1) media supplemented with L-glutamine (1.5 mg/ml), penicillin and streptomycin (0.5mg/ml), gentomycin (100 µg/ml) and 10% (v/v) fetal bovine calf serum, in a humidified incubator at 37°C, with 21% O_2 and 5% CO_2. After 24 hours, the cells were treated with RNAlater for a further 24 hours prior to RNA isolation. All cell culture products were purchased from GIBCO, Life Technologies, UK, unless otherwise stated.
The PKC inhibitor GÖ6983 (Sigma Aldrich, UK), was used at 15uM to inhibit metallothionein 2 (MT2), as PKC inhibition has been shown to inhibit MT2 mRNA expression (Yu et al. 1997). For the specific inhibition of TLR2, this study used a neutralising antibody Mab hTLR2, Clone TL2.1 at 100ng/ml (Invivogen, France) (Schindler & Baichwal 1994). The vehicle control used was at the highest concentration of DMSO used in the inhibitor treatments (0.002% v/v).

4.2.6 Quantitative RT-PCR

RNA was isolated using the MirVana miRNA Isolation Kit (Ambion, UK) according to the manufacturer's instructions. Extracted RNA was assessed using a NanoDrop 2000 UV-Vis Spectrophotometer, measuring absorbance curve at A260/280 and A260/230. An Affinity Script Multiple Temperature cDNA Synthesis Kit (Agilent, UK) was used to reverse transcribe the first strand of cDNA, according to manufacturer's instructions. All samples were amplified in duplicate using the Ultra-Fast SYBR Green QPCR Master Mix III (Agilent, UK) with 5-carboxy-x-rhodamine (ROX) as a reference dye. The annealing temperatures of the primers were optimised at 60°C. The amplification and dissociation were carried out using the RT-qPCR Stratagene MX3005 machine. To determine the difference in mRNA expression, relative quantification using $2^{-\Delta\Delta CT}$ was applied. Several frequently used housekeeping genes were screened (RPL13, TBP, YWHAZ, GAPDH, and β-actin; Table 1A) before selecting RPL13 as the most stably expressed reference gene for the choriodecidual explants, and YWHAZ for the myometrial cell line. Though the geometric mean of reference genes is commonly used to account for the variation in expression of housekeeping genes, RPL13 and YWHAZ were selected as they demonstrated unchanged, stable, mRNA expression across all treatment and control groups. The genes of interest are listed in Table 1B). Statistical analysis used was Wilcoxon signed-rank test; significance was taken as $p<0.05$.

4.3 Results

4.3.1 Network analysis and gene relationships in the choriodecidua

Using the human choriodecidua microarray transcriptomic data, an interactome model was constructed from the BioGRID interaction database using ModuLand to map global interconnecting relationships. The original data identified 796 significant transcriptional changes following labour with a total of 4980 nodes (clusters of genes) and 12914 interactions in the choriodecidua. At the next upper hierarchical level, the nodes clustered to form 71 meta-nodes with 1610 interactions (Figure 2). The nodes, functions and pathways of the top 20 meta-nodes of the choriodecidua are hierarchically illustrated in Table 2. The top meta-node genes indicates a high centrality in the hierarchy of transcriptionally changed genes associated with labour. The genes were dominated by ubiquitin (UBC) and cell division cycle 34 homolog (CDC34; a ubiquitin-conjugating enzyme E2-CDC34) clusters, but also included genes involved in signal transduction in oxidative stress and

longevity e.g. Src homology 2 domain containing transforming protein 1 (SHC1; 4th rank); cell cycle function and repair e.g. breast cancer type 1 (BRCA1; 6th rank); and inflammatory signalling, e.g. suppressor of cytokine signalling (SOCS3; 13th rank). Functional analysis of these regulatory meta-nodes showed that they were involved in cellular responses to stress, apoptosis, circadian clock, DNA repair, adaptive and innate immunity and growth factor signalling (p-value range: $1x10^{-4}$ to $3.9x10^{-7}$) (Table 2). Several nodes also had high hierarchal ranking, for example, the NFκBIA was found within the top meta-node cluster UBC, indicating a high biological interaction in the network (Supplemental table 1).

4.3.2 ClusterONE network analysis of the choriodecidua

From the predicted 71 meta-nodes, 20 candidates were strongly predicted by inclusion of ClusterONE analysis as having high potential in regulating multiple labour-associated downstream events (Supplemental Table 2a). The top downregulated regulatory candidates included: CDK19 (54 nodes), POLR2I (54 nodes), INTS5 (25 nodes), CDC34 (23 nodes), LMO4 (15 nodes) (p-value range = $5x10^{-9}$ to 0.004). The top upregulated regulatory candidates included: VIM (40 nodes), INHBA (41 nodes), CSNK1A1 (37 nodes), KRIT1 (10 nodes) and THBS1 (46 nodes) (Significance of node cluster p-value range =$5.02x10^{-9}$ to 0.035).

4.3.3 Causal network analysis of master regulators in the choriodecidua

The final list of regulator candidates in the choriodecidua with the most interrelationships were VIM, an intermediate filament that increased 1.9 fold during labour; inflammatory activated receptor TLR4 which was increased 3.7 fold; and TNFSF13B, a B-cell activating factor, which was differentially expressed 2.1 fold during labour (Table 3). VIM was associated with 41 downstream genes ($p=1.38x10^{-17}$) with an activation z-score (a calculated direction bias in the regulation of the causal network) (see methods chapter 2.1.5.6 for details), of 3.2 in the network (Table 3); TLR4 had 25 associated downstream genes ($p=4.67x10^{-10}$) and activation z-score of 4.2 (Table 3), and TNFSF13B had 105 downstream genes ($p=9.6x10^{-8}$) and activation z-score of 3.4 (Table 3). Downstream causal analysis supported predicted labour-associated regulatory actions of VIM (Figure 3). The downstream genes of VIM included phospholipidase A2 (PLA2), MMP-1, IL-6, CXCL8 (IL-8) and PTGS2. The downstream genes of TLR4 included IL-6, CXCL-10, TLR2, and PTGS2 (Supplemental Figure 1). The labour-associated downstream genes of TNFSF13B were numerous and included NFkB components, IL-1RN, IL-1A, CXCR4, SOCS3 and multiple chemokines (Supplemental Figure 2).

4.3.4 Network analysis of the myometrium

The myometrial genomic dataset was analysed using the same analysis workflow as for the choriodecidua. From the original 717 myometrial transcripts significantly altered with labour, the BioGRID network model generated a total of 4886 nodes with 12112 interactive relationships in the myometrium. These were represented at the next hierarchical level by 64 meta-nodes and 1277 interactions (Figure 4). The top hierarchical 20 meta-nodes included: cyclin-dependent kinase inhibitor 1A (CDKN1A), UBC and XRCC5 (Table 4). Other labour associated meta-node clusters were involved in signal transduction in oxidative stress e.g. SHC1 (5th rank in ModuLand network),

inflammation and survival e.g. BCL3 B-cell CLL/lymphoma 3 (BCL3; 14th rank), and inflammatory activation e.g. RelB (a NFκB sub unit; 15th rank) (Table 4). The functions and pathways of these meta-nodes were also involved in cellular responses to hypoxia, apoptosis, DNA replication & repair, TGFβ signalling and adaptive immune system (p-value range: $1x10^{-5}$ to $8.9x10^{-7}$) (Table 4). High-ranked nodes in the hierarchy included: heterogeneous nuclear ribonucleoprotein (HNRNPM), which is involved in cytokine-associated pre-mRNA induction; ATP-dependent RNA helicase A (DHX9), a transcriptional regulator; and the transcription factor, CCAAT/enhancer-binding protein alpha (CEBPA) (Table 4; Supplemental Table 3).

4.3.5 ClusterONE network analysis of the myometrium

From the 64 myometrial meta-nodes and 1277 network interactions, 30 highly regulatory candidate genes were identified (Supplemental Table 2b). The down-regulated regulatory candidates included CEBPA (45 nodes), SMAD6 (36 nodes), HDAC9 (42 nodes), GRIA2 (14 nodes), and EMX2 (17 nodes) (p-value range = $2.12x10^{-5}$ to 0.001). The top upregulated regulatory candidates included: UPF2 (40 nodes), BATF (45 nodes), DCP2 (30 nodes), ENG (36 nodes), NKX3-1 (25 nodes) (p-value range = $3.4x10^{-6}$ to $8x10^{-5}$).

4.3.6 Causal network analysis of master regulators in the myometrium

The master-regulator relationships of all 30 myometrial candidates were further explored using causal analysis. The final three prioritised candidates have causal relationships amongst the activated array gene set, but also possess a significant number of labour-associated downstream effects (Table 3). The final candidates included: intracellular ion-regulator, MT2A (or MT2), with a modest fold change of 1.2 during labour, associated with changes in 30 downstream genes ($p=3.24\ x10^{-6}$) and an activation z-score of 3.3 in the network causal analysis (Table 3); RelB with a fold change of 1.4, 123 genes downstream ($5.96x10^{-9}$) and an activation z-score of 2.4 (Table 3); and TLR2 which was upregulated 1.3 fold, with 90 downstream genes ($p=1.13x10^{-6}$) and an activation z-score of 1.3 (Table 3). Analysis of these downstream genes identified several established labour associated genes. Genes downstream of MT2 included estrogen receptor, IL-6, IL-1β, and different NFκB components (Figure 5). Downstream of TLR2 included PTGS2, IL-6, and multiple NFκB components (Supplemental Figure 3) and those downstream of RelB included estrogen receptor, IL-6, IL-1β, and NFκBIA (Supplemental Figure 4). Interestingly, network analysis identified MT2A as downstream of all three myometrial regulator candidates.

4.3.7 Commonality between the master regulators of the choriodecidua and myometrium

To demonstrate the key role of the master regulators in term labour, the relationships between the selected candidates, and their downstream labour associated genes are summarised in Figure 6. Though there are numerous genes regulated by each master regulator, there were downstream genes shared multiple times between the master regulators and between each tissue type. For example, the master regulators of the choriodecidua, VIM and TNSF13B, shared downstream effects on the CXCR4 gene. Amongst the two regulators, VIM and TLR4, there were shared effects on the CCL4 gene. In the myometrium, the master regulators MT2a and RelB, both influenced the

expression of the downstream phosopholipid enzyme PLA2G12A. The master regulators MT2a and TLR2, affected the downstream gene IL-6. Also, some of the downstream genes effected in the *in silico* network are shared across both the choriodecidua and myometrium, including PTGS2, IL-6, NFκB, CXCL10 and TLR2.

The decidual and myometrial network analysis generated highly centralised network relationships and in an analysis of those network connections, the top three meta-nodes identified internal complex interconnections, supporting their high core functions within each tissue (Figure 7). When performing a comparison analysis of the meta-node interrelationships between the choriodecidua and myometrium, a clear biological intersection can be identified with two key meta-node clusters: SHC1 (choriodecidual, 4th central cluster; myometrial 6th central cluster); and PIK3R1 (myometrial, 5th central cluster). Both meta-node clusters were strongly canonically-associated with the adaptive immune system pathways and growth factor signalling, with a number of genetic overlaps between both tissues (4-5 gene clusters), suggesting major roles in the labour process.

4.3.9 Inhibition of master regulators in choriodecidual explants

The function of several putative master regulators within the choriodecidua was blocked using commercially available chemical inhibitors and the downstream effects on mRNA expression of key target genes predicted by network analysis were assessed. After incubating explants of choriodecidual with two inhibitors of VIM, Gö6983 and cytochalasin D, there were no significant changes in the thirteen downstream labour-associated genes assessed, when compared to vehicle control (Figure 8).

4.3.10 Inhibition of master regulators in myometrial cells

Inhibition of the master regulators MT2 and TLR2 in a myometrial cell line altered the expression of several downstream genes associated with labour (Figure 9). Inhibition of MT2 over 24 hours resulted in significant down-regulation of IL-8, IL-6, IL-1α, IL-1β, CD44, PTGER4 and VIM mRNA expression ($p<0.05$-0.01), and upregulated phospholipase PLA2GA mRNA expression ($p<0.05$). Inhibition of TLR2, led to significant down-regulation of IL-8 ($p<0.05$) and a trend for reduced progesterone signalling-related, ZEB2 ($p=0.09$).

4.4 Discussion

Using an unbiased global network analysis, this study has identified gene candidates with central regulatory roles in labour-associated processes. The six candidates are VIM, TLR4 and TNFSF13B in the choriodecidua, and MT2A, TLR2 and RelB in the myometrium. Whilst the mRNA expression of these genes is moderately altered during labour, they possess several important features following network analysis. The master regulators are the topmost regulators with the highest centrality in the whole network of the labour genome, as well as having a significant number of causal relationships with genes associated with labour. These features indicate they have important roles in regulating the labour transcriptome in the choriodecidua and myometrium, and therefore identifies them as putative master regulators of the labour process.

TLR4 and TLR2 have established roles in pregnancy and in parturition (Re & Strominger 2001; O'Brien et al. 2008; Shen et al. 2009; Pawelczyk et al. 2010; Friebe et al. 2011; Montalbano et al. 2013; Agrawal et al. 2013; Lim et al. 2014). The identification of these two TLRs as master regulators in this study provides confidence in the use of predictive *in silico* methods. Our data reinforce existing evidence that TLR4 and TLR2 have an important role in parturition and in addition, demonstrate that their expression has a major role in regulating the expression of numerous genes during labour. The roles of the other candidates VIM, MT2a, TNFSF13B and RelB have not been previously analysed in depth in the labour cascade within the choriodecidua or myometrium.

Vimentin is expressed in many cell types and its role as a master regulator in the choriodecidua during labour is a novel finding. VIM is classically known as a component of intermediate filaments in cytoskeletal organisation. Associated with maintaining cell structure, VIM also has physiological roles in the regulation of cellular organisation, cell polarity, cell to cell adhesion and in cellular motility. VIM is a common marker of epithelial to mesenchymal transitioning (Satelli & Li 2011) and abnormal expression of VIM is associated with advanced cancer and metastasis (Ivaska et al. 2007). In labour, upregulation of VIM protein expression has been reported in the labouring myometrium with proposed roles in sustaining the regularity of muscle contractions (Duquette et al. 2005). The multiple functions of VIM support a regulatory role for this protein during preparation for active parturition. There is a conserved NFκB sequence within the VIM gene promoter (Lilienbaum et al. 1990) suggesting VIM can be regulated by NFκB signalling. Cytokines produced by infiltrating leukocytes induce NFκB signalling, which upregulates PTGS2 expression and prostaglandin synthesis during labour, amongst many other functions (Mittal et al. 2010). The elevated NFκB signalling and decidual and myometrial leukocyte infiltration preceding labour in a rat model, suggests a potential role of decidual VIM towards the preparation for labour (Hamilton et al. 2012). The upregulation of VIM in the decidua during labour may represent signalling between the two uterine layers, potentially through facilitating signals that amplify and maintain myometrial contractions as labour progresses.

The decidual regulatory candidate TLR4 is part of a family of membrane-bound microbial response receptors, well-known to have roles in pregnancy, and in the labour cascade (Re & Strominger 2001; Toshchakov et al. 2002; Shen et al. 2009; Sheldon & Roberts 2010; Pawelczyk et al. 2010; Friebe et al. 2011; Stephen et al. 2014). TLR4 specific signalling requires the surface receptors CD14 and MD2, and is activated through MyD88-dependant (TIRAP) and independent (TRIF/TRAM) pathways to trigger nuclear NFκB signalling (Takeda & Akira 2004). NFκB complex activation is associated with prostaglandin synthesis stimulation, cytokine production, cell growth and differentiation (Lim et al. 2001; Poligone & Baldwin 2001). Though mainly associated with gram negative bacterial activation, the TLR4/ NFκB pathway is also activated in response to non-infection related processes such as tissue trauma, cell death and cellular stress - which are major processes in parturition - due to release of endogenous alarmins (Taniguchi et al. 2009). Constitutive knockout of TLR4 in mice significantly increases length of pregnancy by 13 hours

(Wahid et al. 2015). TLR4 mRNA expression is upregulated in human spontaneous preterm chorioamnion (Kim et al. 2004), myometrial monocytes (Youssef et al. 2009), maternal blood (Pawelczyk et al. 2010), term laboured myometrial monocytes (Youssef et al. 2009), placenta (Ma et al. 2007; Patni et al. 2009), chorioamnion (Kim et al. 2004), choriodecidua (Stephen et al. 2014) and cervix (Hassan et al. 2006), and protein expression is increased in the cord blood of vaginally delivered infants (Shen et al. 2009). In an mouse model of preterm labour induced using LPS, TLR4 receptor inhibition increased the population of regulatory T-cells in the decidua and reduced hypothalamic CRH expression, which is an essential mediator in the hormonal cascade of parturition (Friebe et al. 2011). Suppressing TLR4 receptor function in isolated human decidual cells was associated with a reduction in PTGS2 and cytokine expression (Y. Li et al. 2013), these downstream changes were also predicted by our network analysis.

A member of the tumour necrosis superfamily, TNFSF13B is involved in signalling in inflammation, infection and innate immune responses. Specifically, TNFSF13B is involved in maternal T-helper cell adaptation to the developing fetus (Hunt et al. 2010) and mRNA and protein expression are elevated in the third trimester placenta (Langat et al. 2008). TNFSF13B receptors are expressed in the endometrium and are upregulated during inflammatory periods of endometriosis (Hever et al. 2007). Myocyte TNFSF13B mRNA expression increases following NFkB related p65 overexpression (Khanjani et al. 2011). Transcript variants of TNFSF13B are associated with pregnancy pathologies such as preeclampsia (Fenstad et al. 2010), and spontaneous miscarriage with reduced placental and decidual mRNA and protein expression observed (Guo et al. 2008). TNFSF13B is upregulated partly by TGF-β1 and IFN-γ, and is highly expressed by monocytes and macrophages (Kim et al. 2008), the latter of which are abundant in the decidua in labour. Our network prediction supports the role of decidual TNFSF13B expression in successful labour, as a highly connected regulator of labour-associated genes such as MMPs, tissue inhibitor of metalloproteinases (TIMPS) (Chegini et al. 1999), TGF-β1 and IFN-γ (Kim et al. 2008).

Of the three key regulators of the human myometrium, two were the inflammatory-related molecules TLR2 and RelB. Both participate in cytoplasmic signalling events upstream of NFκB activation. The predicted regulator TLR2 is expressed by many cell types but in pregnancy, expression of TLR2 protein and mRNA is associated with myometrial contractility in normal term labour (O'Brien et al. 2008; Montalbano et al. 2013). Signalling through TLR2 during labour upregulates cytokine production (Re & Strominger 2001; Lim et al. 2014), MMP expression and prostaglandin synthesis (Lappas 2012; Lim et al. 2014) via the MyD88 dependant NFκB pathway (Takeda & Akira 2004; Lim et al. 2014). TLR2 mRNA expression is increased in many reproductive tissues during term labour, including the cervix (Hassan et al. 2006; Dubicke et al. 2010a), placenta (Patni et al. 2009), fetal membranes (Haddad et al. 2006), choriodecidua (Phillips et al. 2014), and is present in increased levels in cord blood at term (Shen et al. 2009). TLR2 has been implicated as a mediator of the labour cascade, as choriodecidual and placental expression is associated with prostaglandin synthesis in preterm and term labouring samples (Shankar et al. 2010; Phillips et al. 2014). Furthermore, homozygous $TLR2^{-/-}$ knockout mice experience extended

pregnancies (labour at D20 vs D19.5; copulation plug as D1) compared to their wild-type counterparts. These mice express reduced levels of labour-related contractile genes and have significantly delayed onset of labour (Montalbano et al. 2013). In our study, an antagonist of TLR2 inhibited the mRNA expression of IL-8 and with a trend for reduced ZEB2 in cultured immortalised myometrial cells. In previous studies, ZEB2 was identified as significantly upregulated in the term labour choriodecidua compared to non-labour (Stephen et al. 2014). ZEB2 is a homeobox gene that regulates GJA1 and OXTR expression, in the myometrium and during term and preterm labour (Renthal et al. 2010; Williams et al. 2012). IL-8 is a pro inflammatory cytokine associated with term and preterm labour in the myometrium, choriodecidua, aminon and amniotic fluids (Kelly et al. 1994; Arntzen et al. 1998; Elliott et al. 2000; Kato et al. 2004; Hamilton et al. 2013; Stephen et al. 2014).

The second master regulator RelB, was not experimentally tested in this study but is a member of the NFκB complex. Studies have shown the NFκB proteins RelA, c-Rel, p52 and p50, are highly expressed in the labouring myometrium compared to term non-labour, however RelB protein was not detected (Chapman et al. 2004). RelB functions in regulating the circadian rhythm, and is part of the IER3 cell stress pathway (Hamidi et al. 2012). Weiner et al 2010 found upregulated myometrial mRNA expression of RelB during labour (Weiner et al. 2010), and this study identified it as being a component of labour associated networks, with high-ranked hierarchy in the labouring myometrium.

The other top predicted myometrial regulator candidate was a metallothionein gene, MT2. MT2 is involved in heavy metal binding and cellular homeostasis (Vašák & Meloni 2011), but is also induced during inflammation (Min et al. 1992), and interacts with progesterone (Slater et al. 1988) and estrogen receptors (Cano-Gauci & Sarkar 1996; Harris et al. 2001). Increased myometrial MT2 mRNA expression has been associated with normal term labour (Helguera et al. 2009) and in the labouring myometrium with failure to progress (Chaemsaithong et al. 2013). Its functional role in normal labour however, has not been investigated. Elevated cytokine production of macrophages is observed in association with low intracellular iron levels (Wang et al. 2009), which are regulated by the heavy metal zinc (Davis & Cousins 2000). Metallothioneins are associated with cellular functions during stress, suggesting an alternative route to inflammatory regulation during labour, via alteration of heavy metal homeostasis. Inhibition of MT2 in this study reduced the basal cell expression of key pro-inflammatory genes IL-1α, -1β, -6, and -8 with established roles in labour in the myometrium, cervix, and fetal membranes (Seghaye et al. 1998; Haddad et al. 2006; Bollapragada et al. 2009; Mittal et al. 2010; Robertson et al. 2010; Khanjani et al. 2012; Lim et al. 2012; Gomez-Lopez et al. 2013; Shynlova et al. 2013a, 2013b) as well as the genes CD44, prostaglandin E receptor PTGER4, and VIM. Thus strengthening the hypothesis that MT2 is a master regulator with therapeutic potential for suppressing the labour cascade.

Although this study did not directly experimentally verify the master regulatory candidates VIM, TLR4 or TNSF13B, they were major predicted master regulators and their significant involvement in cellular processes, such as inflammation, cell to cell signalling and cell movement, may contribute

in regulating the labour cascade. This may be due to the study design – e.g. the use of choriodecidual explants and antibody inhibitor (e.g. TLR4), which may affect the accessibility of the inhibitors to the cellular targets due to tissue penetration, or to ineffective inhibition of the candidate regulators with non-specific targeting of the inhibitors (e.g. PKC inhibitor). Optimisation of the dose regime and concentrations would better elucidate the effectiveness of the chemical inhibitors. Isolation of primary human choriodecidual cells would further help to define the cellular roles of these master regulators and determine the pharmacokinetics and effectiveness of the inhibitors selected. Direct validation of the network analysis genes would be also possible using siRNA-mediated knockdown of each candidate, however, the goal of this study was to investigate potential therapeutics and chemical inhibitors of labour that could be more easily translated to clinical practise. The results of the master regulator inhibition on basal cell of myometrial cells support that the genes identified have regulatory roles, but studies using a labour-stimulated model such as IL-1β is required to determine downstream blockade effectiveness. Using chemical inhibitors to reduce the activity of our selected master candidate of interest do not preclude off target effects. This study used a PKC inhibitor to deplete MT2 activity and VIM function at different doses; though PKC inhibition is not a specific inhibitor of the targets of interest, blocking PKC activity can down-regulate a variety of pathways including expression of PGF2α receptor (Liang et al. 2008) and OXTR expression (Sharkey & Olcese 2007), and increasing cell-cell adhesion and GJA1 connections but not GJA1 expression (Morley et al. 2010).

Summary

This study aimed to identify master regulator genes with significant major downstream pathways associated with labour. We have identified candidates with significant changes in gene expression during labour, where the final candidates are the most central in the whole labour network. This centrality indicates they are the genes with the highest hierarchy over all other gene-gene clustering, protein-gene cluster interactions, and, they have a significant number of labour-associated downstream effects. The regulator candidates were dissimilar between the human choriodecidua and myometrium, but they had common downstream gene components, many of which were inflammatory in nature. Inflammatory genes have been implicated as key transcripts in labour with commonality across preterm, preterm with infection and normal term cases of labour (Weiner et al. 2010). In the decidua, candidate targets also regulate cellular structure, motility and permeability and cellular activation.

The targeting of therapies to multiple tissues within the uterus may be essential, as all uterine tissues must be activated for successful labour to occur. Our comparative network data supports this potential approach of multiple candidate or multiple tissue targeting, defining two highly interconnected regulatory meta-nodes between the choriodecidua and myometrium. While the regulatory candidates were predominantly distinct, two were shared meta-nodes that both regulated functions in adaptive and innate immunity and growth factor signalling. Future work is required to investigate the mechanisms of action of these candidate targets and whether they have therapeutic potential in supressing the labour cascade.

Acknowledgements

We thank the research midwives at St. Mary's Hospital, Manchester for their invaluable help with subject recruitment and tissue collection. This work was funded by The James Tudor Foundation. The MFHRC is supported by Tommy's, an Action Research Endowment Fund, the Manchester BRC and the Greater Manchester CLRN. LKH is a BBSRC David Phillips Research Fellow.

4.5 References

Agrawal, Varkha, Keith Smart, Tamas Jilling, and Emmet Hirsch. 2013. "Surfactant Protein (SP)-A Suppresses Preterm Delivery and Inflammation via TLR2." PloS One 8 (5): e63990. doi:10.1371/journal.pone.0063990.

Aguan, K, J A Carvajal, L P Thompson, and C P Weiner. 2000. "Application of a Functional Genomics Approach to Identify Differentially Expressed Genes in Human Myometrium during Pregnancy and Labour." Mol Hum Reprod 6 (12): 1141–45.

Arntzen, K J, A M Kjøllesdal, J Halgunset, L Vatten, and R Austgulen. 1998. "TNF, IL-1, IL-6, IL-8 and Soluble TNF Receptors in Relation to Chorioamnionitis and Premature Labor." Journal of Perinatal Medicine 26 (1): 17–26.

Beales, I L, and J Calam. 2001. "Inhibition of Carbachol Stimulated Acid Secretion by Interleukin 1beta in Rabbit Parietal Cells Requires Protein Kinase C." Gut 48 (6): 782–89.

Beck, S, D Wojdyla, L Say, A P Betran, M Merialdi, J H Requejo, C Rubens, R Menon, and P F Van Look. 2010. "The Worldwide Incidence of Preterm Birth: A Systematic Review of Maternal Mortality and Morbidity." Bull World Health Organ 88 (1): 31–38. doi:10.2471/BLT.08.062554.

Blencowe, H, S Cousens, M Z Oestergaard, D Chou, A B Moller, R Narwal, A Adler, et al. 2012. "National, Regional, and Worldwide Estimates of Preterm Birth Rates in the Year 2010 with Time Trends since 1990 for Selected Countries: A Systematic Analysis and Implications." Lancet 379 (9832): 2162–72. doi:S0140-6736(12)60820-4 [pii]10.1016/S0140-6736(12)60820-4.

Bollapragada, Shrikant, Refaat Youssef, Fiona Jordan, Ian Greer, Jane Norman, and Scott Nelson. 2009. "Term Labor Is Associated with a Core Inflammatory Response in Human Fetal Membranes, Myometrium, and Cervix." American Journal of Obstetrics and Gynecology 200 (1). Mosby, Inc.: 104.e1–11. doi:10.1016/j.ajog.2008.08.032.

Bukowski, R, G D Hankins, G R Saade, G D Anderson, and S Thornton. 2006. "Labor-Associated Gene Expression in the Human Uterine Fundus, Lower Segment, and Cervix." PLoS Med 3 (6): e169. doi:05-PLME-RA-0327R2 [pii]10.1371/journal.pmed.0030169.

Cano-Gauci, D F, and B Sarkar. 1996. "Reversible Zinc Exchange between Metallothionein and the Estrogen Receptor Zinc Finger." FEBS Letters 386 (1): 1–4.

Chaemsaithong, Piya, Ichchha Madan, Roberto Romero, Nandor Gabor Than, Adi L Tarca, Sorin Draghici, Gaurav Bhatti, et al. 2013. "Characterization of the Myometrial Transcriptome in Women with an Arrest of Dilatation during Labor." Journal of Perinatal Medicine 41 (6): 665–81. doi:10.1515/jpm-2013-0086.

Chan, E Cheng, S Fraser, S Yin, G Yeo, K Kwek, R J Fairclough, and R Smith. 2002. "Human Myometrial Genes Are Differentially Expressed in Labor: A Suppression Subtractive Hybridization Study." The Journal of Clinical Endocrinology and Metabolism 87 (6): 2435–41. doi:10.1210/jcem.87.6.8439.

Chapman, Neil R, G Nicholas Europe-Finner, and Stephen C Robson. 2004. "Expression and Deoxyribonucleic Acid-Binding Activity of the Nuclear Factor kappaB Family in the Human Myometrium during Pregnancy and Labor." The Journal of Clinical Endocrinology and Metabolism 89: 5683–93. doi:10.1210/jc.2004-0873.

Chegini, N, X M Tang, and C Ma. 1999. "Regulation of Transforming Growth Factor-beta1 Expression by Granulocyte Macrophage-Colony-Stimulating Factor in Leiomyoma and Myometrial Smooth Muscle Cells." The Journal of Clinical Endocrinology and Metabolism 84 (11): 4138–43. doi:10.1210/jcem.84.11.6147.

Chio, Chung-Ching, Ying-Hsin Chang, Ya-Wen Hsu, Kwan-Hwa Chi, and Wan-Wan Lin. 2004. "PKA-Dependent Activation of PKC, p38 MAPK and IKK in Macrophage: Implication in the Induction of Inducible Nitric Oxide Synthase and Interleukin-6 by Dibutyryl cAMP." Cellular Signalling 16 (5): 565–75.

Cline, Melissa S, Michael Smoot, Ethan Cerami, Allan Kuchinsky, Nerius Landys, Chris Workman, Rowan Christmas, et al. 2007. "Integration of Biological Networks and Gene Expression Data Using Cytoscape." Nature Protocols 2 (10): 2366–82. doi:10.1038/nprot.2007.324.

Davis, S R, and R J Cousins. 2000. "Metallothionein Expression in Animals: A Physiological Perspective on Function." The Journal of Nutrition 130 (5): 1085–88.

Dubicke, A, P Andersson, E Fransson, E Andersson, A Sioutas, A Malmstrom, E Sverremark-Ekstrom, and G Ekman-Ordeberg. 2010. "High-Mobility Group Box Protein 1 and Its Signalling Receptors in Human Preterm and Term Cervix." J Reprod Immunol 84 (1): 86–94. doi:S0165-0378(09)00523-3 [pii]10.1016/j.jri.2009.09.010.

Duquette, R A, A Shmygol, C Vaillant, A Mobasheri, M Pope, T Burdyga, and Susan Wray. 2005. "Vimentin-Positive, c-Kit-Negative Interstitial Cells in Human and Rat Uterus: A Role in Pacemaking?" Biology of Reproduction 72: 276–83. doi:10.1095/biolreprod.104.033506.

Elliott, C L, D M Slater, W Dennes, L Poston, and P R Bennett. 2000. "Interleukin 8 Expression in Human Myometrium: Changes in Relation to Labor Onset and with Gestational Age." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 43 (5): 272–77.

Esplin, M Sean, M Bardett Fausett, Morgan R Peltier, Steven Hamblin, Robert M Silver, D Ware Branch, Eli Y Adashi, and David Whiting. 2005. "The Use of cDNA Microarray to Identify Differentially Expressed Labor-Associated Genes within the Human Myometrium during Labor." American Journal of Obstetrics and Gynecology 193 (2): 404–13. doi:10.1016/j.ajog.2004.12.021.

Fenstad, Mona H., Matthew P. Johnson, Linda T. Roten, Per A. Aas, Siri Forsmo, Kjetil Klepper, Christine E. East, et al. 2010. "Genetic and Molecular Functional Characterization of Variants within TNFSF13B, a Positional Candidate Preeclampsia Susceptibility Gene on 13q." PLoS ONE 5. doi:10.1371/journal.pone.0012993.

Friebe, Astrid, Alison J Douglas, Emilia Solano, Sandra M Blois, Evelin Hagen, Burghard F Klapp, David A Clark, and Petra C Arck. 2011. "Neutralization of LPS or Blockage of TLR4 Signaling Prevents Stress-Triggered Fetal Loss in Murine Pregnancy." Journal of Molecular Medicine (Berlin, Germany) 89 (7): 689–99. doi:10.1007/s00109-011-0743-5.

Gentleman, Robert C, Vincent J Carey, Douglas M Bates, Ben Bolstad, Marcel Dettling, Sandrine Dudoit, Byron Ellis, et al. 2004. "Bioconductor: Open Software Development for Computational Biology and Bioinformatics." Genome Biology 5 (10): R80. doi:10.1186/gb-2004-5-10-r80.

Germeyer, Ariane, Andrew Mark Sharkey, Mirari Prasadajudio, Robert Sherwin, Ashley Moffett, Karen Bieback, Susanne Clausmeyer, et al. 2009. "Paracrine Effects of Uterine Leucocytes on Gene Expression of Human Uterine Stromal Fibroblasts." Molecular Human Reproduction 15 (1): 39–48. doi:10.1093/molehr/gan075.

Gomez-Lopez, Nardhy, Rodrigo Vega-Sanchez, Marisol Castillo-Castrejon, Roberto Romero, Karen Cubeiro-Arreola, and Felipe Vadillo-Ortega. 2013. "Evidence for a Role for the Adaptive Immune Response in Human Term Parturition." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 69 (3): 212–30. doi:10.1111/aji.12074.

Guo, Wen-jing, Xun Qu, Mei-xiang Yang, Wei-dong Zhang, Lu Liang, Qian-qian Shao, and Bei-hua Kong. 2008. "Expression of BAFF in the Trophoblast and Decidua of Normal Early Pregnant Women and Patients with Recurrent Spontaneous Miscarriage." Chinese Medical Journal 121 (4): 309–15.

Haas, DM, DM Caldwell, P Kirkpatrick, J J McIntosh, and N J Welton. 2012. "Tocolytic Therapy for Preterm Delivery: Systematic Review and Network Meta-Analysis." BMJ 345: e6226.

Haddad, Ramsi, Gerard Tromp, Helena Kuivaniemi, Tinnakorn Chaiworapongsa, Yeon Mee Kim, Moshe Mazor, and Roberto Romero. 2006. "Human Spontaneous Labor without Histologic Chorioamnionitis Is Characterized by an Acute Inflammation Gene Expression Signature." American Journal of Obstetrics and Gynecology 195 (2): 394.e1–24. doi:10.1016/j.ajog.2005.08.057.

Hamidi, Tewfik, Hana Algül, Carla Eliana Cano, Maria José Sandi, Maria Inés Molejon, Marc Riemann, Ezequiel Luis Calvo, et al. 2012. "Nuclear Protein 1 Promotes Pancreatic Cancer Development and Protects Cells from Stress by Inhibiting Apoptosis." The Journal of Clinical Investigation 122 (6): 2092–2103. doi:10.1172/JCI60144.

Hamilton, Sarah A, Y Oomomian, G Stephen, O Shynlova, C L Tower, A Garrod, S J Lye, and R L Jones. 2012. "Macrophages Infiltrate the Human and Rat Decidua during Term and Preterm Labor: Evidence That Decidual Inflammation Precedes Labor." Biol Reprod 86 (2): 39. doi:biolreprod.111.095505 [pii] 10.1095/biolreprod.111.095505.

Hamilton, Sarah A, Clare L Tower, and Rebecca L Jones. 2013. "Identification of Chemokines Associated with the Recruitment of Decidual Leukocytes in Human Labour: Potential Novel Targets for Preterm Labour." PLoS One 8 (2): e56946. doi:10.1371/journal.pone.0056946.

Harris, H, R Henderson, R Bhat, and B Komm. 2001. "Regulation of Metallothionein II Messenger Ribonucleic Acid Measures Exogenous Estrogen Receptor-Beta Activity in SAOS-2 and LNCaPLN3 Cells." Endocrinology 142 (2): 645–52. doi:10.1210/endo.142.2.7952.

Hassan, Sonia S, Roberto Romero, Ramsi Haddad, Israel Hendler, Nahla Khalek, Gerard Tromp, Michael P Diamond, Yoram Sorokin, and John Malone. 2006. "The Transcriptome of the Uterine Cervix before and after Spontaneous Term Parturition." American Journal of Obstetrics and Gynecology 195 (3): 778–86. doi:10.1016/j.ajog.2006.06.021.

Havelock, Jon C, Patrick Keller, Ndaya Muleba, Bobbie A Mayhew, Brian M Casey, William E Rainey, and R Ann Word. 2005. "Human Myometrial Gene Expression before and during Parturition." Biology of Reproduction 72 (3): 707–19. doi:10.1095/biolreprod.104.032979.

Helguera, G, M Eghbali, D Sforza, T Y Minosyan, L Toro, and E Stefani. 2009. "Changes in Global Gene Expression in Rat Myometrium in Transition from Late Pregnancy to Parturition." Physiol Genomics 36 (2): 89–97. doi:10.1152/physiolgenomics.00290.2007.

Hever, Aniko, Richard B Roth, Peter Hevezi, Maria E Marin, Jose A Acosta, Hector Acosta, Jose Rojas, et al. 2007. "Human Endometriosis Is Associated with Plasma Cells and Overexpression of B Lymphocyte Stimulator." Proceedings of the National Academy of Sciences of the United States of America 104 (30): 12451–56. doi:10.1073/pnas.0703451104.

Hunt, S J, J L Pace, and R M Gill. 2010. "Immunoregulatory Molecules in Human Placentas: Potential for Diverse Roles in Pregnancy." International Journal of Developmental Biology 54 (2): 457.

Irizarry, Rafael A, Benjamin M Bolstad, Francois Collin, Leslie M Cope, Bridget Hobbs, and Terence P Speed. 2003. "Summaries of Affymetrix GeneChip Probe Level Data." Nucleic Acids Research 31 (4): e15.

Ivaska, Johanna, Hanna-Mari Pallari, Jonna Nevo, and John E Eriksson. 2007. "Novel Functions of Vimentin in Cell Adhesion, Migration, and Signaling." Experimental Cell Research 313 (10): 2050–62. doi:10.1016/j.yexcr.2007.03.040.

Jung, Jeenah, Aaron W Lifland, Eric J Alonas, Chiara Zurla, and Philip J Santangelo. 2013. "Characterization of mRNA-Cytoskeleton Interactions in Situ Using FMTRIP and Proximity Ligation." PloS One 8 (9): e74598. doi:10.1371/journal.pone.0074598.

Kato, Chikako, Takaya Tamura, Tomohiro Okuda, Hidenori Kojima, Yoshiyuki Kinoshita, and Hideo Honjo. 2004. "Expression of IL-4, IL-8 and IL-18 Messenger RNAs in Maternal Peripheral Blood and Relationships with the HbF-Gamma Chain mRNA in It." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 51 (1): 71–80.

Kelly, R W, P Illingworth, G Baldie, R Leask, S Brouwer, and A A Calder. 1994. "Progesterone Control of Interleukin-8 Production in Endometrium and Chorio-Decidual Cells Underlines the Role of the Neutrophil in Menstruation and Parturition." Hum Reprod 9 (2): 253–58.

Khanjani, Shirin, Mandeep K Kandola, Tamsin M Lindstrom, Suren R Sooranna, Manuela Melchionda, Yun S Lee, Vasso Terzidou, Mark R Johnson, and Phillip R Bennett. 2011. "NF-κB Regulates a Cassette of Immune/inflammatory Genes in Human Pregnant Myometrium at Term." Journal of Cellular and Molecular Medicine 15 (4): 809–24. doi:10.1111/j.1582-4934.2010.01069.x.

Khanjani, Shirin, Vasso Terzidou, Mark R Johnson, and Phillip R Bennett. 2012. "NFκB and AP-1 Drive Human Myometrial IL8 Expression." Mediators of Inflammation 2012 (January): 504952. doi:10.1155/2012/504952.

Kim, Hyun-A, Seong-Hyun Jeon, Goo-Young Seo, Jae-Bong Park, and Pyeung-Hyeun Kim. 2008. "TGF-beta1 and IFN-Gamma Stimulate Mouse Macrophages to Express BAFF via Different Signaling Pathways." Journal of Leukocyte Biology 83 (6): 1431–39. doi:10.1189/jlb.1007676.

Kim, Y M, R Romero, T Chaiworapongsa, G J Kim, M R Kim, H Kuivaniemi, G Tromp, et al. 2004. "Toll-like Receptor-2 and -4 in the Chorioamniotic Membranes in Spontaneous Labor at Term and in Preterm Parturition That Are Associated with Chorioamnionitis." Am J Obstet Gynecol 191 (4): 1346–55. doi:S0002937804007598 [pii]10.1016/j.ajog.2004.07.009.

Krucker, T, G R Siggins, and S Halpain. 2000. "Dynamic Actin Filaments Are Required for Stable Long-Term Potentiation (LTP) in Area CA1 of the Hippocampus." Proceedings of the National Academy of Sciences of the United States of America 97 (12): 6856–61. doi:10.1073/pnas.100139797.

Langat, Daudi L, David A Wheaton, Jeralyn Sue Platt, Travis Sifers, and Joan S Hunt. 2008. "Signaling Pathways for B Cell-Activating Factor (BAFF) and a Proliferation-Inducing Ligand (APRIL) in Human Placenta." The American Journal of Pathology 172: 1303–11. doi:10.2353/ajpath.2008.071139.

Lappas, Martha. 2012. "Nuclear Factor-κB Mediates Placental Growth Factor Induced pro-Labour Mediators in Human Placenta." Molecular Human Reproduction 18 (7): 354–61. doi:10.1093/molehr/gas007.

Li, Yan, Shujuan Zhong, and Ruojin Yao. 2013. "Influence of LPS and Toll-like Receptor 4 Antagonist on Progesterone Receptor, Interleukin-1β, and Cyclooxygenase-2 in Decidual Cells." Journal of Central South University. Medical Sciences 38 (2): 162–68. doi:10.3969/j.issn.1672-7347.2013.02.009.

Liang, Z, S R Sooranna, N Engineer, M Tattersall, S Khanjani, P R Bennett, L Myatt, and M R Johnson. 2008. "Prostaglandin F2-Alpha Receptor Regulation in Human Uterine Myocytes." Mol Hum Reprod 14 (4): 215–23. doi:gan008 [pii]10.1093/molehr/gan008.

Lilienbaum, A, M Duc Dodon, C Alexandre, L Gazzolo, and D Paulin. 1990. "Effect of Human T-Cell Leukemia Virus Type I Tax Protein on Activation of the Human Vimentin Gene." Journal of Virology 64 (1): 256–63.

Lim, J W, H Kim, and K H Kim. 2001. "Nuclear Factor-kappaB Regulates Cyclooxygenase-2 Expression and Cell Proliferation in Human Gastric Cancer Cells." Laboratory Investigation; a Journal of Technical Methods and Pathology 81 (3): 349–60.

Lim, Ratana, Gillian Barker, and Martha Lappas. 2014. "The TLR2 Ligand FSL-1 and the TLR5 Ligand Flagellin Mediate pro-Inflammatory and pro-Labour Response via MyD88/TRAF6/NF-κB-Dependent Signalling." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 71 (5): 401–17. doi:10.1111/aji.12229.

Lim, Sheri, David A MacIntyre, Yun S Lee, Shirin Khanjani, Vasso Terzidou, T G Teoh, and Phillip R Bennett. 2012. "Nuclear Factor Kappa B Activation Occurs in the Amnion prior to Labour Onset and Modulates the Expression of Numerous Labour Associated Genes." PloS One 7 (4): e34707. doi:10.1371/journal.pone.0034707.

Ma, Y, G Krikun, V M Abrahams, G Mor, and S Guller. 2007. "Cell Type-Specific Expression and Function of Toll-like Receptors 2 and 4 in Human Placenta: Implications in Fetal Infection." Placenta 28 (10): 1024–31. doi:10.1016/j.placenta.2007.05.003.

Min, K S, S Mukai, M Ohta, S Onosaka, and K Tanaka. 1992. "Glucocorticoid Inhibition of Inflammation-Induced Metallothionein Synthesis in Mouse Liver." Toxicology and Applied Pharmacology 113 (2): 293–98.

Mittal, Pooja, Roberto Romero, Adi L Tarca, Juan Gonzalez, Sorin Draghici, Yi Xu, Zhong Dong, et al. 2010. "Characterization of the Myometrial Transcriptome and Biological Pathways of Spontaneous Human Labor at Term." J Perinat Med 38 (6): 617–43. doi:10.1515/JPM.2010.097 [doi].

Montalbano, Alina P, Samuel Hawgood, and Carole R Mendelson. 2013. "Mice Deficient in Surfactant Protein A (SP-A) and SP-D or in TLR2 Manifest Delayed Parturition and Decreased Expression of Inflammatory and Contractile Genes." Endocrinology 154 (1): 483–98. doi:10.1210/en.2012-1797.

Morley, Melissa, Claire Jones, Mandeep Sidhu, Vishal Gupta, Suzanne M Bernier, Walter J Rushlow, and Daniel J Belliveau. 2010. "PKC Inhibition Increases Gap Junction Intercellular Communication and Cell Adhesion in Human Neuroblastoma." Cell and Tissue Research 340 (2): 229–42. doi:10.1007/s00441-010-0938-z.

Mor-Vaknin, Nirit, Antonello Punturieri, Kajal Sitwala, and David M Markovitz. 2003. "Vimentin Is Secreted by Activated Macrophages." Nature Cell Biology 5 (1). Nature Publishing Group: 59–63. doi:10.1038/ncb898.

Nepusz, Tamás, Haiyuan Yu, and Alberto Paccanaro. 2012. "Detecting Overlapping Protein Complexes in Protein-Protein Interaction Networks." Nature Methods 9 (5): 471–72. doi:10.1038/nmeth.1938.

Norman, J E, S Bollapragada, M Yuan, and S M Nelson. 2007. "Inflammatory Pathways in the Mechanism of Parturition." BMC Pregnancy Childbirth 7 Suppl 1: S7. doi:1471-2393-7-S1-S7 [pii]10.1186/1471-2393-7-S1-S7.

O'Brien, Margaret, John J Morrison, and Terry J Smith. 2008. "Upregulation of PSCDBP, TLR2, TWIST1, FLJ35382, EDNRB, and RGS12 Gene Expression in Human Myometrium at Labor." Reproductive Sciences (Thousand Oaks, Calif.) 15 (4): 382–93. doi:10.1177/1933719108316179.

Patni, Shalini, Louise P Wynen, Anna L Seager, Gareth Morgan, John O White, and Catherine A Thornton. 2009. "Expression and Activity of Toll-like Receptors 1-9 in the Human Term Placenta and Changes Associated with Labor at Term." Biology of Reproduction 80 (2): 243–48. doi:10.1095/biolreprod.108.069252.

Pawelczyk, Edyta, Bogdan J Nowicki, Michael G Izban, Siddharth Pratap, Nupur A Sashti, Maureen Sanderson, and Stella Nowicki. 2010. "Spontaneous Preterm Labor Is Associated with an Increase in the Proinflammatory Signal Transducer TLR4 Receptor on Maternal Blood Monocytes." BMC Pregnancy and Childbirth 10 (January): 66. doi:10.1186/1471-2393-10-66.

Peterman, E. E., P. 2nd Taormina, M. Harvey, and L. H. Young. 2004. "Go 6983 Exerts Cardioprotective Effects in Myocardial Ischemia/Reperfusion." Journal of Cardiovascular Pharmacology 43 (5): 645–56.

Phillips, Robert J, Michel A Fortier, and Andrés López Bernal. 2014. "Prostaglandin Pathway Gene Expression in Human Placenta, Amnion and Choriodecidua Is Differentially Affected by Preterm and Term Labour and by Uterine Inflammation." BMC Pregnancy and Childbirth 14 (1): 241. doi:10.1186/1471-2393-14-241.

Poligone, B, and A S Baldwin. 2001. "Positive and Negative Regulation of NF-kappaB by COX-2: Roles of Different Prostaglandins." The Journal of Biological Chemistry 276 (42): 38658–64. doi:10.1074/jbc.M106599200.

Re, F, and J L Strominger. 2001. "Toll-like Receptor 2 (TLR2) and TLR4 Differentially Activate Human Dendritic Cells." The Journal of Biological Chemistry 276 (40): 37692–99. doi:10.1074/jbc.M105927200.

Rehman, K S, S Yin, B A Mayhew, R A Word, and W E Rainey. 2003. "Human Myometrial Adaptation to Pregnancy: cDNA Microarray Gene Expression Profiling of Myometrium from Non-Pregnant and Pregnant Women." Mol Hum Reprod 9 (11): 681–700.

Renthal, N E, C C Chen, K C Williams, R D Gerard, J Prange-Kiel, and C R Mendelson. 2010. "miR-200 Family and Targets, ZEB1 and ZEB2, Modulate Uterine Quiescence and Contractility during Pregnancy and Labor." Proceedings of the National Academy of Sciences 107 (48): 20828–33. doi:10.1073/pnas.1008301107.

Robertson, Sarah A, Inge Christiaens, Camilla L Dorian, Dean B Zaragoza, Alison S Care, Anke M Banks, and David M Olson. 2010. "Interleukin-6 Is an Essential Determinant of on-Time Parturition in the Mouse." Endocrinology 151 (8): 3996–4006. doi:en.2010-0063 [pii]10.1210/en.2010-0063.

Rudra, Carole B, Ihunnaya O Frederick, and Michelle A Williams. 2008. "Pre-Pregnancy Body Mass Index and Weight Gain during Pregnancy in Relation to Preterm Delivery Subtypes." Acta Obstetricia et Gynecologica Scandinavica 87 (5): 510–17. doi:10.1080/00016340801996838.

Saito, Rintaro, Michael E Smoot, Keiichiro Ono, Johannes Ruscheinski, Peng-Liang Wang, Samad Lotia, Alexander R Pico, Gary D Bader, and Trey Ideker. 2012. "A Travel Guide to Cytoscape Plugins." Nature Methods 9 (11): 1069–76. doi:10.1038/nmeth.2212.

Satelli, Arun, and Shulin Li. 2011. "Vimentin in Cancer and Its Potential as a Molecular Target for Cancer Therapy." Cellular and Molecular Life Sciences : CMLS 68: 3033–46. doi:10.1007/s00018-011-0735-1.

Schindler, U, and V R Baichwal. 1994. "Three NF-Kappa B Binding Sites in the Human E-Selectin Gene Required for Maximal Tumor Necrosis Factor Alpha-Induced Expression." Molecular and Cellular Biology 14 (9): 5820–31.

Seghaye, M C, W Heyl, R G Grabitz, K Schumacher, G von Bernuth, W Rath, and J Duchateau. 1998. "The Production of pro- and Anti-Inflammatory Cytokines in Neonates Assessed by Stimulated Whole Cord Blood Culture and by Plasma Levels at Birth." Biology of the Neonate 73 (4): 220–27.

Shankar, R, M P Johnson, N A Williamson, F Cullinane, A W Purcell, E K Moses, and S P Brennecke. 2010. "Molecular Markers of Preterm Labor in the Choriodecidua." Reprod Sci 17 (3): 297–310. doi:1933719109353454 [pii]10.1177/1933719109353454.

Shannon, Paul, Andrew Markiel, Owen Ozier, Nitin S Baliga, Jonathan T Wang, Daniel Ramage, Nada Amin, Benno Schwikowski, and Trey Ideker. 2003. "Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks." Genome Research 13 (11): 2498–2504. doi:10.1101/gr.1239303.

Sharkey, James, and James Olcese. 2007. "Transcriptional Inhibition of Oxytocin Receptor Expression in Human Myometrial Cells by Melatonin Involves Protein Kinase C Signaling." The Journal of Clinical Endocrinology and Metabolism 92 (10). Endocrine Society: 4015–19. doi:10.1210/jc.2007-1128.

Sheldon, I M, and M H Roberts. 2010. "Toll-like Receptor 4 Mediates the Response of Epithelial and Stromal Cells to Lipopolysaccharide in the Endometrium." PLoS One 5 (9): e12906. doi:10.1371/journal.pone.0012906.

Shen, Chung-Min, Shih-Chang Lin, Dau-Ming Niu, and Yu Ru Kou. 2009. "Labour Increases the Surface Expression of Two Toll-like Receptors in the Cord Blood Monocytes of Healthy Term Newborns." Acta Paediatrica (Oslo, Norway : 1992) 98 (6): 959–62. doi:10.1111/j.1651-2227.2009.01280.x.

Shynlova, Oksana, Yu-Hui H Lee, Khetsopon Srikhajon, and Stephen J Lye. 2013a. "Physiologic Uterine Inflammation and Labor Onset: Integration of Endocrine and Mechanical Signals." Reproductive Sciences (Thousand Oaks, Calif.) 20 (2): 154–67. doi:10.1177/1933719112446084.

Shynlova, Oksana, Tamara Nedd-Roderique, Yunqing Li, Anna Dorogin, Tina Nguyen, and Stephen J. Lye. 2013b. "Infiltration of Myeloid Cells into Decidua Is a Critical Early Event in the Labour Cascade and Post-Partum Uterine Remodelling." Journal of Cellular and Molecular Medicine 17 (2): 311–24. doi:10.1111/jcmm.12012.

Slater, E P, A C Cato, M Karin, J D Baxter, and M Beato. 1988. "Progesterone Induction of Metallothionein-IIA Gene Expression." Molecular Endocrinology (Baltimore, Md.) 2 (6): 485–91. doi:10.1210/mend-2-6-485.

Smoot, Michael E, Keiichiro Ono, Johannes Ruscheinski, Peng-Liang Wang, and Trey Ideker. 2011. "Cytoscape 2.8: New Features for Data Integration and Network Visualization." Bioinformatics (Oxford, England) 27 (3): 431–32. doi:10.1093/bioinformatics/btq675.

Stephen, Gillian L, Sylvia Lui, Sarah A Hamilton, Clare L Tower, Lynda K Harris, Adam Stevens, and Rebecca L Jones. 2014. "Transcriptomic Profiling of Human Choriodecidua During Term Labor: Inflammation as a Key Driver of Labor." American Journal of Reproductive Immunology (New York, N.Y. : 1989), October. doi:10.1111/aji.12328.

Szalay-Beko, Máté, Robin Palotai, Balázs Szappanos, István a Kovács, Balázs Papp, and Péter Csermely. 2012. "ModuLand Plug-in for Cytoscape: Determination of Hierarchical Layers of Overlapping Network Modules and Community Centrality." Bioinformatics (Oxford, England) 28 (16): 2202–4. doi:10.1093/bioinformatics/bts352.

Takeda, Kiyoshi, and Shizuo Akira. 2004. "TLR Signaling Pathways." Seminars in Immunology 16 (1): 3–9.

Taniguchi, Yoshi, Noriko Yoshioka, Kazue Nakata, Takashi Nishizawa, Hiroyuki Inagawa, and Chie Kohchi. 2009. "Mechanism for Maintaining Homeostasis in the Immune System of the Intestine." Anticancer Res 29 (11): 4855–60.

Toshchakov, Vladimir, Bryan W Jones, Pin-Yu Perera, Karen Thomas, M Joshua Cody, Shuling Zhang, Bryan R G Williams, et al. 2002. "TLR4, but Not TLR2, Mediates IFN-Beta-Induced STAT1alpha/beta-Dependent Gene Expression in Macrophages." Nature Immunology 3 (4): 392–98. doi:10.1038/ni774.

Tsuruta, Daisuke, and Jonathan C R Jones. 2003. "The Vimentin Cytoskeleton Regulates Focal Contact Size and Adhesion of Endothelial Cells Subjected to Shear Stress." Journal of Cell Science 116: 4977–84. doi:10.1242/jcs.00823.

Vašák, Milan, and Gabriele Meloni. 2011. "Chemistry and Biology of Mammalian Metallothioneins." Journal of Biological Inorganic Chemistry. doi:10.1007/s00775-011-0799-2.

Wahid, Hanan H., Camilla L. Dorian, Peck Yin Chin, Mark R. Hutchinson, Kenner C. Rice, David M. Olson, Lachlan M. Moldenhauer, and Sarah A. Robertson. 2015. "Toll-Like Receptor 4 Is an Essential Upstream Regulator of On-Time Parturition and Perinatal Viability in Mice." Endocrinology 156 (10): 3828–41. doi:10.1210/en.2015-1089.

Wang, Lijian, Lynne Harrington, Estela Trebicka, Hai Ning Shi, Jonathan C Kagan, Charles C Hong, Herbert Y Lin, Jodie L Babitt, and Bobby J Cherayil. 2009. "Selective Modulation of TLR4-Activated Inflammatory Responses by Altered Iron Homeostasis in Mice." The Journal of Clinical Investigation 119 (11): 3322–28. doi:10.1172/JCI39939.

Weiner, C P, C W Mason, Y Dong, I A Buhimschi, P W Swaan, and C S Buhimschi. 2010. "Human Effector/initiator Gene Sets That Regulate Myometrial Contractility during Term and Preterm Labor." Am J Obstet Gynecol 202 (5): 474 e1–20. doi:S0002-9378(10)00247-4 [pii]10.1016/j.ajog.2010.02.034.

Williams, K C, N E Renthal, J C Condon, R D Gerard, and C R Mendelson. 2012. "MicroRNA-200a Serves a Key Role in the Decline of Progesterone Receptor Function Leading to Term and Preterm Labor." Proc Natl Acad Sci U S A 109 (19): 7529–34. doi:1200650109 [pii]10.1073/pnas.1200650109.

Wu, A L, J Wang, A Zheleznyak, and E J Brown. 1999. "Ubiquitin-Related Proteins Regulate Interaction of Vimentin Intermediate Filaments with the Plasma Membrane." Mol Cell 4 (4): 619–25. doi:S1097-2765(00)80212-9 [pii].

Youssef, Refaat E, Marie Anne Ledingham, Shrikant S Bollapragada, Neil O'Gorman, Fiona Jordan, Anne Young, and Jane E Norman. 2009. "The Role of Toll-like Receptors (TLR-2 and -4) and Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) in Human Term and Preterm Labor." Reproductive Sciences (Thousand Oaks, Calif.) 16 (9): 843–56. doi:10.1177/1933719109336621.

Yu, Chih Wen, Je Hsin Chen, and Lih Yuan Lin. 1997. "Metal-Induced Metallothionein Gene Expression Can Be Inactivated by Protein Kinase C Inhibitor." FEBS Letters 420 (1): 69–73. doi:10.1016/S0014-5793(97)01489-0.

Zhang, Cuicui, Miaohua Mo, Wenwen Ding, Wenjuan Liu, Dewen Yan, Jianxin Deng, Xinping Luo, and Jie Liu. 2014. "High-Mobility Group Box 1 (HMGB1) Impaired Cardiac Excitation-Contraction Coupling by Enhancing the Sarcoplasmic Reticulum (SR) Ca(2+) Leak through TLR4-ROS Signaling in Cardiomyocytes." Journal of Molecular and Cellular Cardiology 74 (September): 260–73. doi:10.1016/j.yjmcc.2014.06.003.

Figures and tables

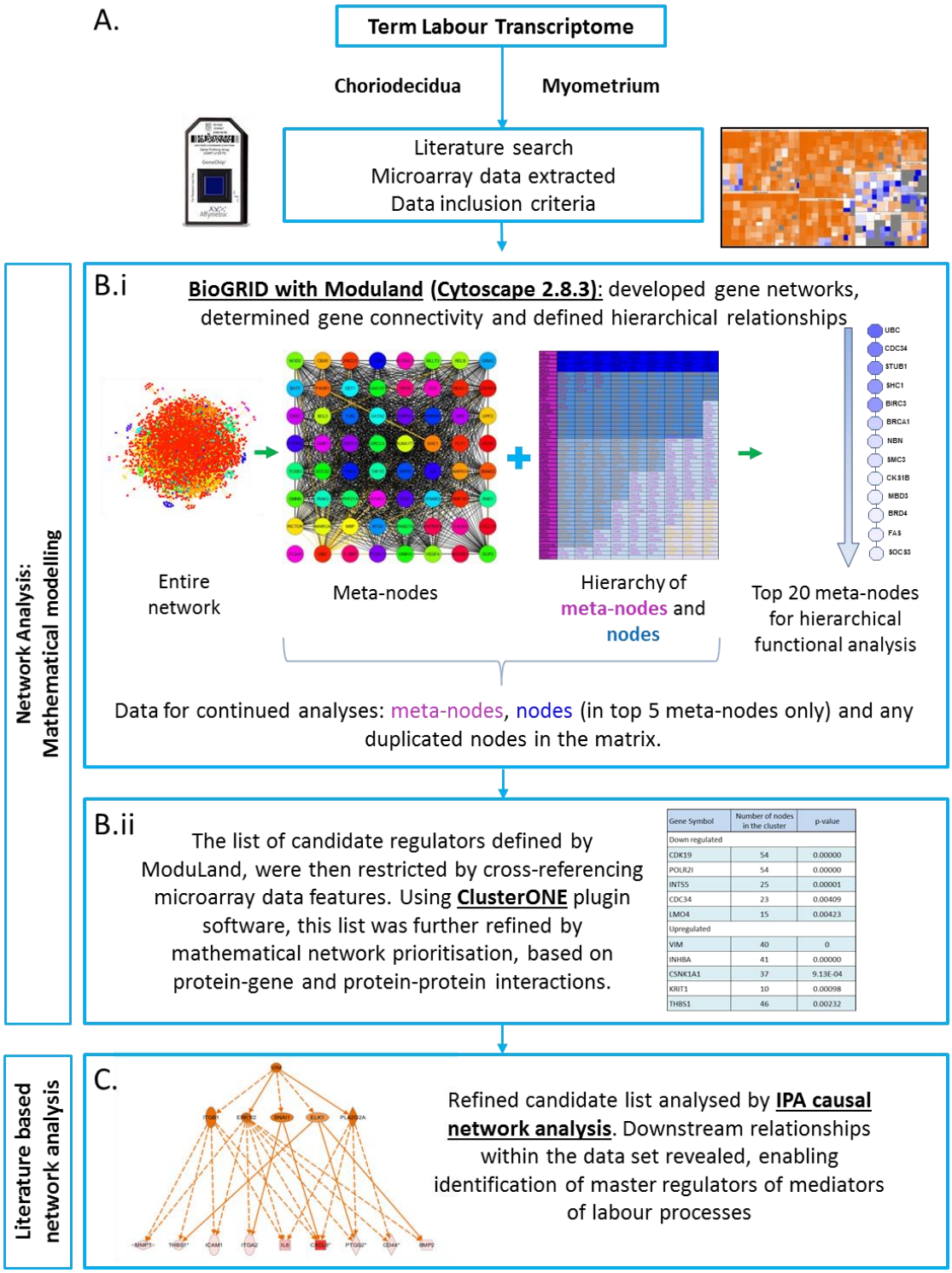


Figure 1. Workflow of data analysis and candidate target identification.

A. The microarray data was extracted and processed using a strict n inclusion criteria of PPLR 0.997 and 0.00001 (choriodecidua) and p<0.02 (myometrium). B. The database BioGRID and network analysis software Cytoscape with i. ModuLand and ii. ClusterONE was used to identify candidate master regulators with high centrality and connectivity. C The relationships of the master regulator candidates with related genes in the network was analysed with causal analysis in the software package Ingenuity Pathway Analysis.

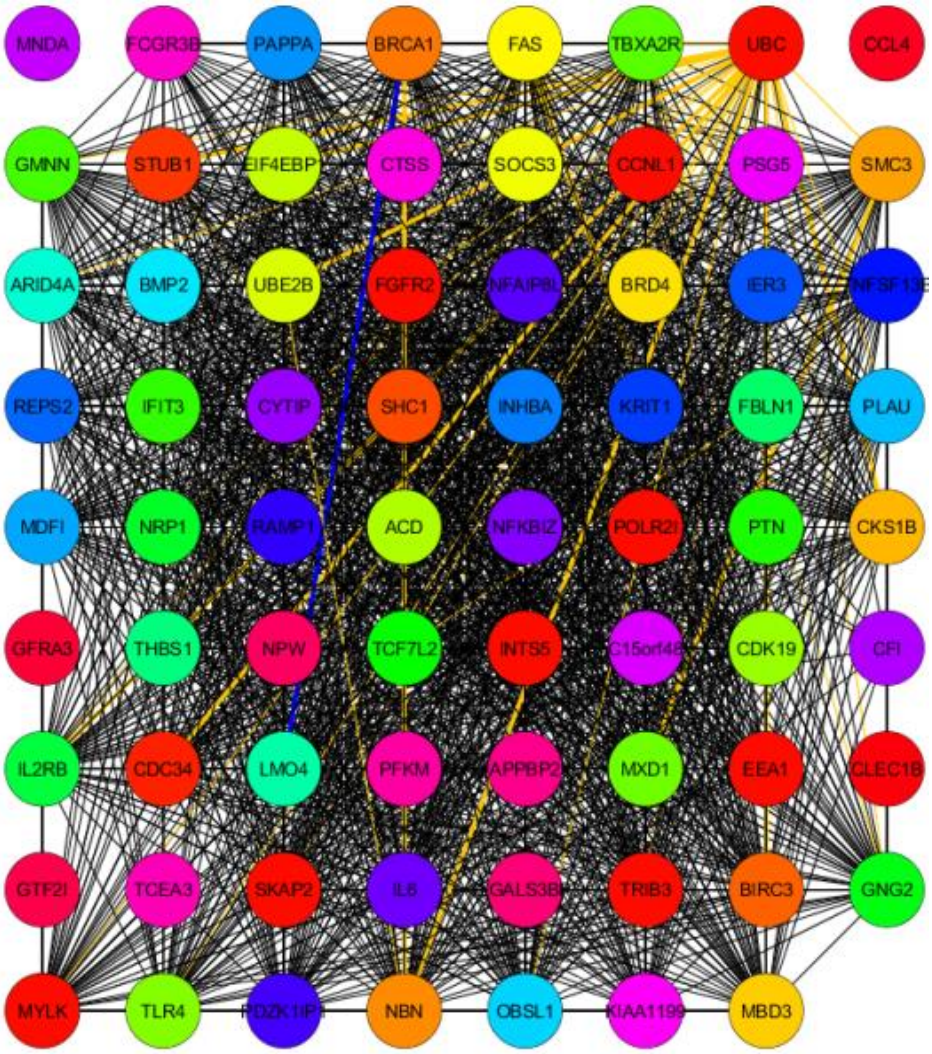


Figure 2. Hierarchical network modelling of the meta-nodes of the human choriodecidua.

The upper level of network clusters assessed using the ModuLand algorithm in Cytoscape 2.8.3. These 71 principle clusters are meta-nodes and the 1610 interactions of active labour in the choriodecidua. The lines indicate interactions between meta-nodes and lower level nodes. The yellow lines indicate highly ranked major interactions.

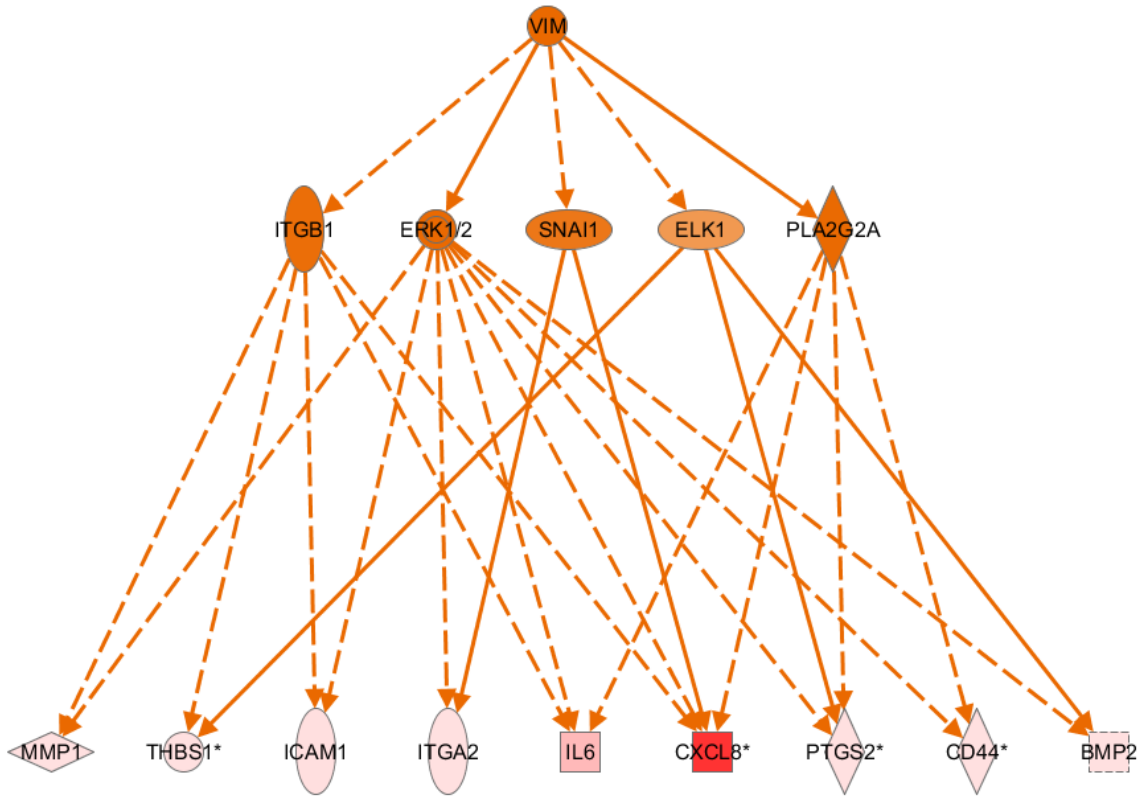


Figure 3. The causal network analysis of vimentin identifying relationships with labour-associated choriodecidual genes downstream.

Vimentin is shown as the master regulator with five lower level regulators. The bottom level is an edited summary of genes affected (full map includes 41 genes). This map shows only the major downstream genes with i) at least two network connections (dashed line) or ii) have multiple associated functions from a single regulator (solid line). Upregulated regulator= orange; upregulated gene=red to pink; The intensity of colour indicates the scale of upregulated expression i.e. upregulation of VIM increased the expression of all the downstream genes indicated, but the expression of CXCL8 was the most affected.

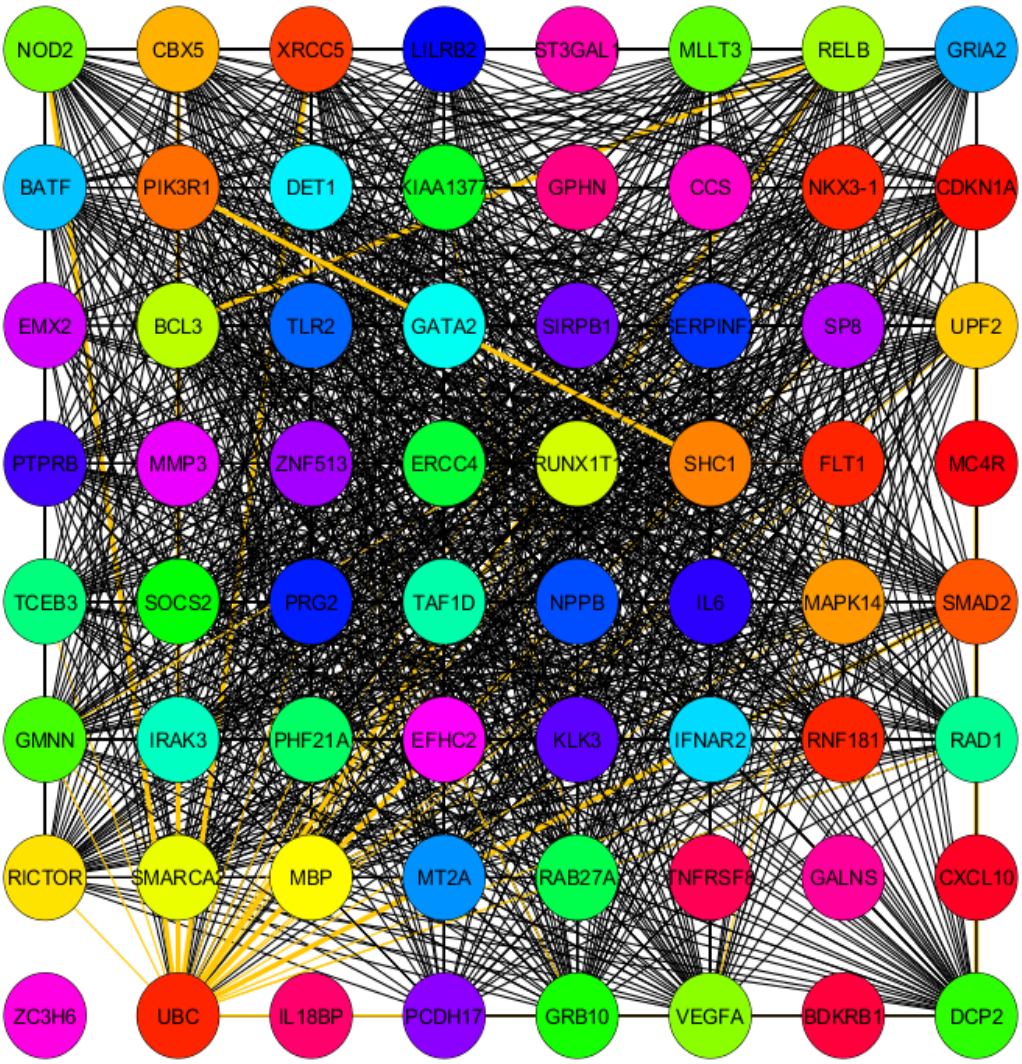


Figure 4. Hierarchical network modelling of metanode-node-gene expression of the human myometrium.

The upper level of network clusters assessed using the Moduland algorithm in Cytoscape 2.8.3. These 64 clusters are meta-nodes identifying all lower level nodes and 1277 interactions. The lines indicate interactions between meta-nodes and lower level nodes. The yellow lines indicate highly ranked major interactions.

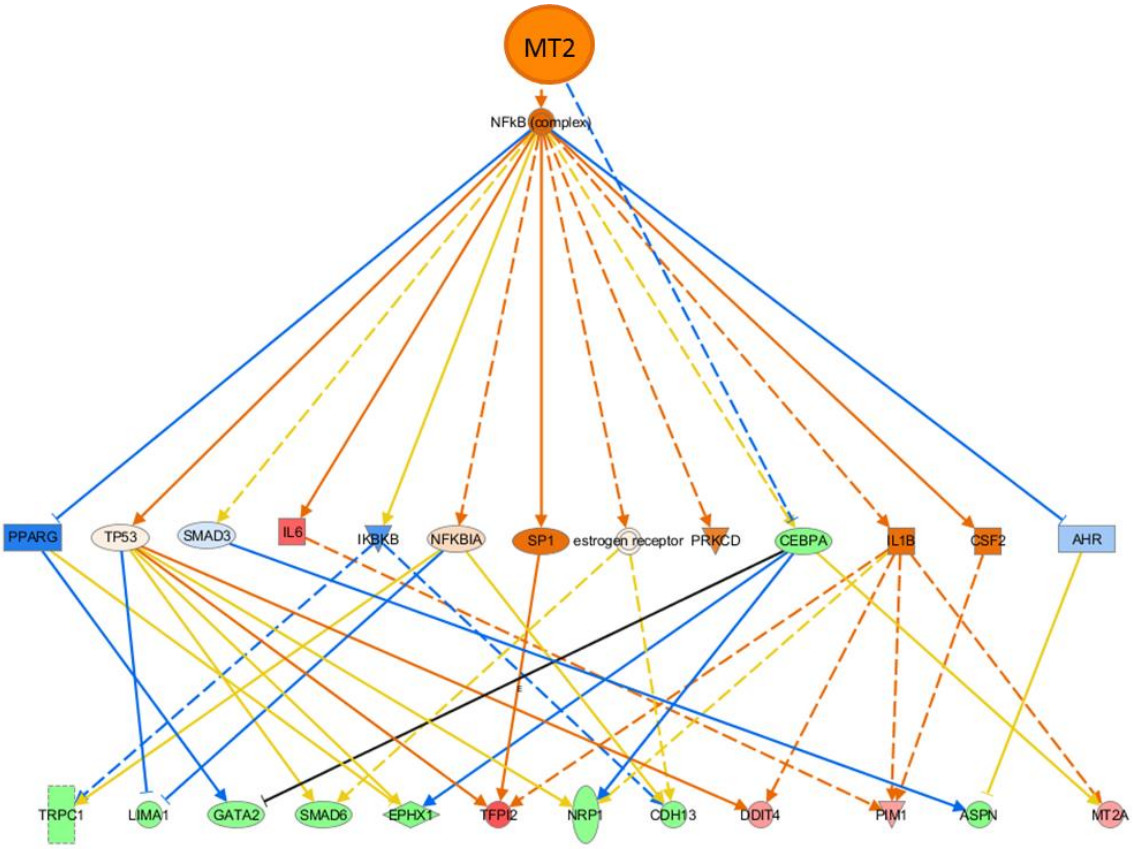


Figure 5. Causal network analysis of metallothionein 2, identifying relationships with labour-associated myometrial genes downstream.

Metallothionein 2 is shown as the top regulator above NFkB. At the next level are 13 lower level regulators. The bottom level is an edited summary of genes affected downstream (full map includes 30 genes). This map shows all the regulators and only the major downstream genes with i) at least two network connections (dashed line) or ii) have multiple associated functions from a single regulator (solid line). Upregulated regulator= orange; downregulated regulator= blue; upregulated gene=red to pink, downregulated gene = green; The intensity of colour indicates the scale of change e.g. the upregulation of MT2 leads to the activation of NFkB complex, inhibiting (blue line) PPARG (dark blue; on the left of the network) which leads to the downregulation of GATA2 (green with blue line) and an unpredicted (yellow line) downregulation of EPHX1 (green).

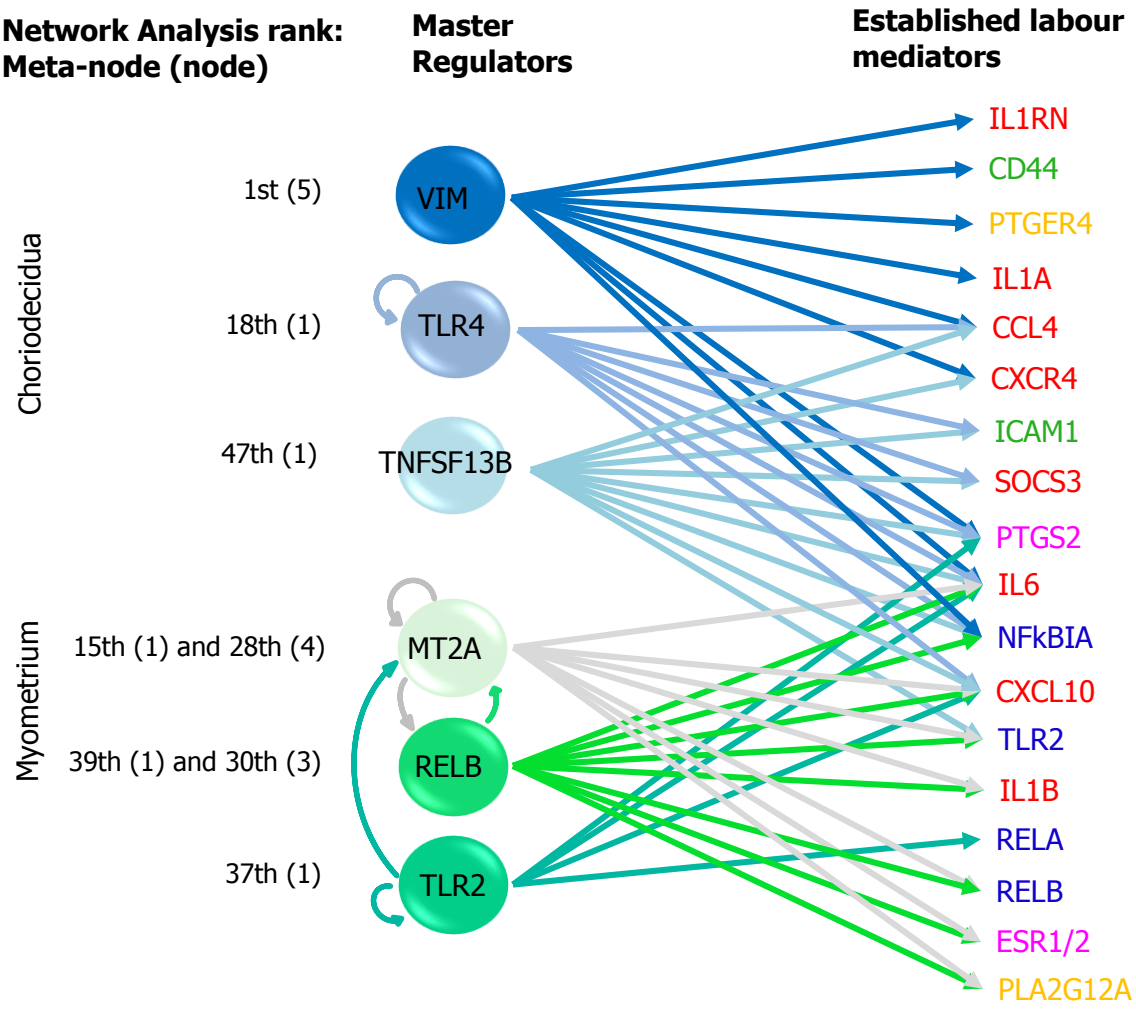
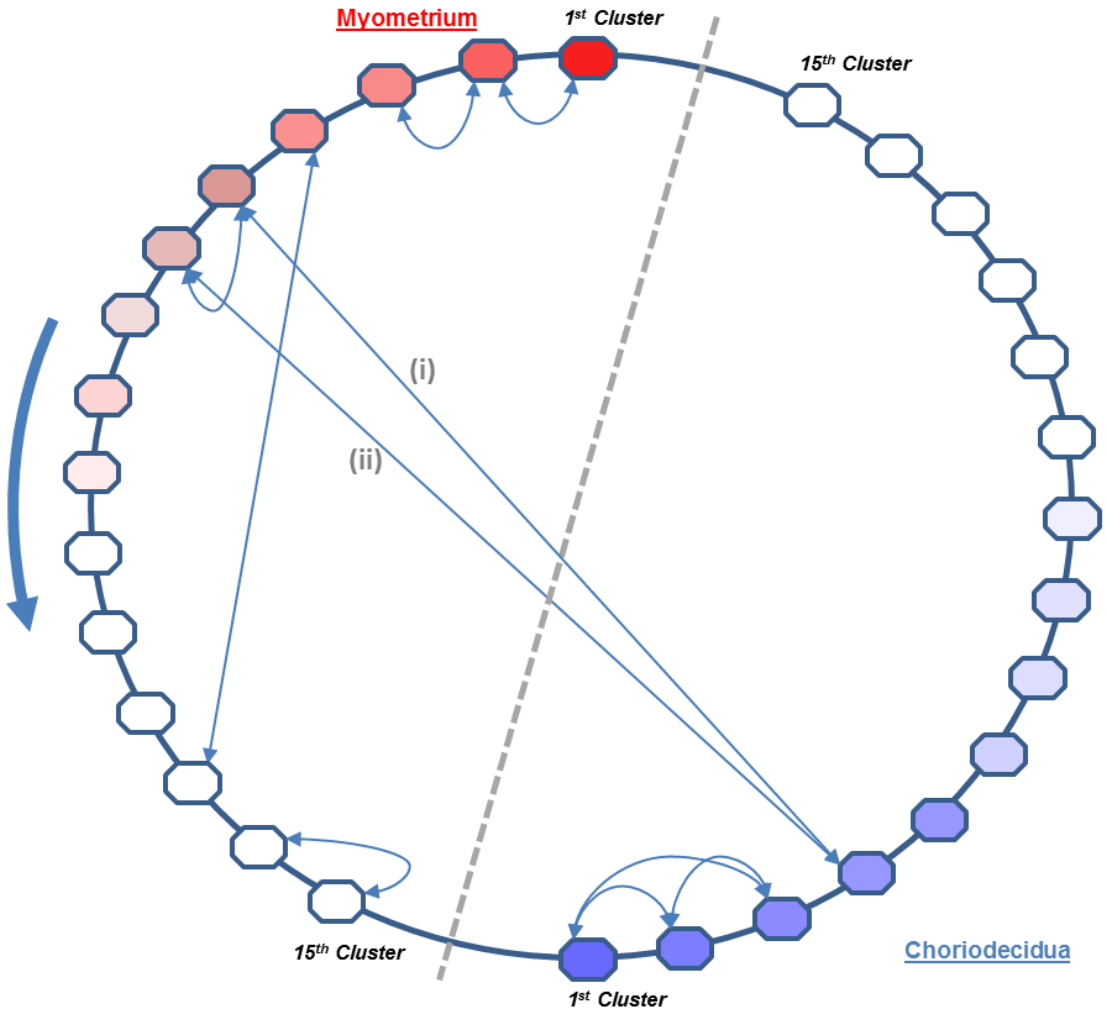


Figure 6. Summary of the identified master regulators, and their predicted effects on labour associated genes of the labouring choriodecidua and myometrium, with their centrality and causal relationships.

The left-side identifies the relationship within the network by the meta-node rank and location within the meta-node (node). The middle are the identified master regulators with arrows indicating a regulatory relationship, with the upper three are from the choriodecidua (blue), and the bottom three from the myometrium (green). The right-side are genes that are well-established to change in expression during labour. The labour mediators are colour-coded according to their functions and include inflammatory-associated (red), eicosanoids (yellow), cellular adhesion (green), estrogen (pink), NFkB complex pathway (blue). Several of these downstream genes overlap between the identified master regulators, and some are shared between the master regulators and between the two tissue types.



Intersection	Myometrium Related Cluster	Choriodecidua Related Cluster	Number of Genes in Overlap
(i)	**PIK3R1** (5th)	**SHC1** (4th)	**4**
(ii)	**SHC1** (6th)	**SHC1** (4th)	**5**

Figure 7. Connective relationships between the network models based on the metanode-node-gene relationships between the human choriodecidua and myometrium.

Clusters are represented as octagons and arranged hierarchically by colour intensity in an anticlockwise direction (marked by rank; see table 2 and 4). The biologically common relationships between the meta-node clusters are marked by two headed arrow, where they share at least 3 clusters of genes (nodes). Dotted grey line separates the different network models. The intersection demonstrates two major meta-nodes sharing a significant number of nodes and genes: PIK3R1 and SHC1.

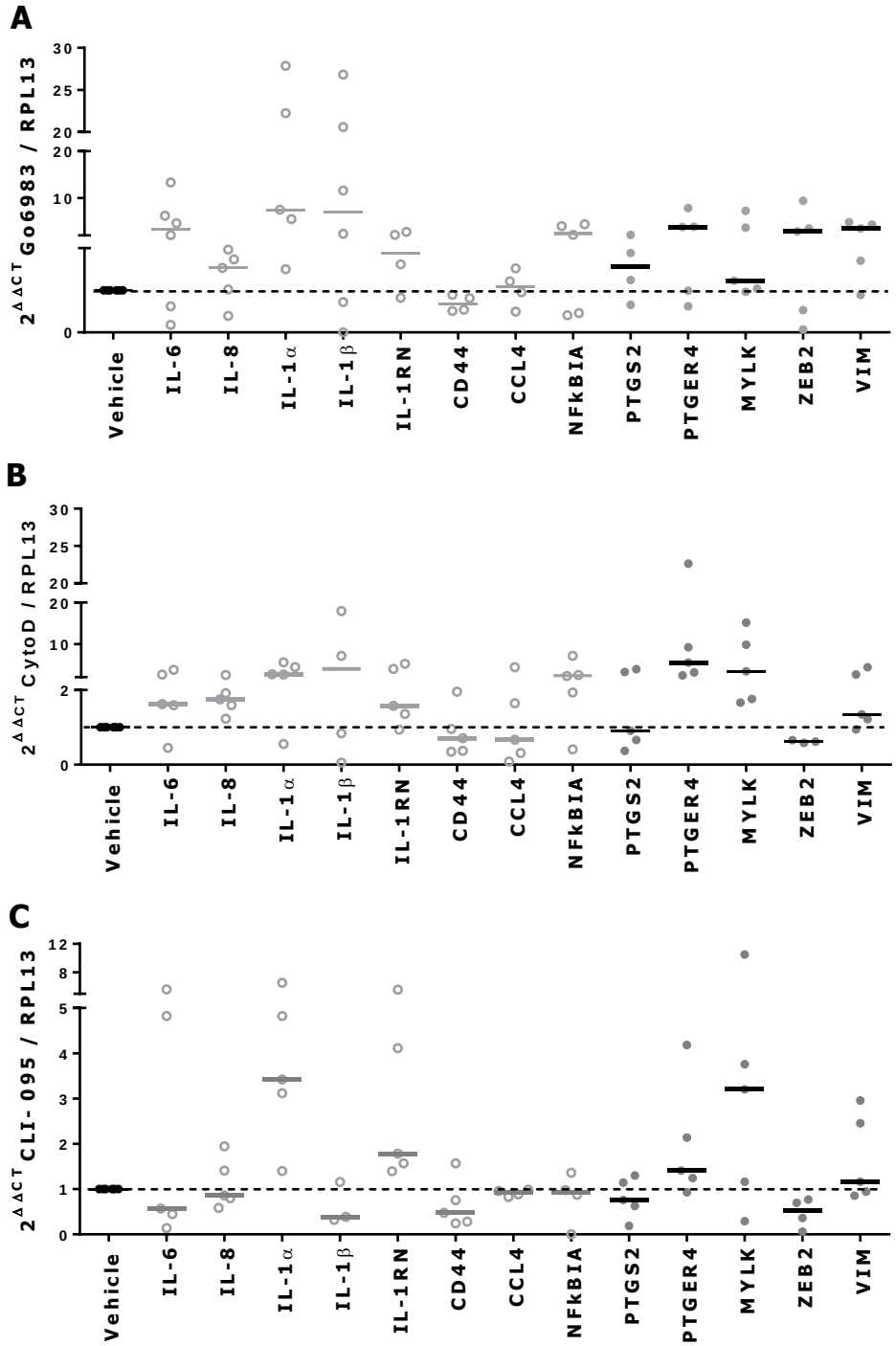


Figure 8. Choriodecidual expression of downstream labour-associated genes after the inhibition of master regulators.

Inhibition of VIM with A. Go6983 and B. cytochalsinD. Inhibition of TLR4 with C. CLI-095 (n=4-6). All results are expressed using the comparative $2^{\Delta\Delta CT}$ method, to the vehicle control (DMSO 0.002% v/v) and normalised to RPL13 reference gene. Inflammatory-related regulators are in light grey. Genes well-known to be associated with the labouring process in dark grey. Medians are shown.

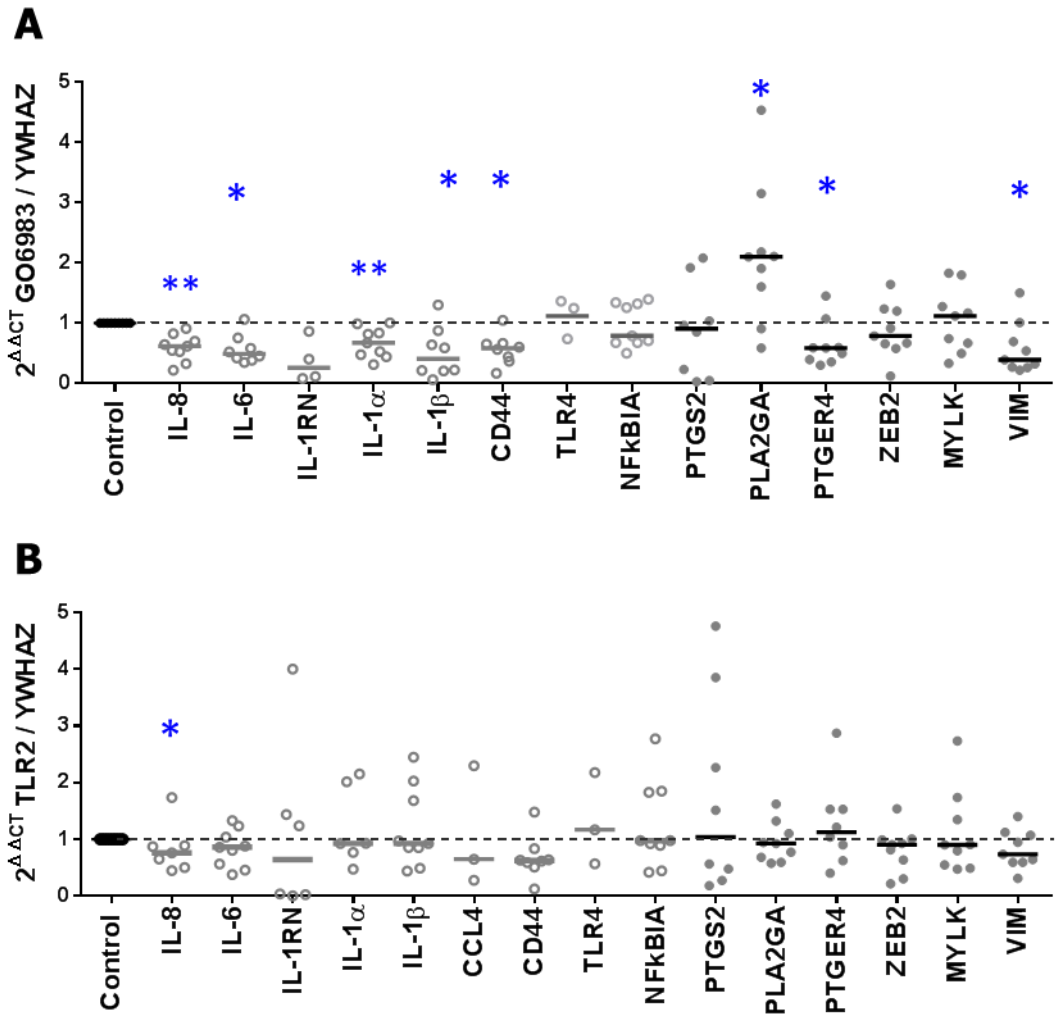


Figure 9. Myometrial cell expression of downstream labour-associated genes after the inhibition of master regulators.

A. Metallothionein inhibitor (G6983) and B. TLR2 neutralising antibody (Mab-hTLR2) (n=8). All results are expressed using the comparative $2^{\Delta\Delta CT}$ method, to the appropriate control: vehicle (DMSO 0.002% v/v) or control (media) respectively, and normalised to YWHAZ reference gene. Inflammatory genes are in light grey. Genes well-known to be associated with the labouring process in dark grey. Medians are shown. Significance at $p \leq 0.05$ using Wilcoxon-signed rank test (*$p \leq 0.05$; **$p \leq 0.01$).

Table 1. Primers used for changes in mRNA expression.

A. reference genes analysed for stable expression. B genes of interest selected from the array and from the downstream genes of the network analysis.

A

Gene	Forward 5'	Reverse 3'	Accession number
TBP	CACGAACCACGGCACTGATT	TTTTCTTGCTGCCAGTCTGGAC	NM_003194
YWHAZ	ACTTTTGGTACATTGTGGCTTCAA	CCGCCAGGACAAACCAGTAT	NM_003406
β-actin	ATGTGGCCGAGGACTTTGATT	AGTGGGGTGGCTTTTAGGATG	NM_001101
RPL13	CTTCTCGGCCTGTTTCCGTAG	CGAGGTTGGCTGGAAGTACC	NM_012423

B

Gene	Forward primer 5'	Reverse primer 3'	Accession number
CCL4	CAGCACCAATGGGCTCAGA	CACTGGGATCAGCACAGACT	NM_002984
CD44	CTGCCGCTTTGCAGGTGTA	CATTGTGGGCAAGGTGCTATT	NM_000610.3
IL1-RN	CGGGTGCTACTTTATGGGCA	GGTCGGCAGATCGTCTCTAA	NM_000577
IL-1α	TGGTAGTAGCAACCAACGGGA	ACTTTGATTGAGGGCGTCATTC	NM_000575.3
IL-1β	CTCGCCAGTGAAATGATGGCT	GTCGGAGATTCGTAGCTGGAT	NM_000576.2
IL-6	CCTGAACCTTCCAAAGATGGC	TTCACCAGGCAAGTCTCCTCA	NM_000600
IL-8	CACCGGAAGGAACCATCTCACT	TGGGGACACCTTTTAGCATC	NM_000584.3
MYLK	CCCGAGGTTGTCTGGTTCAAA	GCAGGTGTACTTGGCATCGT	NM_053025
NFKBIA	CCAACTACAATGGCCACACGTGTCTACA	GAGCATTGACATCAGCACCCAAGG	NM_020529
PLA2G4A	TACCAGCACATTATAGTGGAGCA	GCTGTCAGGGGTTGTAGAGAT	NM_024420
PTGER4	CATCATCTGCGCCATGAGTGT	GCTTGTCCACGTAGTGGCT	NM_000958
PTGS2	CGATGCTCATGCTCTTCGC	GGGAGACTGCATAGATGACAGG	NM_000956.3
TLR4	AGACCTGTCCCTGAACCCTAT	CGATGGACTTCTAAACCAGCCA	NM_138554.4
VIM	AGTCCACTGAGTACCGGAGAC	CATTTCACGCATCTGGCGTTC	NM_003380.3
ZEB2	TCTGTAGATGGTCCAGTGAAGA	GTCACTGCGCTGAAGGTACT	NM_001171653.1

Table 2. The functions, pathways and top core nodes that form each of the major meta-nodes of the choriodecidual network map.

The left most column are the top 20 meta-nodes. The middle column lists the first 10 core nodes that cluster to form the meta-node. The right column describes the functions and canonical pathways of the cluster. The colours are arbitrary but are used here to indicate a ranking in hierarchical clustering based on interactions (dark colour = high clustering interactions indicating possible regulatory functions, to light colour = lower interactions). The highest ranked meta-nodes include: ubiquitin, CDC34 and STUB1. Within these meta-nodes the core nodes also highly ranked in interaction, they include NFKBIA, NOTCH1 and VIM. This table represents only part of the 71 meta-nodes with 1610 interactions.

Cluster	Core nodes of cluster	Hierarchy of Functions and Pathways
UBC	**UBC**, NFKBIA, RUVBL2, NOTCH1, VIM, UBE2D3, RPS4X, ARRB1, RPSA, XPO1	Cellular responses to stress, Apoptosis, Circadian clock, DNA repair ($p<1.0X10^{-4}$). G1/S DNA damage checkpoint ($p<3.4x10^{-6}$)
CDC34	**CDC34**, UBE2D3, UBC, RBX1, ARRB1, MDM2, CUL1, RUVBL2, NOTCH1, NRF1	Cellular responses to hypoxia, Apoptosis, Circadian clock, DNA repair ($p<1.0X10^{-4}$). G1/S DNA damage checkpoint ($p<2.0x10^{-7}$)
STUB1	**STUB1**, UBC, UBE2D3, ARRB1, HSP90AA1, HSPA4, UBE2D1, CSNK1A1, PARK7, TP53	Cellular responses to hypoxia, Apoptosis, Circadian clock, DNA repair ($p<1.0X10^{-4}$). G1/S DNA damage checkpoint ($p<2.1x10^{-7}$)
SHC1	**SHC1**, GRB2, LCP2, EGFR, ERBB3, CBL, PAG1, SOS1, ERBB2, GRAP2	Adaptive and innate immunity ($p<3.8x10^{-6}$), Growth factor signalling ($p<3.9x10^{-7}$)
BIRC3	**BIRC3**, TRAF2, TNFAIP3, USP53, UBE2D2, BCL10, RIPK1, PEG3, TRAF6, DIABLO	Innate immunity ($p<1.4x10^{-6}$)
BRCA1	**BRCA1**, BRIP1, BACH1, NBN, LMO4, MLH1, RPA1, PMS2, HNRNPA2B1, EZR	DNA repair ($p<2.1x10^{-7}$)
NBN	**NBN**, MRE11A, RAD50, H2AFX, MDC1, CALR, ATM, USP53, BACH1, EP300	Cellular senescence ($p<1.5x10^{-5}$), DNA repair ($p<1.8x10^{-9}$)
SMC3	**SMC3**, RAD21, SMC1A, CASP4, STAG1, ANKRD28, S100A9, PDS5A, STAG2, CLU	Cell cycle ($p<1.3x10^{-4}$)
CKS1B	**CKS1B**, CDK2, SKP2, CDK1, MCM2, SKP1, CDKN1B, COPS6, CCNA2, CCNG2	G1/S transition ($p<6.5x10^{-9}$), DNA replication ($p<7.5x10^{-7}$)
MBD3	**MBD3**, HDAC1, BCL11B, HDAC2, MTA2, MBD2, MXD1, ATF3, RBBP4, ARID4A	Chromatin organisation ($p<5.6x10^{-8}$)
BRD4	**BRD4**, CDK9, AFF4, CCNT2, ELL2, CCNT1, MLLT3, MLLT1, AFF1, HEXIM1	TGFB signalling ($p<7.8x10^{-5}$)
FAS	**FAS**, FADD, DAXX, FASLG, KRIT1, FAF1, CASP10, CASP8, TGFB2, CFLAR	Apoptosis ($p<8.2x10^{-10}$)
SOCS3	**SOCS3**, JAK2, CSF2RB, TCEB1, PRLR, PTPN11, TCEB2, IL2RB, IFNGR1, JAK1	Cytokine signalling ($p<1.3x10^{-7}$)
UBE2B	**UBE2B**, RAD18, UBR2, UBR3, UBA1, CTNNB1, UBQLN1, DSTN, PCNA, CPLX1	Adaptive immune system ($p<3.5x10^{-2}$)
EIF4EBP1	**EIF4EBP1**, MTOR, EIF4E, RPTOR, EIF2C2, LRRK2, ATM, SLMAP, PPP2R4, LRPAP1	MTOR signalling ($p<1.0x10^{-8}$), Cellular response to heat stress ($p<1.6x10^{-4}$)
ACD	**ACD**, POT1, TINF2, CALD1, DBNL, PGM1, ANXA4, SARS, ACOT7, IL1RN	Telomere maintenance, glycogen breakdown ($p<8.0x10^{-4}$)
CDK19	**CDK19**, MED9, MED16, MED28, MED19, MED26, MED29, MED12, MED18, CDK8	Metabolism of lipids ($p<6.0x10^{-9}$)
TLR4	**TLR4**, MYD88, TLR2, TIRAP, LY96, TICAM2, TOLLIP, TLR1, SRC, SYK	Cytokine & TLR signalling ($p<5.0x10^{-3}$)
MXD1	**MXD1**, MAX, SIN3A, AKT1, MLX, ARID4A, SAP30, VDR, KDM5A, HDAC2	Chromatin modification ($p<6.0x10^{-3}$)
TBXA2R	**TBXA2R**, GNAQ, GNA13, RAB11A, PTGIR, PRKCA, PSME3, KCNMA1, PSMA7, GNB1	Regulation of insulin secretion, WNT signalling ($p<2.7x10^{-5}$)

Table 3. The primary master regulator candidates of the labour transcriptome in choriodecidua and myometrium from network analysis and microarray analysis.

The final output in the table demonstrates the final master regulator gene candidates with the following criteria: significant transcriptional changes (defined by microarray), network centrality (Moduland), *in silico* predicted protein-gene interaction (ClusterONE) and association with known labour-associated downstream genes (Causal Network Analysis).

		Microarray		Moduland network analysis		ClusterONE analysis		Causal network analysis		
	Gene	Fold change	PPLR	Metanode (& node rank)	Repeated node	Nodes in cluster	p-value	z-score	Down-stream genes	p-value
Choriodecidua	VIM	1.9	0.999	1st (5)	-	40	$1.3x10^{-9}$	3.2	41	$1.4x10^{-17}$
	TLR4	3.7	0.9997	18th (1)	-	20	0.009	4.2	25	$4.7x10^{-10}$
	TNFSF13B	2.1	0.997	47th (1)	-	5	0.042	3.4	105	$9.6x10^{-8}$
Myometrium	MT2A	1.2	0.0058	37th (1)	-	11	0.048	3.3	30	$3.24x10^{-6}$
	TLR2	1.3	0.0101	39th (1)	30th (3)	8	0.03	1.3	90	$1.13x10^{-6}$
	RELB	1.4	0.0193	15th (1)	28th (4)	27	0.012	2.4	123	$5.96x10^{-9}$

Table 4. The functions, pathways and top core nodes that form each of the major meta-nodes of the myometrial network map.

The left most column are the top 20 hierarchically interactive clusters. The middle column lists the first 10 core nodes that form the meta-node. The right column describes the functions and canonical pathways of the cluster. The colours are arbitrary but were used to indicate a ranking in hierarchical interactions (dark colour = high clustering and interactions to light pink = lower cluster with interactions). The highest ranked meta-nodes with the greatest number of interactions over all clusters were CDKN1A, UBC and XRCC5. Within these meta-nodes the core nodes were also highly ranked including DHX9, HNRNPM and CEBPA.

Cluster	Core nodes of cluster	Hierarchy of Functions and Pathways
CDKN1A	**CDKN1A**, UBC, YWHAQ, CDK2, PCNA, GAPDH, UBE2D3, HNRNPM, DHX9, SET	Cellular response to hypoxia, Apoptosis, DNA replication & repair ($p<1.0x10^{-5}$). G1/S checkpoint ($p<5.0x10^{-10}$)
UBC	**UBC**, VCP, YWHAQ, UBE2D3, HNRNPM, ITCH, CD81, PSMC2, NCL, DHX9	Cellular response to hypoxia, Apoptosis, DNA replication & repair ($p<1.0x10^{-5}$).
XRCC5	**XRCC5**, XRCC6, BAX, DHX9, FMNL1, UBC, NCL, CEBPA, CBX5, SUMO2	Cellular responses to hypoxia, Apoptosis, DNA replication & repair ($p<1.0x10^{-5}$).
SMAD2	**SMAD2**, SMAD4, SMAD6, HDAC9, HDAC1, RUNX1, TGIF1, SMURF2, ENG, TGFBR1	TGFB signalling ($p<1.9x10^{-11}$).
PIK3R1	**PIK3R1**, CBL, SHC1, BLNK, SLA, IRS1, GRB2, SYN1, CD28, EGFR	Adaptive immune system ($p<8.9x10^{-7}$). Growth factor signalling ($p<2.8x10^{-6}$)
SHC1	**SHC1**, GRB2, EGFR, BLNK, PIK3R1, CAV1, SYN1, ABL2, ERBB2, SOS1	Adaptive immune system ($p<8.5x10^{-4}$). Growth factor signalling ($p<1.3x10^{-5}$)
MAPK14	**MAPK14**, ATF2, RPS6KA5, MBP, MAPKAPK2, OBSL1, MAP2K6, MAP2K3, MKNK1, PHC2	Cellular senescence ($p<4.2x10^{-6}$. Innate immune system ($p<3.9x10^{-4}$)
CBX5	**CBX5**, CBX3, TRIM28, CHAF1A, HIST3H3, HDAC9, SMARCA4, CBX1, DNMT3B, MIS12	WNT signalling ($p<8.9x10^{-2}$)
UPF2	**UPF2**, RBM8A, UPF1, DCP2, UPF3B, SMG1, MAGOH, EIF4A3, RNPS1, UPF3A	Nonsense mediated decay ($p<1.2x10^{-15}$)
RICTOR	**RICTOR**, MTOR, FKBP1A, MAPKAP1, AKT1, MLST8, HSPA4, PREX1, RPTOR, PDIA3	Cellular response to heat stress ($p<8.5x10^{-5}$). Growth factor signalling ($p<1.0x10^{-5}$)
MBP	**MBP**, ELK1, MAPK1, MAPK3, LRRK2, MAPK8, MAPK14, MAPK9, PRKCA, UBE2I	Cellular senescence ($p<3.4x10^{-7}$). Innate immune system ($p<1.3x10^{-5}$). RAF/MAP kinase cascade ($6.6x10^{-4}$)
SMARCA2	**SMARCA2**, SMARCC1, PHF10, SMARCB1, SMARCC2, SMARCE1, SMARCD1, SMARCA4, ACTL6A, ARID1A	Chromatin modification ($p< 3.3x10^{-13}$)
RUNX1T1	**RUNX1T1**, NCOR1, HDAC9, HDAC1, RUNX1, HEY2, CBFA2T2, SIN3A, NCOR2, HDAC3	Chromatin modification ($p< 1.2x10^{-4}$). Circadian clock ($p<7.9x10^{-3}$)
BCL3	**BCL3**, MAP3K8, NFKB1, RELB, NFKB2, NFKBIZ, RELA, TRAF2, KAT5, MAP2K1	Innate immune system ($p<1.5x10^{-3}$). Cytokine signalling ($p<7.4x10^{-4}$)
RELB	**RELB**, BCL3, NFKB2, MAP3K8, RELA, DAXX, DPF2, NFKB1, GSK3B, NFKBIZ	Cytokine signalling ($p<4.0x10^{-3}$)
VEGFA	**VEGFA**, NRP1, KDR, PGF, FLT1, CRYAB, TGFBR2, HNRNPD, HNRNPL, CTGF	Signalling by VEGF ($p<4.4x10^{-7}$)
NOD2	**NOD2**, NLRC4, RIPK2, MAP3K7, CASP1, RNF31, Sharpin, XIAP, RBCK1, SUGT1	Cytokine & TLR signalling ($p<6.7x10^{-4}$)
MLLT3	**MLLT3**, CDK9, DOT1L, AFF1, MLLT1, AFF4, AFF3, ELL, CCNT1, BCOR	TGF signalling & RNA Pol II transcription ($p<1.0x10^{-3}$)
GMNN	**GMNN**, CDT1, CDC20, AURKA, NCF1, KAT7, REPIN1, IMPDH1, CDH1, FZR1	Cell cycle & DNA replication ($p<1.0x10^{-3}$)
DCP2	**DCP2**, EDC4, DCP1B, MMS19, CAPN2, FANCD2, TRMT2A, POLA2, IKBKAP, INTS7	Deadenylation-dependent mRNA decay ($p<7.8x10^{-4}$)

Supplemental table 1. Nodal details of the choriodecidua network map demonstrating the top ten nodes that form each of the major meta-nodes.

The left most column are the meta-nodes. The colours are arbitrary but are used here to indicate a hierarchical ranking in interactions. The upper top left corner are nodes with the greatest regulatory function, and therefore major downstream biological effects (red, orange, purple colours). There is a decrease in biological rank as the table progresses towards the bottom right corner of the table (yellow and green colours). There are 71 meta-nodes of the labouring human choriodecidua. Blue arrows indicate descent of hierarchical rank the highest ranked meta-nodes: UBC and CDC34, and nodes: NFKBIA and UBE2D3.

UBC	NFKBIA	RUVBL2	NOTCH1	VIM	UBE2D3	RPS4X	ARRB1	RPSA	XPO1
CDC34	UBE2D3	UBC	RBX1	ARRB1	MDM2	CUL1	RUVBL2	NOTCH1	NRF1
STUB1	UBC	UBE2D3	ARRB1	HSP90AA1	HSPA4	UBE2D1	CSNK1A1	PARK7	TP53
SHC1	GRB2	LCP2	EGFR	ERBB3	CBL	PAG1	SOS1	ERBB2	GRAP2
BIRC3	TRAF2	TNFAIP3	USP53	UBE2D2	BCL10	RIPK1	PEG3	TRAF6	DIABLO
BRCA1	BRIP1	BACH1	NBN	LMO4	MLH1	RPA1	PMS2	HNRNPA2B1	EZR
NBN	MRE11A	RAD50	H2AFX	MDC1	CALR	ATM	USP53	BACH1	EP300
SMC3	RAD21	SMC1A	CASP4	STAG1	ANKRD28	S100A9	PDS5A	STAG2	CLU
CKS1B	CDK2	SKP2	CDK1	MCM2	SKP1	CDKN1B	COPS6	CCNA2	CCNG2
MBD3	HDAC1	BCL11B	HDAC2	MTA2	MBD2	MXD1	ATF3	RBBP4	ARID4A
BRD4	CDK9	AFF4	CCNT2	ELL2	CCNT1	MLLT3	MLLT1	AFF1	HEXIM1
FAS	FADD	DAXX	FASLG	KRIT1	FAF1	CASP10	CASP8	TGFB2	CFLAR
SOCS3	JAK2	CSF2RB	TCEB1	PRLR	PTPN11	TCEB2	IL2RB	IFNGR1	JAK1
UBE2B	RAD18	UBR2	UBR3	UBA1	CTNNB1	UBQLN1	DSTN	PCNA	CPLX1
EIF4EBP1	MTOR	EIF4E	RPTOR	EIF2C2	LRRK2	ATM	SLMAP	PPP2R4	LRPAP1
ACD	POT1	TINF2	CALD1	DBNL	PGM1	ANXA4	SARS	ACOT7	IL1RN
CDK19	MED9	MED16	MED28	MED19	MED26	MED29	MED12	MED18	CDK8
TLR4	MYD88	TLR2	TIRAP	LY96	TICAM2	TOLLIP	TLR1	SRC	SYK
MXD1	MAX	SIN3A	AKT1	MLX	ARID4A	SAP30	VDR	KDM5A	HDAC2
TBXA2R	GNAQ	GNA13	RAB11A	PTGIR	PRKCA	PSME3	KCNMA1	PSMA7	GNB1
GMNN	CDT1	CDC20	ARNT	CDH1	HDAC11	NCF1	IMPDH1	CDKN2A	REPIN1
IFIT3	IFIT2	IFIT1	IFIT5	SNRPA	PABPC4	LIN28B	SNRPB2	SNRPF	SNRPC
PTN	SRGN	UBQLN1	SGTA	CHD3	BAG6	TAC3	RIT1	TAF1D	LAMA4
TCF7L2	CTNNB1	PTPRF	JUP	PSEN1	CEBPA	CTBP1	NLK	ZBTB33	DSC3
GNG2	GNB1	GNB2	GNB3	GNB4	GNB5	TBXA2R	ATP4A	GNAI1	GNB2L1
NRP1	VEGFA	PRRG4	TGFBR1	TGFBR2	RPL27	TGFBR3	SRRM1	CSNK2A2	PDGFB
IL2RB	JAK1	HGS	IFNGR1	PLA2G4A	SOCS1	STAT3	STAT1	SHB	CISH
EEA1	RAB5A	RABEP1	PDCD6IP	NSF	TNK2	GF1R	CFTR	BMI1	RABAC1
FBLN1	NID1	ATN1	NELL1	CACNA1A	GFI1B	MPG	LAMC1	SKIL	TAF9
THBS1	COL3A1	LRP1	PLG	COL1A1	ITGB3	PDGFB	SOX2	ITGB1	TGFB1
POLR2I	POLR2A	MED26	INTS5	DCUN1D1	CSH2	POLR2D	WDR48	TBCD	RECQL5
LMO4	LDB1	RBBP8	PRRG4	ITSN1	PLK1	KPNA1	SEC24D	TINAGL1	RPS6KA6
INTS5	INTS10	POLR2D	PPP2CB	OBFC2B	POLR2B	POLR2C	ZNF687	POLR2J	ZNF592
ARID4A	RB1	SAP30	BRMS1	SIN3A	SAMSN1	SIRT2	HIST2H3C	CDK4	RBL1
TRIB3	ATF4	GRN	LTBP4	GIT1	HLA-B	CDC25A	PRMT5	SETDB1	TIAF1
FGFR2	FGF1	FGF7	STAT3	ITGA5	PPM1A	FRS2	S100A13	STAT5B	PGR
BMP2	BMPR1A	BMPR2	BMPR1B	ACTR2	COL2A1	TRIB3	CRTAP	HLA-B	CDC25A
OBSL1	MAPK14	CUL7	ALB	TBKBP1	PASK	KCNE4	CAMKK2	FBXW8	TSGA10
PLAU	PLAUR	HRAS	CEBPG	BTK	SERPINB2	ALAS1	PLAT	LRP2	ELAVL1
MDFI	PHLDA1	EBI3	GDF15	GOSR1	DUSP6	CANX	KRTAP4-12	RPL14	DNPEP
PAPPA	PRG2	SKIL	RBM48	SMAD9	PLG	SPARCL1	CHD3	SMAD3	SMAD2
INHBA	ACVR2A	ACVR2B	ACVR1	ERBB2IP	PKP4	ARVCF	ERBB2	LMO2	SIRT3
REPS2	EPN1	GTF3A	AP2A1	AMPH	EPS15	RALBP1	AR	CDK1	CXCR2
IER3	MAPK1	PPP2R5C	PML	ITGB6	PRNP	PPP2R4	DUSP6	MAPK3	SLC1A2
KRIT1	ITGB1BP1	RGS4	RAP1B	RCHY1	GNAI1	FAS	SERPINA1	ERBB3	RPN2
CCNL1	CDK11A	JMJD6	CSNK2A2	ISG15	CARS	GADD45A	APP	BRD4	NEDD8
TNFSF13B	TNFRSF13B	TNFRSF13C	TNFSF13	TNFSF12	APP	XPO1	OSM	SNX21	MBNL1
SKAP2	FYN	PRAM1	HCK	PTK2B	PAG1	MCAM	KHDRBS1	FGFR2	SRC
MYLK	CALM1	CTTN	MLC1	ALK	HDAC4	SRC	CSRP3	METAP2	MYF6
RAMP1	CALCRL	SLC31A2							
PDZK1IP1	PDZK1	UTP14A	COPS6	KLHL24	LYZ	ZEB2			
TNFAIP8L1	FBXW5	GRK5	DVL3	LYZ	APP	MAP4	USP25	NME2	SMAD6
IL6	IL6R	SH3GL2	LCP2	PLCG1					
NFKBIZ	STAT3	NFKB2	LAMB2	NDUFA13	IVNS1ABP	FGFR2	IL2RB	SCAF11	BRD4
CYTIP	CYTH1	SOCS1	UBC						
CFI	C3	GLP1R							
MNDA	PRMT7								
C15orf48	TSSK3	ABL2							
PSG5	TIE1	PRPF40A							
KIAA1199	PLXNA2	DCUN1D1							
CTSS	CST5								
FCGR3B	APCS	IGHG1							

Supplemental Table 2. ClusterONE choriodecidual and myometrial prioritisation of candidates of interest from the Moduland network analysis and BioGRID interactions.

The secondary mathematical analysis used protein-protein and gene protein interactions.

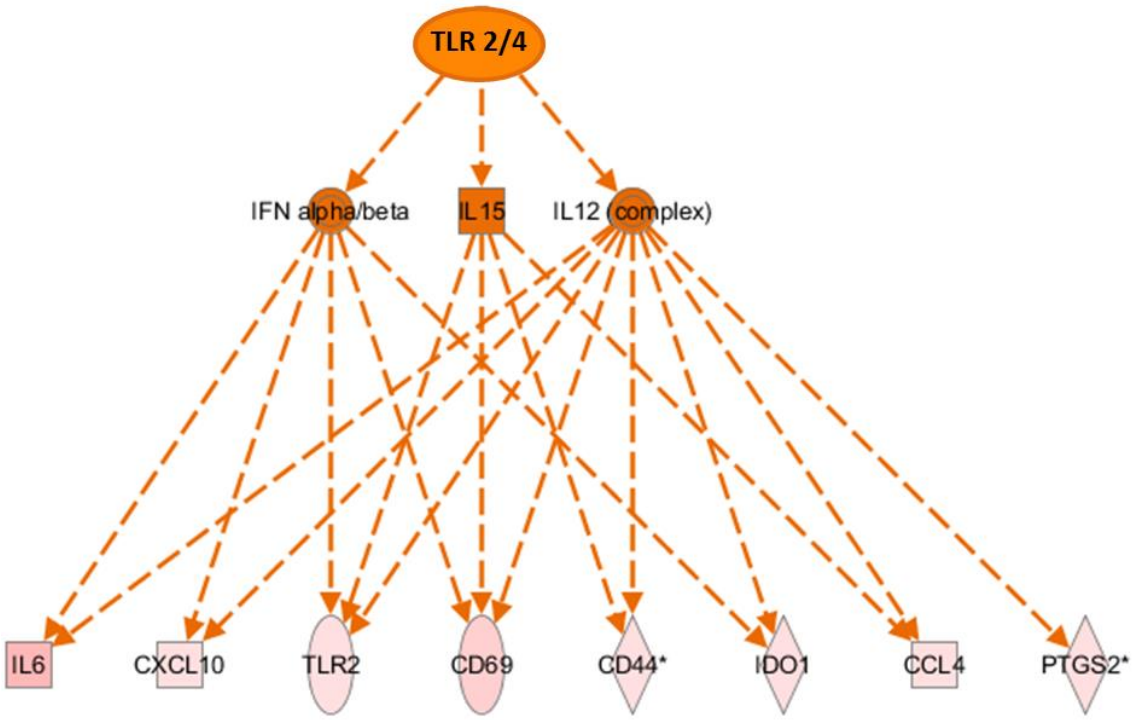
A. Choriodecidua and B. myometrium. The output in the table represents all genes with significant transcriptional changes in expression (array) and significant centrality within the genomic network (Moduland network analysis), and additionally, possesses predicted networks of protein-gene interaction (ClusterONE).

A

Gene	Number of nodes in the cluster	p-value
Down regulated		
CDK19	54	5.02×10^{-9}
POLR2I	54	1.33×10^{-5}
INTS5	25	1.33×10^{-5}
CDC34	23	0.00409
LMO4	15	0.00423
BRD4	28	0.00871
TBXA2R	20	0.03565
Upregulated		
VIM	40	1.27×10^{-9}
INHBA	41	4.72×10^{-8}
CSNK1A1	37	0.00091
KRIT1	10	0.00098
THBS1	46	0.00232
IFIT3	48	0.00286
TLR4	20	0.00922
MNDA	4	0.01032
BMP2	8	0.01179
GNAI1	29	0.01460
CTSS	3	0.02617
TNFSF13B	5	0.04254
EEA1	10	0.04623

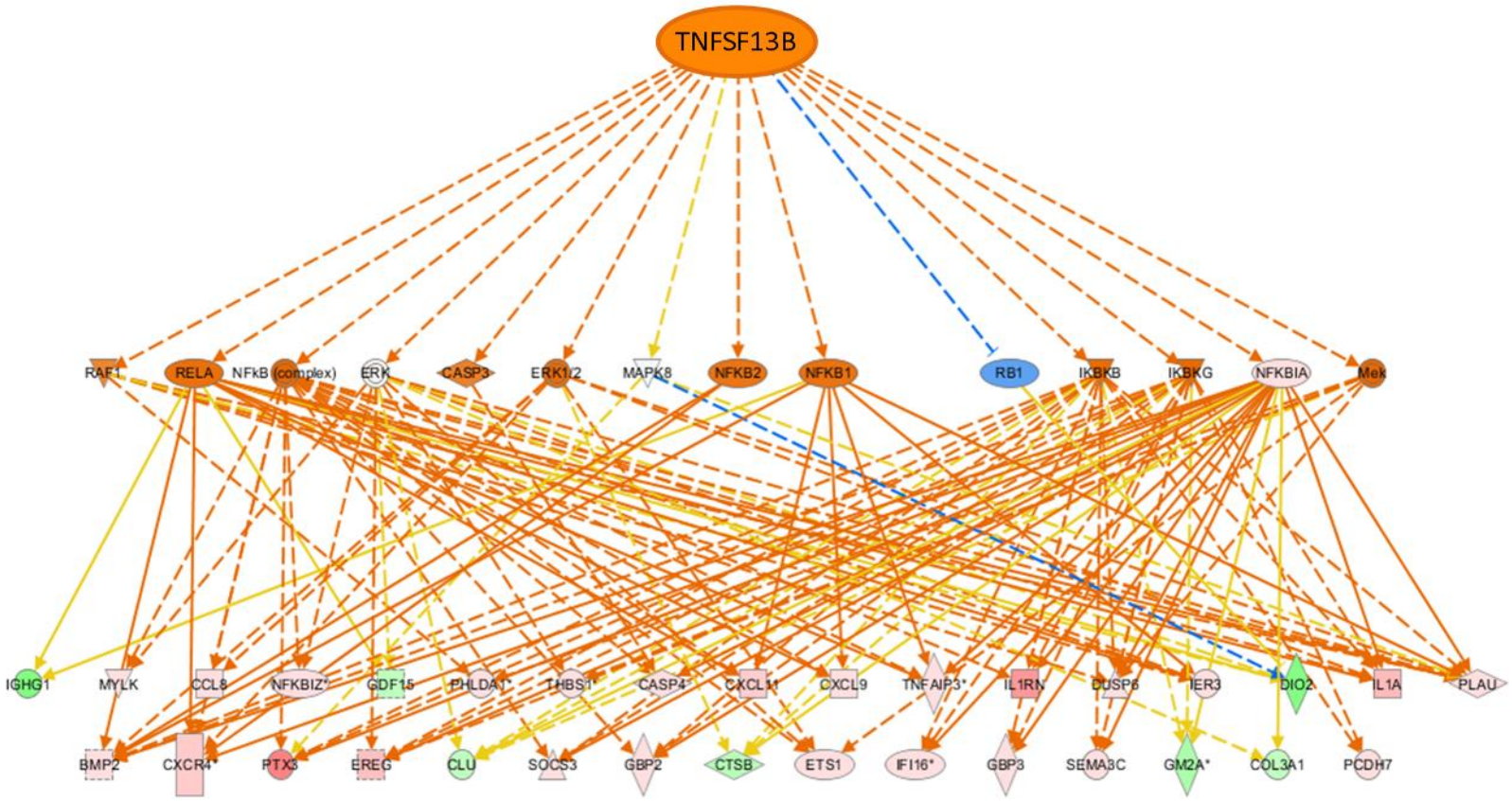
B

Gene	Number of nodes in the Cluster	p-value
Down regulated		
CEBPA	45	2.12×10^{-5}
SMAD6	36	5.69×10^{-5}
HDAC9	42	0.00012
GRIA2	14	0.00131
EMX2	17	0.00156
NRP1	20	0.00366
RUNX1T1	44	0.01026
MBP	26	0.01952
Upregulated		
UPF2	40	3.41×10^{-6}
BATF	45	2.12×10^{-5}
DCP2	30	2.24×10^{-5}
ENG	36	5.69×10^{-5}
NKX3-1	25	8.39×10^{-5}
SET	41	0.00043
RNF181	45	0.00069
TAF1D	23	0.00081
RAB27A	14	0.00133
FLT1	20	0.00366
VEGFA	20	0.00366
ZNF513	32	0.00541
RUNX1	44	0.01026
RELB	27	0.01245
IRAK3	13	0.01474
MLX	8	0.03044
TLR2	8	0.03044
ABL2	15	0.03675
FMNL1	30	0.04422
MT2A	11	0.04891



Supplemental figure 1. Causal network analysis of the TLR2/4 complex identifying relationships with labour-associated choriodecidual genes downstream.

TLR2/4 is shown as the top regulator with three lower level regulators. The bottom level is a summary of genes affected downstream (full map includes 25 genes). This map shows the major downstream genes with at least two network connections (dashed line). Upregulated expression of regulators = orange; upregulated expression of genes = pink. The intensity of colour indicates the degree of upregulated expression.

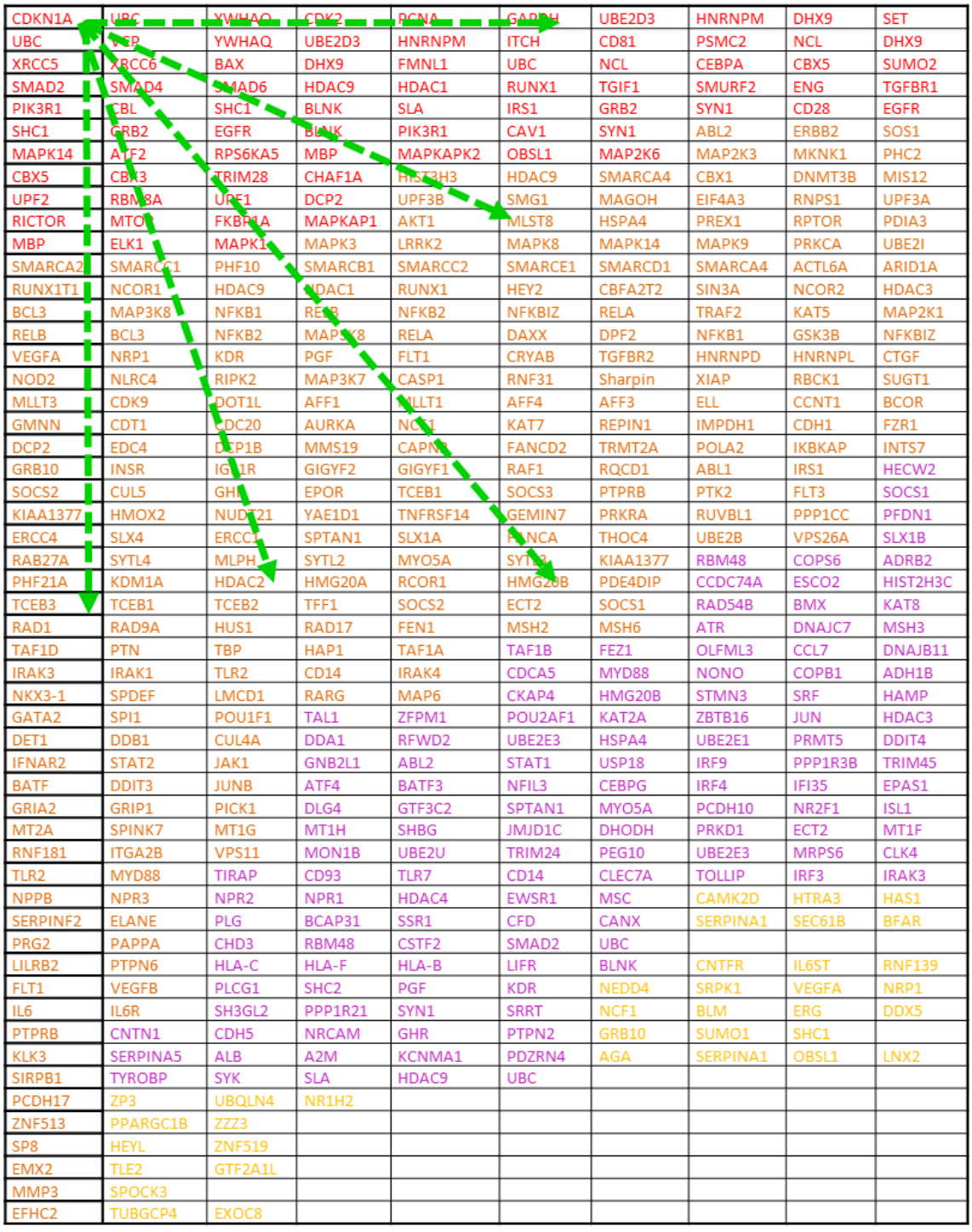


Supplemental figure 2. Causal network analysis of TNFSF13B identifying relationships with labour-associated choriodecidual genes downstream.

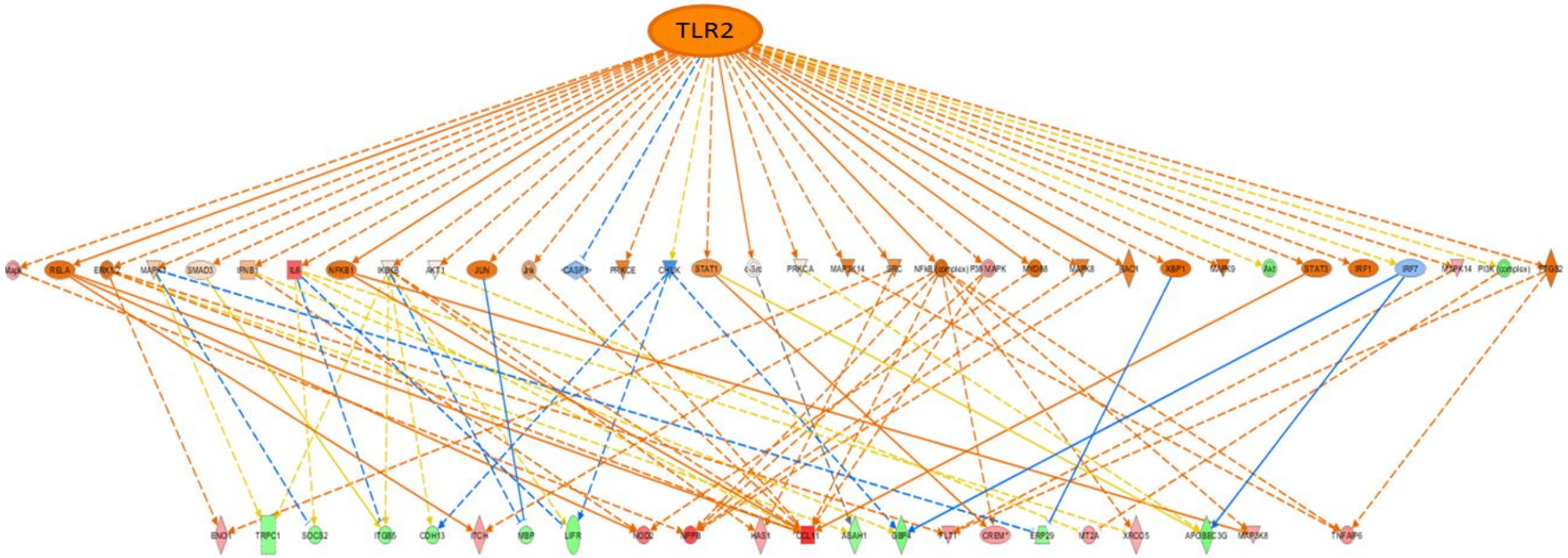
TNFSF13B is shown as the top regulator with 14 lower level regulators. The bottom level is a summary of genes affected downstream (full map includes 105 genes). This map shows all the regulators and only the major downstream genes with i) at least two single network connections (dashed line) or ii) have multiple associated functions from a single regulator (solid line). Upregulated regulator = orange, upregulated gene = pink; Downregulated regulator = blue, downregulated gene = green where the intensity of colour indicates the degree of up or down regulated expression. Lines indicate activation (orange) or inhibition (blue) or the change was not as predicted in literature (yellow).

Supplemental table 3. Node details of the myometrium network map demonstrating meta-nodes and their top ten nodes.

The left most column are meta-nodes. The colours indicate a hierarchical ranking. The upper top left corner of nodes have the greatest hierarchical ranking, and therefore major downstream biological effects (red, orange, purple colours). There is a decrease in biological rank as the table progresses towards the bottom right corner of the table (yellow and green colours). There are 64 meta-nodes of the labouring human myometrium. Arrows indicate descent of hierarchical rank the highest ranked meta-nodes CDKN1A and ubiquitin and nodes: UBC and VCP.

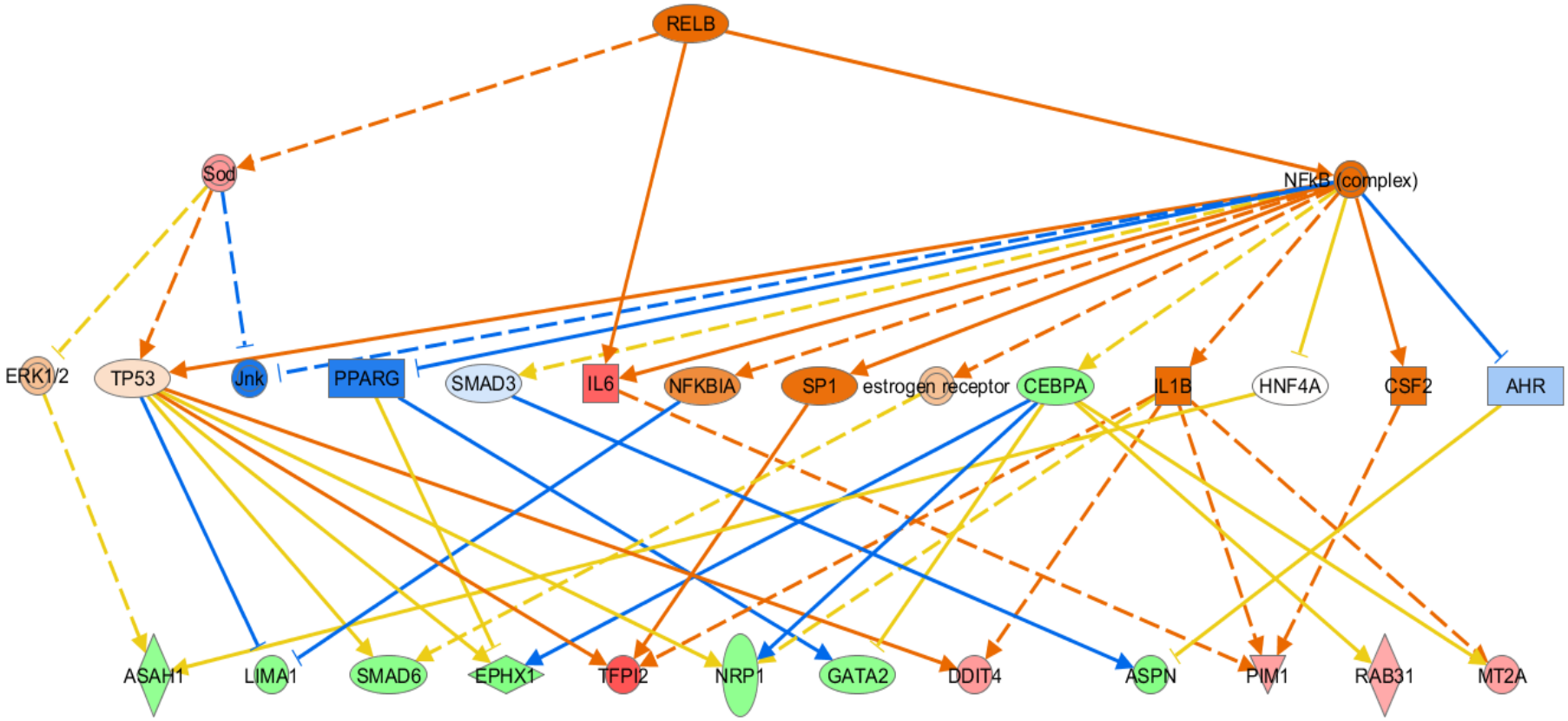


CDKN1A	UBC	YWHAQ	CDK2	PCNA	GAPDH	UBE2D3	HNRNPM	DHX9	SET
UBC	VCP	YWHAQ	UBE2D3	HNRNPM	ITCH	CD81	PSMC2	NCL	DHX9
XRCC5	XRCC6	BAX	DHX9	FMNL1	UBC	NCL	CEBPA	CBX5	SUMO2
SMAD2	SMAD4	SMAD6	HDAC9	HDAC1	RUNX1	TGIF1	SMURF2	ENG	TGFBR1
PIK3R1	CBL	SHC1	BLNK	SLA	IRS1	GRB2	SYN1	CD28	EGFR
SHC1	GRB2	EGFR	BLNK	PIK3R1	CAV1	SYN1	ABL2	ERBB2	SOS1
MAPK14	ATF2	RPS6KA5	MBP	MAPKAPK2	OBSL1	MAP2K6	MAP2K3	MKNK1	PHC2
CBX5	CBX3	TRIM28	CHAF1A	HIST2H3	HDAC9	SMARCA4	CBX1	DNMT3B	MIS12
UPF2	RBM8A	UPF1	DCP2	UPF3B	SMG1	MAGOH	EIF4A3	RNPS1	UPF3A
RICTOR	MTOR	FKBP1A	MAPKAP1	AKT1	MLST8	HSPA4	PREX1	RPTOR	PDIA3
MBP	ELK1	MAPK1	MAPK3	LRRK2	MAPK8	MAPK14	MAPK9	PRKCA	UBE2I
SMARCA2	SMARCC1	PHF10	SMARCB1	SMARCC2	SMARCE1	SMARCD1	SMARCA4	ACTL6A	ARID1A
RUNX1T1	NCOR1	HDAC9	HDAC1	RUNX1	HEY2	CBFA2T2	SIN3A	NCOR2	HDAC3
BCL3	MAP3K8	NFKB1	RELB	NFKB2	NFKBIZ	RELA	TRAF2	KAT5	MAP2K1
RELB	BCL3	NFKB2	MAP3K8	RELA	DAXX	DPF2	NFKB1	GSK3B	NFKBIZ
VEGFA	NRP1	KDR	PGF	FLT1	CRYAB	TGFBR2	HNRNPD	HNRNPL	CTGF
NOD2	NLRC4	RIPK2	MAP3K7	CASP1	RNF31	Sharpin	XIAP	RBCK1	SUGT1
MLLT3	CDK9	DOT1L	AFF1	MLLT1	AFF4	AFF3	ELL	CCNT1	BCOR
GMNN	CDT1	CDC20	AURKA	NCL	KAT7	REPIN1	IMPDH1	CDH1	FZR1
DCP2	EDC4	DCP1B	MMS19	CAPN1	FANCD2	TRMT2A	POLA2	IKBKAP	INTS7
GRB10	INSR	IGF1R	GIGYF2	GIGYF1	RAF1	RQCD1	ABL1	IRS1	HECW2
SOCS2	CUL5	GH1	EPOR	TCEB1	SOCS3	PTPRB	PTK2	FLT3	SOCS1
KIAA1377	HMOX2	NUDT21	YAE1D1	TNFRSF14	GEMIN7	PRKRA	RUVBL1	PPP1CC	PFDN1
ERCC4	SLX4	ERCC1	SPTAN1	SLX1A	FANCA	THOC4	UBE2B	VPS26A	SLX1B
RAB27A	SYTL4	MLPH	SYTL2	MYO5A	SYTL3	KIAA1377	RBM48	COPS6	ADRB2
PHF21A	KDM1A	HDAC2	HMG20A	RCOR1	HMG20B	PDE4DIP	CCDC74A	ESCO2	HIST2H3C
TCEB3	TCEB1	TCEB2	TFF1	SOCS2	ECT2	SOCS1	RAD54B	BMX	KAT8
RAD1	RAD9A	HUS1	RAD17	FEN1	MSH2	MSH6	ATR	DNAJC7	MSH3
TAF1D	PTN	TBP	HAP1	TAF1A	TAF1B	FEZ1	OLFML3	CCL7	DNAJB11
IRAK3	IRAK1	TLR2	CD14	IRAK4	CDCA5	MYD88	NONO	COPB1	ADH1B
NKX3-1	SPDEF	LMCD1	RARG	MAP6	CKAP4	HMG20B	STMN3	SRF	HAMP
GATA2	SPI1	POU1F1	TAL1	ZFPM1	POU2AF1	KAT2A	ZBTB16	JUN	HDAC3
DET1	DDB1	CUL4A	DDA1	RFWD2	UBE2E3	HSPA4	UBE2E1	PRMT5	DDIT4
IFNAR2	STAT2	JAK1	GNB2L1	ABL2	STAT1	USP18	IRF9	PPP1R3B	TRIM45
BATF	DDIT3	JUNB	ATF4	BATF3	NFIL3	CEBPG	IRF4	IFI35	EPAS1
GRIA2	GRIP1	PICK1	DLG4	GTF3C2	SPTAN1	MYO5A	PCDH10	NR2F1	ISL1
MT2A	SPINK7	MT1G	MT1H	SHBG	JMJD1C	DHODH	PRKD1	ECT2	MT1F
RNF181	ITGA2B	VPS11	MON1B	UBE2U	TRIM24	PEG10	UBE2E3	MRPS6	CLK4
TLR2	MYD88	TIRAP	CD93	TLR7	CD14	CLEC7A	TOLLIP	IRF3	IRAK3
NPPB	NPR3	NPR2	NPR1	HDAC4	EWSR1	MSC	CAMK2D	HTRA3	HAS1
SERPINF2	ELANE	PLG	BCAP31	SSR1	CFD	CANX	SERPINA1	SEC61B	BFAR
PRG2	PAPPA	CHD3	RBM48	CSTF2	SMAD2	UBC			
LILRB2	PTPN6	HLA-C	HLA-F	HLA-B	LIFR	BLNK	CNTFR	IL6ST	RNF139
FLT1	VEGFB	PLCG1	SHC2	PGF	KDR	NEDD4	SRPK1	VEGFA	NRP1
IL6	IL6R	SH3GL2	PPP1R21	SYN1	SRRT	NCF1	BLM	ERG	DDX5
PTPRB	CNTN1	CDH5	NRCAM	GHR	PTPN2	GRB10	SUMO1	SHC1	
KLK3	SERPINA5	ALB	A2M	KCNMA1	PDZRN4	AGA	SERPINA1	OBSL1	LNX2
SIRPB1	TYROBP	SYK	SLA	HDAC9	UBC				
PCDH17	ZP3	UBQLN4	NR1H2						
ZNF513	PPARGC1B	ZZZ3							
SP8	HEYL	ZNF519							
EMX2	TLE2	GTF2A1L							
MMP3	SPOCK3								
EFHC2	TUBGCP4	EXOC8							



Supplemental figure 3. Causal network analysis of TLR2 identifying relationships with labour-associated myometrial genes downstream.

TLR2 is shown as the top regulator with 34 lower level regulators. The bottom level is a summary of genes affected downstream (full map includes 90 genes). This map shows all the regulators and only the major downstream genes with i) at least two single network connections (dashed line) or ii) have multiple associated functions from a single regulator (solid line). Upregulated regulator = orange, upregulated gene = pink; Down-regulated regulator = blue, down-regulated gene = green where the intensity of colour indicates the degree of up or down regulated expression. Lines indicate



Supplemental figure 4. Causal network analysis of RELB identifying relationships with labour-associated myometrial genes downstream.

RELB is shown as the top regulator with 2 lower level regulators. The bottom level is a summary of genes affected downstream (full map includes 123 genes). This map shows all the regulators and only the major downstream genes with i) at least two single network connections (dashed line) or ii) have multiple associated functions from a single regulator (solid line). Upregulated regulator = orange, upregulated gene = pink; Down-regulated regulator = blue, down-regulated gene = green where the intensity of colour indicates the degree of up or down regulated expression. Lines indicate activation (orange) or inhibition (blue) or the change was not as predicted in literature (yellow).

5.0 Results chapter

Phenotyping the uterine transcriptome preceding and during active labour in the mouse using *in silico* network and *in vitro* analyses

Sylvia Lui[1], Cyntia Duval[4], Lynda K. Harris[1, 3], Adam Stevens[2], Rebecca L. Jones[1]

Affliations:

[1]Maternal and Fetal Health Research Centre, Institute of Human Development, University Of Manchester, UK [2]Institute of Human Development, University Of Manchester, UK. [3]School of Pharmacy, University Of Manchester, UK. [4]Sainte-Justine Hospital Research Center, Department of Obstetrics and Gynecology, University of Montreal, Canada.

Keywords:

Decidua, Myometrium, Network analysis, Microarray

Abstract

Coordination of labouring processes are essential for successful parturition where abnormal or activity is associated with preterm and obstructed labour. Both conditions increase the risk of fetal mortality and range in severity of morbidities. Elucidating the coordination and regulation of the established normal term processes leading to active labour is essential in understand labour disorders. This study aimed to identify master regulators in the myometrium and decidua in a mouse model at E16.5, E17.5, E18.5 and during active labour (E19). The transcriptome was validated using qRT-PCR and characterised by pathway and network analysis using Ingenuity and Cytoscape 2.8.3 software, with additional gene barcoding techniques for absolute expression at each gestational/labour stage. Preliminary *in vitro* master regulator inhibition studies in a mouse smooth muscle cell line (MOVAS) were also performed.

The differential expression of the decidua is characterised by enrichment in cell death and survival, inflammatory response and the haematological system and cell pathways represented in LXR/RXR activation, acute phase response and coagulation system. However the decidual pattern in the number of genes, type of functional changes, and in the diversity of pathways activated, prior to labour was initiated by as early as E17.5. The gene activity and functional diversity continues through to E18.5, with the largest number of increased gene activity at E19.0. The myometrium demonstrates enrichment in tissue development, cellular assembly and cell morphology, with and cellular signalling in integrin, IGF and chemokine pathways. These are shared at E17.5 and E18.5 until 12 hours prior to labour, with the number of genes climbing sharply between E18.5 and active labour at E19. At E19, the genes are overrepresented in LXR/RXR activation, acute phase response and WNT signalling. In the decidua, IRAK4 and TIRAP were identified as master regulators with highly connected networks in both the relative and absolute expression of genes analysis. In the myometrium, TLR2/4 complex formed network significance. qRT-PCR validated OXTR, CXCR4, MMP11, IRAK4, SLIT2, AQP5, PTGS2, CXCL12, GJA1, and TLR4 between the decidua and myometrium ($p<0.05$ all Wilcoxon-signed rank test).

These analyses identified high-level master regulators of active labour in decidua and myometrium. The decidua is highly functional in the stages preceding labour where the myometrium is active at term and labour. Further investigations are required to test the therapeutic potential of blocking labour activity.

5.1 Introduction

Successful parturition involves amplification of multiple pathways that sensitise and coordinate the uterine tissues to deliver the fetus. Aberrant activity and coordination of these processes are likely to underlie problems in timely delivery, including preterm labour, obstructed labour and failure of onset of spontaneous labour. Preterm delivery is the leading cause of neonatal morbidity and mortality (Blencowe et al. 2012; Chang et al. 2012), whilst dysfunctional or obstructed labour increases adverse risks to mother and infant, requiring clinical intervention including induction of labour or caesarean delivery (Karaçam et al. 2014; Neal et al. 2015). A greater understanding of the mechanisms initiating and driving labour and the key regulatory processes is vital to improve clinical management of abnormal labour.

Studying the transition from pregnancy to active labour is challenging in humans, thus animal models provide an invaluable means of characterising the mechanisms underlying the activation of the labour cascade. The genetic information and availability of knock out mouse models have assisted in identifying some of the regulatory processes involved, including inflammatory signalling via toll-like receptor (TLR) 2 (Montalbano et al. 2013) and TLR4 signalling (Wahid et al. 2015), prostaglandin synthesis and action via cyclooxygenase-1 (COX-1; PTGS-1 gene) and -2 (COX-2 or PTGS-2 gene) (Dinchuk et al. 1995; Lim et al. 1997; Gross et al. 1998) and increased prostaglandin $F_{2\alpha}$ receptor (FP) (Sugimoto et al. 1997), and cell to cell communication via connexion 43 gap junction protein (GJA1 gene) expression (Doring et al. 2006). Ablation of these genes in mice either significantly delayed or prevented the onset of parturition, indicating non-redundant roles in the labour process. However, normal parturition is preserved following knockout of other genes strongly associated with labour, including prostaglandin E (PGE_2) receptors EP1, EP2, EP3 (Esaki et al. 2010), cytokines interleukin (IL)-1 (Hirsch et al. 2006) and IL-6 (Sakurai et al. 2012), and oxytocin receptor (OXTR) (Takayanagi et al. 2005), demonstrating our incomplete understanding of the involvement of these established mediators of labour, and their actions in association with multiple other factors expressed during labour.

Transcriptomic approaches have been utilized to identify global gene changes associated with labour in mice and rats; however, only two murine studies included functional pathway analysis of the differentially expressed genes and there is a scarcity of data utilising network modelling of transcriptomic data in any partition studies. Complementary microarray studies have demonstrated widespread alterations in myometrial gene expression during labour in the mouse, and activation of multiple pathways, including G protein signalling, inflammatory and immune-related signalling, regulators of cell cycle, cell growth and differentiation, cytoskeletal cellular transformation, cell junctions, and translational regulators, in addition to those governing myometrial contraction (Bethin et al. 2003; Salomonis et al. 2005). Inflammation and NFκB activation have been implicated as having an important role in labour, in both infection-associated preterm labour and in non-infectious models of preterm (Romero et al. 1991; Lockwood et al. 1994; Romero et al. 1994; Lim et al. 2012; Kim et al. 2012; Shynlova 2013a; Heng et al. 2014; Shynlova et al. 2014; MacIntyre et al. 2014) and term labour (Bulmer et al 1988; Norwitz et al. 1991; Thomson et al.

1999; Osman et al. 2003; M. Yuan et al. 2009; Weiner et al. 2010; Hamilton et al. 2012; Hamilton et al. 2013; Shynlova et al. 2013b). How inflammatory pathways interrelate with other regulatory pathways is unclear, and whether their activation is a cause or consequence of labour is still unknown.

The decidua plays a critical role in fertility and establishment of pregnancy, and there is an increasing recognition of a regulatory role for the decidua in labour. In murine models, immune cells infiltrate the decidua prior to labour (Hamilton et al. 2012; Shynlova et al. 2013a), at an earlier time and by a greater magnitude than is observed in the myometrium (Hamilton et al. 2012). Uterine-specific deletion of tumour related protein 53 (Trp53) in the mouse has been shown to induce preterm birth, coincident with abnormal decidualisation and irregular prostaglandin synthesis activity and secretion of labour-associated PGF2α (Hirota et al. 2010). These changes could be rescued by PTGS2 inhibition, or with the mTORC1 inhibitor rapamycin, or by the creation of a secondary knockout, TP53-related cell cycle kinase inhibitor (CDKN1A). This implicates normal decidual function in governing pregnancy length and parturition, and reinforces the importance of cell cycle control as a contributing mechanism (Hirota et al. 2010; Hirota et al. 2011).

The current study was undertaken to characterise the labour and pre-labour transcriptome phenotype in the murine decidua and myometrium, to identify the biological pathways and key regulators involved in the initiation and progression of labour, and to explore potential overlapping and discrete roles of the two uterine layers. To do this, genome wide transcriptomic analysis of the mouse myometrium and decidua was performed during active labour (E19), and in the preceding three days (E16.5, E17.5, E18.5) of pregnancy, followed by detailed *in silico* and *in vitro* analyses. The aims of the study were: 1) to identify the temporal transition in the transcriptome during pregnancy and leading up to active labour in the decidua and myometrium; 2) to identify master regulators of the labour process in the two uterine layers; and 3) to assess the predicted targets of master regulators by manipulating their activity *in vitro* and assessing the effect on downstream gene expression.

5.2 Methods

5.2.1 Animal tissue collection

Animals were kept in accordance with the UK Animals Scientific Procedures Act 1986. Pregnant female C57BL/6 mice between 8 to 12 weeks old were housed under a 12h light/dark cycle at 21–23 °C with food (Beekay Mouse Diet, Bantin & Kingman, Hull, UK) and water provided *ad libitum*. The first day of pregnancy was determined by the detection of a copulation plug and designated embryonic day (E0.5). Active labour and parturition occurred on the evening of E18.5, designated E19.0, after the delivery of the first pup with umbilical cord still attached.

To investigate the transcriptomic changes leading up to and during active labour uterine tissue was collected at: E16.5, E17.5, E18.5 and E19.0; each group representing mid-late pregnancy, late pregnancy, 12 hours before labour and active labour respectively (N=6 dams each). For the tissue culture experiments, a separate group of mice were used at pregnancy day E18.5 (N=6). Mice

were culled by cervical dislocation and tissues harvested by sampling two implantation sites randomly from the uterine horns. The tissues were quickly submerged in RNAlater (Ambion, UK) for 24 to 48 hours at 4^{o}C. Before storage at -80^{o}C, fetal tissues were removed and the decidua paretalis and myometrium separated under a dissecting microscope for microarray hybridisation and PCR analysis; or intact uterine tissues, with fetal tissues removed, were placed in culture medium for *in vitro* analyses.

5.2.2 RNA isolation

RNA was isolated following manufacturer's instructions using the MirVana miRNA Isolation Kit, RNeasy miniprep kit (Life Technologies, UK). RNA was assessed using NanoDrop 2000 UV-Vis Spectrophotometer, measuring absorbance curves at A260/280 and A260/230. For the microarray, individual myometrial and decidual RNA samples collected from N=6 mice per group were pooled, for each gestational age studied; pooled samples were assessed again for RNA quality using the Agilent 2200 TapeStation system for a RNA integrity number equivalent (RIN^{e}) score (Schroeder et al. 2006). Microarray samples within this study had a RIN^{e} of 8.3 to 9.1.

5.2.3 Microarray hybridisation

The samples were hybridised in duplicate onto GeneChip Mouse Genome 430 2.0 Array according to manufacturer's instructions. The chip detects over 39,000 transcripts. Normalisation was performed using multi-mgMos (Liu et al. 2005) with a robust multivariate analysis (RMA) which is a Bayesian method of probe-level error measurement (Liu et al. 2006). This normalisation method accounts for experimental variation in fluorescent dye labelling, and includes robust local regression for the intensity and spatial labelling of each individual spot (Yang et al., 2002). Resulting .cel files were analysed with Propagating Uncertainty in Microarray Analysis (PUMA) from the Affymetrix Microarray Suite 5.0 software (Irizarry et al. 2003). PUMA was used to generate a probability of positive log ratio (PPLR) which is a probability of low certainty for significance of difference in gene expression between the arrays (Liu et al. 2006). The PPLR was also used as a significance threshold cut-off ($0.997>PPLR<1x10^{-5}$) for use in pathway and network analysis. The samples were also analysed using frozen RMA (fRMA), an approach that uses all known data on the 430 Mouse Genechip to calculate presence or absence of gene expression to a high level of confidence (McCall et al. 2010; Langfelder et al. 2011). The presence of expression of each probe signal in our data was mapped to an estimated z-score, a calculation of the average distribution of all the multiple probe signals of each gene within all cell types of the tissue. The distribution Z-score cutoff was >2.5 (Zilliox and Irizarry 2007; McCall et al. 2010; McCall et al. 2014).

5.2.4 Quantitative RT-PCR

Reverse transcription was performed according to manufacturer's instructions using the Affinity Script Multiple Temperature cDNA Synthesis Kit (Agilent, UK). Primers were purchased from Eurofins, Germany, except for GAPDH, ARG1 and IL-1β (Qiagen, UK). The annealing temperatures of the primers were optimised at 60^{o}C (Table 1). Samples were amplified in duplicate using the Ultra-Fast SYBR Green QPCR Master Mix III (Agilent, UK) with 5-carboxy-x-rhodamine (ROX) as a reference dye, using the RT-qPCR Stratagene MX3005 machine (Agilent, UK). An amplification with

a single peak in the dissociation curve was accepted as a single product. To determine relative expression of mRNA, this study used $2^{-\Delta CT}$ for difference in expression patterns between two groups, or $2^{-\Delta\Delta CT}$ to compare groups to an untreated control. Validation of the microarray was normalised to GAPDH, all other PCR data were normalised to the reference gene TBP, and expressed as treatment as compared to control. Several reference genes were screened (TBP, YWHAZ, GAPDH, and MBP were selected as expression was unchanged in the array); TBP and GAPDH were identified as the most stably expressed gene for reference for each experiment. Statistical analysis was undertaken using GraphPad Prism 6.0; a Mann-Whitney U-test for was used to analyse changes in gene expression between two groups and a Wilcoxon signed-rank test was used to compared expression to an untreated control. Significance was reached when $p<0.05$.

5.2.5 Functional pathway analysis of active labour

To organise the large volume of data from the microarray of E19 for pathway and network analysis, we used the relative expression to non-labouring gestational age at E16.5, with a threshold of 1.3 fold change and statistical threshold cut off of a PPLR score of $\leq 1x10^{-5}$ and ≥ 0.997, indicating a very low probability of uncertainty in the data (Stephen et al. 2014). The enriched functions and pathways detected from the changes in gene expression were analysed using Ingenuity pathway analysis software (IPA) and presented according to significance using Right-sided Fishers exact test (hypergeometric test).

5.2.6 Network analysis of active labour

Using the network analysis workflow previously developed to analyse the labour transcriptome in human pregnancies (Lui et al. 2015 manuscript in preparation – chapter 4), a robust network analysis was performed. In brief, all known interactions were referenced using the Biological General Repository for Interaction Datasets (BioGRID) database (BioGRID: http://thebiogrid.org). The hierarchy of clusters in the genomic interactions were identified using the ModuLand algorithm (Szalay-Beko et al. 2012), a Cytoscape 2.8.3 plugin (Shannon et al. 2003; Smoot et al. 2011). The hierarchy of network clusters were then cross referenced back to the array for genes with a significant difference between labour and non-labour. To provide additional confidence and prioritise highly ranked gene clusters, a second plugin algorithm for Cytoscape, CLusterONE was applied to the dataset (Nepusz et al. 2012). This resulted in a list of "master regulators" of the labour transcriptome, ranked by the hierarchy of network clusters; these were then examined for downstream effects in the network, using causal analysis in Ingenuity Pathway Analysis software, and then the list was further refined based on regulation of the most downstream mediators associated with labour.

5.2.7 Absolute presence of genes in the stages leading up to and during active labour

Data were also interrogated to identify the gene profile at each stage of labour from E16.5 to active labour E19 using Gene Barcoding. The gene barcoding method removes the need for relative expression between samples, so each gene in every microarray (i.e. gestational stages) can be analysed individually. The calculated output produced is a binary outcome - expressed or not expressed - allowing the detection of all actual genes present, or absent, with a high level of

certainty (McCall et al. 2011; McCall et al. 2014). The presence of these genes will be referred to as the absolutely expressed genes, and these were compared across gestational stages in the lead up to labour for similarities and differences using the Venn diagram software from Bioinformatics and Evolutionary Genomics (http://bioinformatics.psb.ugent.be/software). The resulting categorically expressed genes for each time point were then analysed using the IPA software for pathway and causal analysis, and Cytoscape plugin ModuLand, was used as described for the relative expression data.

5.2.8 Mouse cell and tissue culture

All cell culture products were purchased from GIBCO, Life Technologies, UK, unless otherwise stated. Immortalised mouse aortic smooth muscle cells, MOVAS (ATCC® CRL-2797™), were used for *in vitro* investigation of the effect of inhibiting the master regulators (IRAK, TIRAP, TLR4), on the expression of predicted downstream genes. The cells were cultured in DMEM media containing L-glutamine (1.5 mg/ml), geneticin (200µg/ml) and 10% (v/v) fetal bovine calf serum, in a humidified incubator at 37°C, with 21% O_2 and 5% CO_2 for at least two passages from frozen before experimental treatments. MOVAS cells were seeded in a 6-well plate at $8x10^4$ per well, with two wells per treatment. The cells were cultured with inhibitors (Table 2) or vehicle control (DMSO, 0.002% v/v to match the highest concentration used in inhibitor experiments) for 24h or 48h. Cells were then incubated with RNAlater for 24 hours at 4°C prior to RNA isolation and qRT-PCR analysis.

Full thickness (i.e. myometrium and decidua parietalis) uterine explants from C57BL/6 mice at E18.5 were cultured for 24 hours in DMEM/F12 with L-glutamine (1.5 mg/ml), penicillin and streptomycin (0.5mg/ml), and 10% (v/v) fetal bovine calf serum. Explants were treated with inhibitors against master regulators (Table 2) or vehicle control (DMSO, 0.002% v/v) for 24 hours with treatment every 12 hours. After treatment, the explants were incubated in RNA later and processed for PCR analysis.

5.3 Results

5.3.1 Global differential expression of genes at active labour

There were 2549 genes differentially expressed in the term active labour decidua parietalis at E19.0 compared to non-labour at E16.5. Of these, 785 were down regulated and 1764 upregulated with fold change in expression ranging from -233 to 572. The myometrium demonstrated 1146 changes, with 489 genes down-regulated and 657 upregulated. The fold change in expression ranged between -458 and 28.

Several genes with previously identified labour associated function, and/or those with significant changes in expression, were selected from the microarray for validation in individual samples of decidua and myometrium. In the decidua, 11 genes were used for validation; of these, expression changes in 9 genes validated in the same direction as the microarray (Figure 1A). In both the microarray and PCR, OXTR, CXCR4, MMP11, IRAK4, and SLIT2 were upregulated ($p<0.05$ Wilcoxon-signed rank test), AQP5 down-regulated ($p<0.05$ Wilcoxon-signed rank test), whilst IL-

1β, and GAB1 were unchanged (these were unchanged in the array and were selected as internal controls). Two genes (ARG1 nor CXCL12), whose expression were significantly increased in the pooled microarray analysis samples, were not significantly altered in expression in individual PCR amplified samples. However, both genes exhibited a trend for upregulation as observed in the array ($p=0.06$ and $p=0.09$ respectively, Wilcoxon-signed rank test).

In the myometrium, altered expression of 9 of the 14 genes at E19.0 was validated by PCR and three more genes exhibited a fold change in expression that did not reach statistical significance (Figure 1B). The validated genes include PTGS2, OXTR, GJA1, CXCL12, IL1F8, MMP11, SLIT2, and TLR4, which were upregulated, and AQP5 which was down-regulated (all $p<0.05$). OXTR, CXCR4 and IRF8 were significantly upregulated in the pooled samples used for the array, but not in the individual samples used for PCR analysis ($p=0.06$). The genes ARG1 ($p=0.15$) and IL-1β ($p=0.69$) did not validate as upregulated genes.

5.3.2 Pathway analysis of genes differentially expressed in preparation for labour in the decidua and myometrium

Analysis of the biological functions of differentially expressed genes in the decidua at E17.5, 36 hours before labour, revealed down-regulation of metabolic cellular pathways and alterations in cell survival (Heat map, Figure 2A). At E18.5, 12 hours before labour, the differentially expressed genes cluster into immune and inflammation responses, which together with cellular signalling pathways, were upregulated, whilst cancer functions (including cell proliferation and migration) were suppressed. During active labour at E19, differentially expressed genes were overwhelmingly upregulated and clustered in cell survival, chemotaxis and immune responses, identifying an overlap with the biological functions at E18.5 but distinct from E17.5.

In the myometrium at 36 hours before labour (E17.5) (Heat map, Figure 2B) the genes differentially expressed relative to E16.5 were primarily categorised into tissue development, cell movement and cell maintenance type functions. At 12 hours before labour (E18.5), the biological functions overlap with E17.5 in terms of tissue development cell movement and assembly, but there was a significant group of upregulated genes with functions relating to skeletal and muscular development. By active labour (E19) the main functions were cellular movement and cell development, and also the immune/haematological system.

The major canonical pathways of the differentially expressed genes in the decidua are shown in figure 3. These are consistent with the biological functions shown in the heatmap, e.g. on E17.5 related to E16.5, major down-regulated pathways were: LXR/RXR activation, acute phase response signalling, coagulation system, nitric oxide and reactive oxygen species in macrophages (Figure 3A). By E18.5, the differentially expressed genes were a mix of up and down-regulated genes and the canonical pathways included LXR/RXR activation, cell cycle control of chromosomal replication, and acute phase response signalling, coagulation system (Figure 3B). At active labour E19, the canonical pathways were governed by a pattern of up and down regulated genes, again in LXR/RXR activation, inflammatory pathways, and activity in the coagulation system (Figure 3C).

At E17.5, the genes of the myometrium were characterised by upregulated genes and were found in canonical signalling pathways related to integrins, IGF-1, G-beta gamma, ephrin receptor and gap junction (Figure 4A). At E18.5 the cellular signalling was also enriched for IGF-I and integrin signalling, and also VEGF signalling (Figure 4B). By active labour at E19.0, the canonical pathways included genes that were both up and down-regulated and identified in LXR/RXR activation, acute phase response signalling, and wnt/β-catenin signalling (Figure 4C).

5.3.3 Comparative analysis of the absolute uterine gene expression in the days leading up to labour.

The characterisation of the functional changes occurring at active labour prompted further analysis of the stages of pregnancy leading up to labour. All genes expressed were compared to identify commonalties or discrete gene expression at each individual stage from E16.5 to E19.0 in the decidua and myometrium, to determine distinguishing features leading up to labour (Figure 5). In the decidua, 654 genes were common to all four days studied, representing genes important for normal decidual function (Figure 5A) (Gene set A). A total of 129 genes (Gene set B) were transiently expressed between 12-36 hours before labour (i.e. on E17.5 and E18.5), but are not expressed during active labour. There were 330 genes (Gene set C) expressed 12-36 hours before labour (on E17.5 and E18.5), and their expression is maintained during labour (E19). In the 12 hours preceding labour and during labour (i.e. on E19), 620 genes (Gene set D) were expressed and therefore may be presumed to be those that mediate the labour process.

These sets of genes leading up to and during active labour, were subjected to pathway analysis to determine the major biological functions, and also, the causal networks for major upstream regulators of each stage (Table 3). The genes consistently expressed in the decidua during mid to late pregnancy prominently featured pathways and specific genes governing normal decidual function, including cholesterol biosynthesis and metabolism, maintenance of cell-cell junctions, coagulation cascade and STAT3 signalling. Causal analysis identified progesterone, peroxisome proliferator-activated receptor gamma (PPARγ) and SMAD2 as major upstream regulators (Table 3 A). Transient upregulation of calcium transporters, acute phase response, prolactin and TLR signalling occurred 12-36 hours prior to labour (Table 3B). Key genes within these pathways were glucocorticoid receptor, TLR1, suppressor of cytokine signalling 4 (SOCS4) and IL-33. Major causal regulators included TNF receptor associated factor (TRAF), phospholipase C (PLC-β) proteins and zinc finger e-box binding homeobox 1 (ZEB1). Prominent pathways activated in the 12-36 hours prior to and during active labour were cell cycle control, cancer pathways, growth hormone and macrophage function (Table 3C). Multiple cyclins and cyclin dependent kinases (CDK) were apparent in these pathways, together with regulators of apoptosis (B-cell CLL/lymphoma 2 (BCL2) and caspase 3 (CASP3), growth factors and inflammatory mediators. There was considerable overlap in the pathways and major genes in the active labour gene set (Table 3D), with the addition of Wnt signalling (including Wnt7a, frizzled receptors, β-catenin) and DNA repair (breast cancer type 1 susceptibility protein (BRAC1), and recombination protein a (Rad51). However, the top upstream causal regulators of the two gene sets were distinct. Estradiol, CDKs and TRIM65

were highly significant causal regulators of the genes that were expressed prior to labour, whilst ZEB1, IkB kinase complex, syncytin 1 and prostaglandin E1 (PGE1) were major causal regulators of the active labour gene set.

In the myometrium, 643 genes (Gene set A) were expressed over mid-late pregnancy (Figure 5B). At 12 to 36 hours before labour (Gene set B), 257 genes were transiently expressed. The genes expressed in the lead up to labour (12 to 36 hours) and were maintained during active labour include 192 genes (Gene set C). A total of 411 genes (Gene set D) were differentially expressed exclusively at 12 hours before and during active labour at E19.

The pathway analysis of the myometrial pregnancy-related genes, constantly expressed over late gestation were involved normal smooth muscle cell function, eNOS signalling and cell-cell junctions, including myosin, actin, fibronectin, endothelin and angiotensin receptor (Table 4A). Regulators of apoptosis (CASP3, BCL2 and Bax) were also prominent. The top upstream causal regulators included PTGS2, tumour necrosis factor (TNF) and hypoxia inducible factor 1α (HIF1A). In the 12-36 hours prior to labour, genes involved in calcium signalling and cell turnover were transiently expressed, including calcium transporters, phospholipases, integrins and cyclins (Table 4B). The main causal upstream regulators of these genes included cytokines/growth factors (interferon, hepatocyte growth factor (HGF) and oncostatin M), and the JAK1 pathway. Prominent pathways activated in the 12-36 hours prior to and during active labour were molecular mechanisms of cancer and growth factor / hormone signalling (Table 4C). Key genes included FOXO1, multiple oncogenes and signalling molecules. Causal analysis identified diverse regulators, including heat shock protein (HSP) 70, TNF family members and Stearoyl-CoA desaturase-1 (SCD1). During active labour there was a marked expression of inflammatory and immunological pathways, including NFκB, IL-6, TLR and IFN signalling, together with macrophage and granulocyte function and cell turnover pathways (Table 4D). Key genes in these pathways included SMADs, chemokines, MMPs and TLR. The top causal regulators include Pentraxin 3 (PTX3), Cathepsin B (CTSB), SMAD3 and CD11c.

The dynamic temporal changes in late pregnancy of some of the key pathways identified in the decidua are represented graphically in Figure 6. Cholesterol biosynthesis is a major signalling pathway during mid-late pregnancy, but it decreased rapidly in significance towards active labour. At 36 hours prior to labour to 12 hours before labour, temporal changes in glucocorticoid receptor signalling indicates it has a role at all stages of pregnancy and labour but this significance gradually increases towards active labour. At E17.5 to 19.0, the identified pathways included cyclins and cell cycle regulation and insulin receptor signalling; these increased in significance over time from late-pregnancy towards active labour. At active labour, the macrophage / monocyte function and NFκB signalling had elevated significance leading up to active labour.

In the myometrium (Figure 7), temporal analysis of Wnt-β-catenin signalling and tight junction signalling expression highlighted their role in normal myometrial function but not in active labour. At 12-36 hours prior to labour, phospholipase C signalling and Oncostatin M signalling were

predominately involved in late pregnancy with little significance in the earlier pregnancy-related or later active labour stages. The pathways at E17.5 to E19 included BMP signalling pathway and renin-angiotensin signalling. BMP signalling exhibited rising significance in signalling towards labour, and renin-angiotensin signalling is maintained at all pregnancy-related periods and labour but reduced in active labour signalling. Glucocorticoid receptor signalling indicates significant temporal signalling rising at late pregnancy towards active labour, whereas NFκB signalling dramatically increases exclusively during active labour.

5.3.4 Network analysis of differentially expressed genes during active labour

Network analysis comparisons were performed on the transcriptome to uncover potential master regulators of active labour in the decidua and myometrium. The 2549 differentially expressed decidual labour genes were used to generate network models, and this hierarchy was used to identify and prioritise master regulators. In the decidua, the cluster at the top of the generated hierarchy contained a total of 2870 nodes (Figure 8A & B). At the next hierarchical level, they generated 69 meta-nodes that connected 1882 interactions, representing all the interactions identified at level 0 (Figure 8A & C). In the myometrium, the 1146 differentially expressed genes clustered to form 2217 nodes and 2700 interactions at the first level (Figure 9A & B). At the next hierarchical level the genes generated 34 nodes with 408 interactions (Figure 9A & C).

Master regulator candidates in the decidua are summarised in Table 5A. These were further refined to select the final master regulators, which possess high interconnectivity within the whole system of labour, but also influence numerous downstream genes that include a number of established labour-associated genes. IRAK4 was identified in both algorithms indicating high interactions and centrality. Using causal analysis, IRAK4 formed two networks, with an activation score within the whole system of labour of 3.82 ($p<0.0025$) (Table 5B; Supplemental Figure 1). The second master regulator selected was TIRAP which had an activation score of 2.19 in the system (Table 5B; Supplemental Figure 2).

In the myometrium, master regulator candidates identified after the robust network analysis are summarised in Table 6A. The final master regulator candidates with the highest interconnectivity and several established labour-associated gene downstream were the TLR2/4 complex and WNT5a (Table 6B; Supplemental Figure 3 and 4).

5.3.5 *In vitro* chemical manipulation of the master regulators

To investigate whether inhibition of the master regulators altered the expression of downstream labour-associated genes, we utilised an immortalised mouse aortic smooth muscle cell line (MOVAS) and identified chemical inhibitors of our master regulators (Table 2). After initial optimisation (not shown), a single dose of inhibitor was applied to MOVAS cells for 24h. We demonstrated that chemical inhibition of the predicted master regulators TLR4 and WNT5a could selectively moderate expression of predicted downstream genes (Figure 10). Inhibition of TLR4 resulted in a significant decrease in expression of network predicted gene CCL9 and an increase in expression of CASP3 ($p=0.03$ both), with a trend towards an increase in IKBKB and CXCL12 also

observed (p=0.06) (Figure 10C). The WNT5a inhibitor BOX 5 significantly increased CCL6 (p=0.03) and there was a trend towards an increase for IRAK4 (p=0.06) (Figure 10D). Inhibition of IRAK1/4 had no significant effect on downstream gene expression (Figure 10A). TIRAP inhibitor peptide demonstrated promising results with patterns of reduction in many of the network predicted downstream genes (Figure 10B). However with only n=3 in the current study, further exploration of the effect of inhibiting TIRAP is required.

After 48 hours, IRAK4 inhibition led to upregulation of EGR1, CXCL12, FOS and IKBKB ($p<0.05$) (Figure 11A), and TIRAP inhibition resulted in a decrease in expression of several genes including GJA1, CXCL12, CCL9, FOS and GNA14 (Figure 11B). Inhibition of TLR4 led to upregulation of CXCL12, TLR4, FOS and DUSP10 ($p<0.05$) and a trend towards a change in mRNA expression of PTGS2 (p=0.06) (Figure 11C). Incubation of cells with BOX5, a specific inhibitor of WNT5a, had no significant effects on any of the genes assessed for change. Expression of CASP3 however did demonstrate a moderate reduction with a trend for decrease (p=0.06) (Figure 11D).

Chemical inhibition of the selected master regulators in uterine explants resulted in no significant changes in downstream gene expression from pregnant mice at E18.5 (data not shown).

5.3.6 Network analysis of the absolute uterine gene expression in the days leading up to labour

The original candidates from the relative expression analysis i.e. E16.5 to E19, were mapped to ModuLand analysis of the absolute gene expression datasets (gene barcode) (Table 7). Network analysis of the final master regulators demonstrated that IRAK4 was a strong regulatory candidate in the decidua, with additional network significance in the absolute ModuLand analysis (Table 7A). IRAK4 is a master regulator with hierarchical significance from 60 hours prior to and during labour (E16.5 to E19) (DEC A) and also has significance at 12 hours prior to labour at (E18.5 to E19) (DEC D). IRAK4 was also found with high centrality in the myometrium at the same stages leading towards labour as the decidua, though the meta-node hierarchy is higher in the decidua. IRAK4 also forms different networks in the decidua (found in TIRAP and IRAK4), compared to the myometrium (found in PELI2 and PELI1). TIRAP was also found to be a strong master regulator candidate, with hierarchical significance in the decidua during pregnancy 60 hours prior to, and at active labour (E16.5 to E19) (DEC A) and the centrality is maintained at stages closer to active labour (E18.5 to E19) (DEC D) (Table 7A). TIRAP was found in multiple meta-node clusters in the decidua (MAP3K5, TIRAP, IRAK4) and was also identified in the myometrium in the meta-node cluster TLR2. In the myometrium (Table 7B), the original master regulator complex TLR2/4 was mapped to the absolute ModuLand networks but as individual genes. TLR2 was a master regulator with hierarchical significance at E18.5 to E19 (MYO D) 12 hours before and during active labour and was mapped to two different meta-node clusters PELI1 and TLR2. However, it was also mapped with high hierarchical significance in the decidua in the stages leading up to and including active labour (E16.5 to E19) (DEC A) and 12 hours before labour (E18.5 to E19) (DEC D). The myometrial master regulator TLR4 and Wnt5a could only be mapped to the decidual absolute ModuLand networks.

5.4 Discussion

Pathway analysis of relative gene expression in the decidua and myometrium

To our knowledge, our study is the first to explore the globally expressed genes, the cellular pathways activated and the hierarchical functional gene networks formed in the normal progression of pregnancy from E16.5 to active labour at E19 in the mouse decidua and myometrium. The biological functions of the gene profile at active labour in the decidua and myometrium, indicate an upregulation in cell survival, cell movement and haematological system, supporting the role of immune cell infiltration and immune activity at labour. Leukocyte infiltration and cytokine presence have been previously found at term labour in the mouse myometrium (Bethin et al. 2003; Zhao et al. 2007; Shynlova et al. 2013a) and mouse and rat decidua (Hamilton et al. 2012; Shynlova et al. 2013b). In human labour, inflammatory genes IL-1, -6, and -8 are upregulated at term pregnancy (Osmers et al. 1995; Rehman et al. 2003) and in term labour in the myometrium (Elliott et al. 2000; O'Brien et al. 2008; Mittal et al. 2010; Mittal et al. 2011), chorioamnion (Haddad et al. 2006), and placenta (Peng et al. 2011). These studies support the role of inflammation at term pregnancy and labour in a non-infection related conditions.

Our data provide further evidence for a role for inflammation in the days preceding labour in the decidua at 12 hours, and at 36 hours before labour. Acute phase response and coagulation feature highly in the top canonical pathways at 36 hours and 12 hours before labour, with an inflammatory dominance 12 hours prior to labour and in active labour. The inflammatory gene activation just prior to labour in this study, supports a previous observation of extensive leukocyte infiltration in the rat decidua 12 hours before parturition (Hamilton et al. 2012). In the relative expression, the top pathways in the days preceding labour in the decidua included liver X receptor LXR/RXR activation, which was maintained through mid-late pregnancy to labour. LXR/RXR pathways regulate lipid metabolism and leukocyte homeostasis (Calkin & Tontonoz 2010), and are involved in attenuating synthesis of PTGS-2, IL-6 and MMP-9 expression (Hong & Tontonoz 2008). The LXR/RXR pathway appears to have dual roles in inflammation, indicating it may play a role in mediating appropriate inflammatory signals during normal term labour. The LXR/RXR pathway was also identified as an important canonical pathway in human choriodecidual during normal term labour (Stephen et al. 2014).

In a previous study of the mouse myometrium, Salomonis et al, 2005 identified that mouse myometrial genes from mid to term pregnancy, clustered into biological functions of prostaglandin synthesis, cell growth and extracellular matrix, contractile function and DNA transcription. The canonical pathways included contraction, cytoskeletal remodelling and estrogen signalling and transcription (Salomonis et al. 2005). In our study, the myometrial genes were associated cellular assembly and organismal development at 12 hours, and then at 36 hours before labour. The pathways included integrins, IGF-1, and gap junction signalling, supporting anatomical development of the myometrium during mid to late pregnancy. Integrins mediate intracellular signalling with a wide number of roles mediating cell motility, cell adhesion, cell polarity, cell death

and inflammation to name a few (Gille and Swerlick 1996; van Kooyk et al. 1998; Lévesque and Simmons 1999). Growth factors, such as IGF-I and EGF, stimulate myocyte hypertrophy and increased uterine tension (Shynlova et al. 2007a; Shynlova et al. 2010b; Liu et al. 2013), which increases extracellular matrix proteins and integrin expression in the uterus to accommodate the developing fetus (Salomonis et al. 2005; Shynlova et al. 2009). At these gestational stages, the gene expression across late pregnancy is unsurprisingly dominated by functions associated with maintenance of pregnancy.

In contrast to the decidua, inflammatory pathways only featured in the myometrium during active labour. The presence of inflammatory activity at active labour in the myometrium has been associated with myometrial contractile-signalling amplification (Seibert and Masferrer 1994; Gross et al. 2000; Chevillard et al. 2007; Terzidou et al. 2011; MacIntyre et al. 2014; Phillips et al. 2014) and remodelling activity (Blanks et al. 2007; Yellon et al. 2009; Lirussi et al. 2010). The top canonical pathways at E19 are involved in LXR/RXR activation, acute phase response, coagulation signalling, and granulocyte adhesion and diapedesis, mirroring the type of immune activation seen in the decidua during active labour. This suggests that in the myometrium, inflammatory pathways are important during active labour, but not in the preceding stages, arguing against inflammation as a driving force for myometrial labour activity. In another study, pathway analysis in the rat uterus identified immune responses and WNT activation of NFκB as major cellular pathways at term labour (Girotti & Zingg 2003). We also found prominent changes in WNT-signalling, nitric oxide and reactive oxygen species, in the myometrium during active labour. The canonical WNT/β-catenin pathways are involved in many cell types and functions during embryonic cell fate and cancer development, including tissue regeneration, migration and proliferation (Blagodatski et al. 2014). Expression of multiple Wnt family members have been reported in the myometrium (Gorowiec et al. 2011), human fetal membranes (Bollapragada et al. 2009) and cervix (Dobyns et al. 2015) at term, where its activity is associated with prostaglandin production (Yuan et al. 2012). Our identification of nitric oxide as a major cellular activity during active labour is supported by its role in promoting prostaglandin synthesis at high concentrations with elevated levels contributing to LPS induced mouse preterm birth (Cella et al. 2010). The involvement of reactive oxygen species during NFκB signalling is associated with signalling in pro-inflammation, proteases, and prostaglandin expression (Lappas et al. 2003).

Pathway analysis of absolute gene expression in the decidua and myometrium

We refined our original analysis to determine the gene profile in the decidua and myometrium on each day leading up to labour, by detecting all genes that were expressed at each time point, and analysing across the gestational stages for contributing mechanisms that lead up to active labour, rather than a relative expression compared to E16.5. Using this technique, this study identified significant alterations in gene expression that transition in between each stage of pregnancy to active labour. We could identify genes constitutively expressed by the uterine tissues on each day of mid-late pregnancy, late pregnancy and active labour, and those that were switched on or off as the uterus prepares for labour. This approach provides a wealth of data about the potential drivers

of the labour process in each uterine tissue layer. Our investigation into the functional characteristics of the transcriptome temporally expressed during pregnancy and active labour, demonstrated there were very different functions in the different stages leading up to, and during, labour between both tissues.

In decidua, we have defined genes governing normal decidual function across mid to late pregnancy, consistent with roles in steroid hormone and eicosanoid biosynthesis and growth factor signalling. Inflammatory processes are evident in the 12-36 hours prior to labour, especially in the cohort of genes that are maintained during active labour. These include genes regulating macrophage function and TLR signalling. Interestingly, transient preparatory events include glucocorticoid receptor signalling – implying an early priming role of glucocorticoids for labour, whilst changes in cell cycle are a major feature of preparation for and active labour. These studies help to define a sequence of events in decidual function, reinforcing that inflammatory changes are an early event, followed by modulation of cell cycle and cell death accompanied by inflammation during active labour. Causal analysis strongly predicted established labour mediators including PGE_1, progesterone and estrogen, but also those not yet associated with the regulation of labour, including cyclin dependent kinases, homeobox genes and syncytin. The identification of the steroid hormones progesterone and estrogen as sequential upstream regulators of normal decidual gene function and preparation for labour respectively is consistent with our knowledge of endocrine regulation of labour.

This study has identified a novel function of the homeobox gene ZEB1 as a causal regulator of decidual function in late pregnancy, supported by the known roles of ZEB genes in the myometrium. Previous studies have indicated ZEB1/2 suppression is involved in contractile associated sensitisation of the myometrium via increased prostaglandin and cytokine synthesis (Williams et al. 2012), and expression of GJA1 and OXTR through progesterone function (Renthal et al. 2010). Cyclin dependant kinases are inactive until the units complex with a cyclin subunit to mediate different stages of the cell cycle. Progesterone is a dual regulator/activator of cyclin dependent kinases inhibitors (Owen et al. 1998) and estrogen receptor β upregulates cyclin D1 in the presence of a selective estrogen receptor blocker (Sauvé et al. 2009), indicating a role of cell cycle of during pregnancy and timing of active labour. Specific inhibitors of cyclin kinases (P21) have been shown to regulate decidua growth and function during pregnancy and normal induction of labour (Hirota et al. 2010), indicating abnormal function of the decidual cell cycle contributes to timing of labour. Identification of syncytin strongly supports the global approach to understanding the contributions of signals from multiple sources in regulating normal labour. This placental specific retrovirus is involved in the formation of the syncytiotrophoblast which, is responsible for the placental production of corticotropin-releasing hormone (Riley et al. 1991). The identification of syncytin suggests a significant placental contribution to the regulation of labour, through hormonal modulation either through the decidual-placental contact or indirectly via maternal circulation, as it is released from the placenta into maternal blood (Mi et al. 2000). The regulation of labour may also be through syncytin altering immune cell responses, as it has also been shown to upregulate inflammatory maternal leukocyte production of IL-2, -6, -8, -10, chemokines CCL2-5, and CXCL1

(Holder et al. 2012), but suppress Th1-type cytokine production of CXCL10, TNF-α and IFN-γ (Tolosa et al. 2012).

Genes governing normal myometrial function unsurprisingly heavily featured those regulating smooth muscle cell development and behaviour, with regulatory molecules including PTGS2, HIF1α and TNFα. Stress related responses to hypoxia have been shown to be triggered in the progression of pregnancy from hypertrophy and uterine stretch from the developing fetus (Shynlova et al. 2010a). HIF1α in particular has been shown to be expressed in the human labouring myometrium (Mittal et al. 2010; Chaemsaithong et al. 2013), supporting our network analysis in identifying it as a causal regulator of pregnancy. The pathways detected across the gestational stages in preparation for labour were similar to the relative expression analysis, with pathways involved in activation of smooth muscle cell function, regulation of cell turnover and growth factor signalling. Some of the genes included Wnt/frizzled receptor genes and multiple oncogenes. This supports the relative expression analysis identification of the Wnt-signalling pathway and suggests that the regulation of cell growth and death is a critical event for onset of labour. The topmost regulators of these pathways and genes included growth factors HGF and Oncostatin M (OSM), and hsp70. In the pregnant rat myometrium, expression of HGF is associated with IL-1β upregulation via progesterone receptor regulation during pregnancy (Lee et al. 2012b). OSM has been previously identified as differentially expressed at labour in the human myometrium and cervix (Bollapragada et al. 2009). Supporting our analysis of OSM as a top regulator in the stages preceding labour, its presence was identified in maternal blood in women with threatened preterm labour that delivered with 48 hours (Heng et al. 2014). Heat shock proteins (hsp70, 40, 90, p23) play a role in progesterone function by forming subunit complexes in progesterone to progesterone receptor interactions (Kosano et al. 1998).
The pathways defined using absolute gene expression correlate comprehensively with those defined using relative gene expression changes and contribute to our knowledge of the temporal processes occurring during labour. They reinforce that completely distinct priming events occur in decidua and myometrium and that the changes in the decidua are earlier and are far more extensive. Moreover, this study adds important knowledge to the debate regarding the role of inflammation in labour and whether it plays a causal role. Our data confirm that inflammatory processes are highly active during labour, and support the hypothesis that inflammation is an important priming event in the decidua but not the myometrium.

There were also common master regulators identified in the myometrium by relative expression that were more of decidual origin in the absolute analysis. The Wnt pathway frequently appeared as an important regulator in the myometrium, and functioned as key genes and upstream causal regulators in the decidua. The identification of the placental-specific regulator syncytin in the decidua, suggest signals from the developing fetus and placenta may be facilitated through the decidua, but this is a tentative suggestion. These observations contribute to the idea that there is crosstalk between the two uterine layers, with labour being potentially regulated by signals from the decidua. The role of the decidua as a mediator of myometrial responses for the onset of

labouring processes has been previously suggested. As the decidua is a major site of prostaglandin synthesis (Makino et al. 2007; Phillips et al. 2011), is a mediator of progesterone function (Blanks & Brosens 2012), and there is decidual immune cell infiltration prior to labour and at cell populations far greater than those that are found in the myometrium at labour (Hamilton et al. 2012). There is further evidence in a mouse model with a uterine deletion of p53. This deletion resulted in reduced decidualisation and abnormal elevated decidual expression of PTGS2. The increased PTGS2 was not observed in the myometrium but resulted in preterm labour (Hirota et al. 2010).

The volume of genetic data describing term and term labour in the myometrium has repeatedly demonstrated there are genes that are not directly associated with contractile generation, but are involved in a wide range of pathways that contribute to the labour cascade. However, to generate a meaningful understanding of the genes and pathways in the context of the labour system, in-depth analysis of the relationships between the differentially genes and interconnections of the networks they form was required. Ultimately characterising the phenotype of signals leading towards labour will help identify the prominent components and mechanisms that affect the biology of labour.

In vitro analysis of the master regulators

To identify master regulators of the labour transcriptome, we analysed the relative expression of active labour to a non-labouring stage at E16.5. The relative comparison identified several genes that were predicted upstream regulators with hierarchically high relationships and highly significant interconnections in the gene networks. The final candidates included IRAK4 and TIRAP in the decidua, and TLR2/4 complex and Wnt5a in the myometrium. IRAK4 and TIRAP are essential co-factors in interleukin and TLR signalling, consistent with the inflammatory phenotype of the decidua. The inhibition of these upstream regulators of major inflammatory was predicted to down-regulate multiple labour-associated signals. WNT5a is a non-canonical signalling pathway, involved in cell to cell contact, adhesion, differentiation and cell movement in cancer invasion and metastasis (Kikuchi et al. 2012). Activation of TLRs with TNFα and IL-6 can induce Wnt5a activation, but through multiple different signalling cascades (such as JAK/STAT, TGFβ, NFκB and MAP3K7) (Katoh & Katoh 2009). Wnt5a also has roles in regulating cellular metabolism in cancer (Sherwood et al. 2014), and endothelial expression of MMPs (Masckauchán et al. 2006). The multiple roles of Wnt5a, and its identification as a major regulator of labour-associated targets suggested a potential for effective suppression of downstream targets.

The chemical inhibition of each individual master regulator IRAK4, TIRAP, TLR4 and Wnt5a had some effect on the basal expression of our predicted genes, but these were limited. At 24h IRAK4 inhibition generated no differences downstream, but the preliminary results of TIRAP inhibition are supportive of it being a prospective upstream regulator of labour-associated mediators. TLR4 inhibition decreased mRNA expression of the network predicted downstream chemokine CCL9 at 24h, but produced a contrary direction of change in many of the other genes analysed at 24h. The

WNT5a inhibitor BOX 5 also generated an opposite direction of expected change in mRNA expression, with increased downstream gene expression of predicted downstream genes CCL6 and IRAK4. At 48 hours, both IRAK4 and TLR4 inhibition increased the expression of many of the downstream genes, contrary to the network prediction. The inhibitor BOX5 significantly reduced CASP3 expression, positively validated the predicted decrease in this gene. The preliminary results of TIRAP demonstrated encouraging inhibition of the predicted downstream network.

Although two of the targeted master regulators generated an unexpected pattern of change, it is encouraging that the inhibitors generated a response in an unstimulated cell model, and altered expression of specific genes predicted from causal network analysis. Investigation of the effects using a stimulated labour model is required to better represent the changes in gene expression occurring during labour. To further improve experimental outcome, drug-target inhibition interactions could assist the targeting of our upstream predicted regulators. There are vast numbers of pharmacological agents available for our candidate regulators and the ones in this study were selected based on their ability to block each of the individual candidates. However, the chemical interactions against our total candidate network were unknown. Drug to target effects are shown to interact differently on the biology, the functional pathways and governing biological network. There are available techniques to map chemical interaction to target networks, which would better identify effectiveness in altering the downstream pathways and therefore reducing experimental time in identifying an appropriate reagent (Spiro et al. 2008; Tang et al. 2013; Martínez-Jiménez and Marti-Renom 2015; Seal et al. 2015). Additional work in selecting appropriate inhibitors through target-drug interaction analysis and would help identify appropriate therapies. Studies combining genomic and network analysis with drug-target analysis in cancer development identified a series of specific inhibitors that effectively reduced cancer cell viability by at least 50%, compared to other cell-viability suppression compounds that maintained cell viability at 73% (Jeon et al. 2014). Using network analysis and siRNA ablation, another study validated only 63% of downstream targets to a transcription factor (Qian et al. 2003). The study identified that the relationship between regulators and targets are multifaceted, and selective targeting of multiple levels of a network in order to abolish/promote the activity of specific pathways is required. This is apparent in molecular investigation as transcription factors promote the expression of a whole network of genes, and those genes are also regulated by more than one transcription factor (Qian et al. 2003). Our network analysis indicated that the master regulators have associated lower level regulators in the network and inclusion of these in experimental inhibition may develop a better response downstream. For example, combining the inhibition of Wnt5a and TLR signalling may result in more potent inhibition due to the inflammatory association of Wnt5a signalling. The combination of inhibiting the TLR2 and/or 4 together with eicosanoid signalling would target an earlier stage of a labour-associated cascade via upstream interruption of inflammatory and prostaglandin signalling.

It has been previously shown that inhibition of a significantly expressed inflammatory signalling pathway in the labour cascade can reduce the rates of preterm labour. In a mouse model of

preterm labour induced by a gram negative bacteria LPS, the use of a broad spectrum chemokine inhibitor reduced the rates of preterm birth by 36% (Shynlova et al. 2014). This study supports that the blockade of multiple key pathways can prevent labour. However, the partial reduction of preterm labour also emphasises that targeting of multiple or upstream pathways is probably required to more effectively block the labour cascade.

Summary

The dynamic interactions and relationships were investigated between uterine genes during the initiation of labour and in active labour, using pathway analysis, and network analyses of changes in gene expression to assess the hierarchy of molecular mechanisms involved. These analyses identified high-level master regulators of active labour in decidua and myometrium, which possessed strong regulatory effects on downstream networks of labour-associated mediators. As a pre-requisite to future translational studies to therapeutically promote or suppress the labour process, manipulation of these regulatory targets *in vitro* tested their potential effectiveness in suppressing or activating labour-associated genes.

Using functional genomics this study has found there are substantial changes in genes, signalling pathways and regulatory clusters during pregnancy, prior to labour and at active labour in both the myometrium and decidua. A limitation of human studies is the ability to investigate samples that are in active labour and a mouse model allows the investigation of the processes that preceding and activates the processes for parturition. Using the decidua paretalis provides a model that removes the potential effect of fetal placental tissue or contaminating invasive trophoblast cells.

Functional enrichment studies in human uterine tissues identify inflammation driven responses in laboured myometrium and fetal membranes (Arthur et al. 2008; Helguera et al. 2009; Elmes et al. 2015). Our data indicates the inflammatory pathways are highly involved prior to the onset of labour during pregnancy in the decidua, but are only present in the myometrium during active labour. Temporal activity plays an important role as there are gene and functional activity maintained throughout gestation and labour in both tissues. These may not define labour, but participate in supporting or contributing to the whole process of successful labour. More importantly, there is a significant diversity of functions occurring across pregnancy and in the lead up towards labour, especially in the decidua. The decidua is highly active at an earlier gestational stage and this activity continues through to active labour. The myometrium is moderately active with functional activity involved in smooth muscle maintenance, and cell to cell signalling. Major changes in myometrial activity with involvement of major inflammatory signalling peaked during active labour. The decidual function at stages preceding labour, and the detection of a myometrial regulator as network-related in the decidua, suggests a potential functional cross-talk between the two uterine layers for the activation of the labour cascade.

More detailed *in vitro* studies are required for the therapeutic potential of the *in silico* predicted genes. The evidence for the decidual predicted master regulators is encouraging with IRAK4 and TIRAP, both with major pathway significance and high network regulation. The initial results of this

inhibition study using a single target in an unstimulated system alter the response in downstream predicted genes. Though the direction of change is in contrast to the network, the basal cell results indicate a labour-model stimulation would produce a greater magnitude of effects. The targeting of the master regulator and one of its related regulators in the network would also demonstrate the effect on network in the system of labour.

Acknowledgements:

We thank the Genomic Technologies and Bioinformatics Core Facilities in the Faculty of Life Sciences at the University of Manchester for providing technical support and analysis with regard to Affymetrix arrays. We extend our thanks to the midwives in St Mary's Hospital for their assistance with patient recruitment, and to the women for donating tissue samples to research. This study was funded by the James Tudor Foundation, Tommy's the Baby Charity and Ferring. LKH is a BBSRC David Phillips Research Fellow. The Maternal and Fetal Health Research Centre is supported by funding from Tommy's the Baby Charity, and Action Medical Research Endowment, the Manchester Biomedical Research Centre and the Greater Manchester Comprehensive Research Network.

5.5 References

Arthur, Patrice, Michael J Taggart, Barbara Zielnik, Susan Wong, and Bryan F Mitchell. 2008. "Relationship between Gene Expression and Function of Uterotonic Systems in the Rat during Gestation, Uterine Activation and Both Term and Preterm Labour." J Physiol 586 (Pt 24): 6063–76. doi:10.1113/jphysiol.2008.164004.

Bethin, K E, Y Nagai, R Sladek, M Asada, Y Sadovsky, T J Hudson, and L J Muglia. 2003. "Microarray Analysis of Uterine Gene Expression in Mouse and Human Pregnancy." Mol Endocrinol 17 (8): 1454–69. doi:10.1210/me.2003-0007.

Blagodatski, Artem, Dmitry Poteryaev, and Vladimir L Katanaev. 2014. "Targeting the Wnt Pathways for Therapies." Molecular and Cellular Therapies 2 (1). BioMed Central Ltd: 28. doi:10.1186/2052-8426-2-28.

Blanks, A M, and J J Brosens. 2012. "Progesterone Action in the Myometrium and Decidua in Preterm Birth." Facts, Views & Vision in ObGyn 4 (3): 33–43.

Blanks, A M, A Shmygol, and S Thornton. 2007. "Preterm Labour. Myometrial Function in Prematurity." Best Pract Res Clin Obstet Gynaecol 21 (5): 807–19. doi:S1521-6934(07)00045-4 [pii]10.1016/j.bpobgyn.2007.03.003.

Blencowe, H, S Cousens, M Z Oestergaard, D Chou, A B Moller, R Narwal, A Adler, et al. 2012. "National, Regional, and Worldwide Estimates of Preterm Birth Rates in the Year 2010 with Time Trends since 1990 for Selected Countries: A Systematic Analysis and Implications." Lancet 379 (9832): 2162–72. doi:S0140-6736(12)60820-4 [pii]10.1016/S0140-6736(12)60820-4.

Bollapragada, Shrikant, Refaat Youssef, Fiona Jordan, Ian Greer, Jane Norman, and Scott Nelson. 2009. "Term Labor Is Associated with a Core Inflammatory Response in Human Fetal Membranes, Myometrium, and Cervix." American Journal of Obstetrics and Gynecology 200 (1). Mosby, Inc.: 104.e1–11. doi:10.1016/j.ajog.2008.08.032.

Bulmer, J N, D Pace, and A Ritson. 1988. "Immunoregulatory Cells in Human Decidua: Morphology, Immunohistochemistry and Function." Reprod Nutr Dev 28 (6B): 1599–1613.

Calkin, Anna C, and Peter Tontonoz. 2010. "Liver X Receptor Signaling Pathways and Atherosclerosis." Arteriosclerosis, Thrombosis, and Vascular Biology 30 (8): 1513–18. doi:10.1161/ATVBAHA.109.191197.

Cella, M, M G Farina, A P Dominguez Rubio, G Di Girolamo, M L Ribeiro, and A M Franchi. 2010. "Dual Effect of Nitric Oxide on Uterine Prostaglandin Synthesis in a Murine Model of Preterm Labour." Br J Pharmacol 161 (4): 844–55. doi:10.1111/j.1476-5381.2010.00911.x.

Chaemsaithong, Piya, Ichchha Madan, Roberto Romero, Nandor Gabor Than, Adi L Tarca, Sorin Draghici, Gaurav Bhatti, et al. 2013. "Characterization of the Myometrial Transcriptome in Women with an Arrest of Dilatation during Labor." Journal of Perinatal Medicine 41 (6): 665–81. doi:10.1515/jpm-2013-0086.

Chang, H H, J Larson, H Blencowe, C Y Spong, C P Howson, S Cairns-Smith, E M Lackritz, et al. 2012. "Preventing Preterm Births: Analysis of Trends and Potential Reductions with Interventions in 39 Countries with Very High Human Development Index." Lancet. doi:S0140-6736(12)61856-X [pii]10.1016/S0140-6736(12)61856-X [doi].

Chevillard, Grégory, Anna Derjuga, Dominic Devost, Hans H Zingg, and Volker Blank. 2007. "Identification of Interleukin-1beta Regulated Genes in Uterine Smooth Muscle Cells." Reproduction (Cambridge, England) 134 (6): 811–22. doi:10.1530/REP-07-0289.

Dinchuk, J E, B D Car, R J Focht, J J Johnston, B D Jaffee, M B Covington, N R Contel, V M Eng, R J Collins, and P M Czerniak. 1995. "Renal Abnormalities and an Altered Inflammatory Response in Mice Lacking Cyclooxygenase II." Nature 378 (6555): 406–9. doi:10.1038/378406a0.

Dobyns, Abigail E, Ravi Goyal, Lauren Grisham Carpenter, Tom C Freeman, Lawrence D Longo, and Steven M Yellon. 2015. "Macrophage Gene Expression Associated with Remodeling of the Prepartum Rat Cervix: Microarray and Pathway Analyses." PloS One 10 (3): e0119782. doi:10.1371/journal.pone.0119782.

Doring, B, Oksana Shynlova, Prudence Tsui, Dominik Eckardt, Ulrike Janssen-Bienhold, Franz Hofmann, Susanne Feil, et al. 2006. "Ablation of connexin43 in Uterine Smooth Muscle Cells of the Mouse Causes Delayed Parturition." J Cell Sci 119 (Pt 9): 1715–22. doi:jcs.02892 [pii]10.1242/jcs.02892.

Elliott, C L, D M Slater, W Dennes, L Poston, and P R Bennett. 2000. "Interleukin 8 Expression in Human Myometrium: Changes in Relation to Labor Onset and with Gestational Age." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 43 (5): 272–77.

Elmes, Matthew, Alexandra Szyszka, Caroline Pauliat, Bethan Clifford, Zoe Daniel, Zhangrui Cheng, Claire Wathes, and Sarah McMullen. 2015. "Maternal Age Effects on Myometrial Expression of Contractile

Proteins, Uterine Gene Expression, and Contractile Activity during Labor in the Rat." Physiological Reports 3 (4). doi:10.14814/phy2.12305.

Esaki, Yoshiyasu, Youxian Li, Daiji Sakata, Chengcan Yao, Eri Segi-Nishida, Toshiyuki Matsuoka, Kazuhiko Fukuda, and Shuh Narumiya. 2010. "Dual Roles of PGE2-EP4 Signaling in Mouse Experimental Autoimmune Encephalomyelitis." Proceedings of the National Academy of Sciences of the United States of America 107 (27): 12233–38. doi:10.1073/pnas.0915112107.

Gille, J, and R A Swerlick. 1996. "Integrins: Role in Cell Adhesion and Communication." Annals of the New York Academy of Sciences 797 (October): 93–106.

Girotti, Milena, and Hans H Zingg. 2003. "Gene Expression Profiling of Rat Uterus at Different Stages of Parturition." Endocrinology 144 (6): 2254–65. doi:10.1210/en.2002-0196.

Gorowiec, Marta R, Rob D Catalano, Jane E Norman, Fiona C Denison, and Henry N Jabbour. 2011. "Prokineticin 1 Induces Inflammatory Response in Human Myometrium: A Potential Role in Initiating Term and Preterm Parturition." Am J Pathol 179 (6): 2709–19. doi:S0002-9440(11)00846-7 [pii]10.1016/j.ajpath.2011.08.029.

Gross, G A, T Imamura, C Luedke, S K Vogt, L M Olson, D M Nelson, Y Sadovsky, and L J Muglia. 1998. "Opposing Actions of Prostaglandins and Oxytocin Determine the Onset of Murine Labor." Proc Natl Acad Sci U S A 95 (20): 11875–79.

Gross, G, T Imamura, S K Vogt, D F Wozniak, D M Nelson, Y Sadovsky, and L J Muglia. 2000. "Inhibition of Cyclooxygenase-2 Prevents Inflammation-Mediated Preterm Labor in the Mouse." Am J Physiol Regul Integr Comp Physiol 278 (6): R1415–23.

Haddad, Ramsi, Gerard Tromp, Helena Kuivaniemi, Tinnakorn Chaiworapongsa, Yeon Mee Kim, Moshe Mazor, and Roberto Romero. 2006. "Human Spontaneous Labor without Histologic Chorioamnionitis Is Characterized by an Acute Inflammation Gene Expression Signature." American Journal of Obstetrics and Gynecology 195 (2): 394.e1–24. doi:10.1016/j.ajog.2005.08.057.

Hamilton, Sarah A, Y Oomomian, G Stephen, O Shynlova, C L Tower, A Garrod, S J Lye, and R L Jones. 2012. "Macrophages Infiltrate the Human and Rat Decidua during Term and Preterm Labor: Evidence That Decidual Inflammation Precedes Labor." Biol Reprod 86 (2): 39. doi:biolreprod.111.095505 [pii] 10.1095/biolreprod.111.095505.

Hamilton, Sarah A, Clare L Tower, and Rebecca L Jones. 2013. "Identification of Chemokines Associated with the Recruitment of Decidual Leukocytes in Human Labour: Potential Novel Targets for Preterm Labour." PLoS One 8 (2): e56946. doi:10.1371/journal.pone.0056946.

Helguera, G, M Eghbali, D Sforza, T Y Minosyan, L Toro, and E Stefani. 2009. "Changes in Global Gene Expression in Rat Myometrium in Transition from Late Pregnancy to Parturition." Physiol Genomics 36 (2): 89–97. doi:10.1152/physiolgenomics.00290.2007.

Heng, Yujing Jan, Craig Edward Pennell, Hon Nian Chua, Jonathan Edward Perkins, and Stephen James Lye. 2014. "Whole Blood Gene Expression Profile Associated with Spontaneous Preterm Birth in Women with Threatened Preterm Labor." PloS One 9 (5): e96901. doi:10.1371/journal.pone.0096901.

Hirota, Y, J Cha, M Yoshie, T Daikoku, and S K Dey. 2011. "Heightened Uterine Mammalian Target of Rapamycin Complex 1 (mTORC1) Signaling Provokes Preterm Birth in Mice." Proc Natl Acad Sci U S A 108 (44): 18073–78. doi:1108180108 [pii]10.1073/pnas.1108180108.

Hirota, Yasushi, Takiko Daikoku, Susanne Tranguch, Huirong Xie, Heather B Bradshaw, and Sudhansu K Dey. 2010. "Uterine-Specific p53 Deficiency Confers Premature Uterine Senescence and Promotes Preterm Birth in Mice." The Journal of Clinical Investigation 120 (3): 803–15. doi:10.1172/JCI40051.

Hirsch, E, Y Filipovich, and M Mahendroo. 2006. "Signaling via the Type I IL-1 and TNF Receptors Is Necessary for Bacterially Induced Preterm Labor in a Murine Model." Am J Obstet Gynecol 194 (5): 1334–40. doi:S0002-9378(05)02449-X [pii]10.1016/j.ajog.2005.11.004.

Holder, Beth S., Clare L. Tower, Karen Forbes, Melissa J. Mulla, John D. Aplin, and Vikki M. Abrahams. 2012. "Immune Cell Activation by Trophoblast-Derived Microvesicles Is Mediated by Syncytin 1." Immunology 136 (2): 184–91. doi:10.1111/j.1365-2567.2012.03568.x.

Hong, Cynthia, and Peter Tontonoz. 2008. "Coordination of Inflammation and Metabolism by PPAR and LXR Nuclear Receptors." Current Opinion in Genetics and Development 18 (5): 461–67. doi:10.1016/j.gde.2008.07.016.

Irizarry, Rafael A, Benjamin M Bolstad, Francois Collin, Leslie M Cope, Bridget Hobbs, and Terence P Speed. 2003. "Summaries of Affymetrix GeneChip Probe Level Data." Nucleic Acids Research 31 (4): e15.

Jeon, Jouhyun, Satra Nim, Joan Teyra, Alessandro Datti, Jeffrey L Wrana, Sachdev S Sidhu, Jason Moffat, and Philip M Kim. 2014. "A Systematic Approach to Identify Novel Cancer Drug Targets Using Machine

Learning, Inhibitor Design and High-Throughput Screening." Genome Medicine 6 (7): 57. doi:10.1186/s13073-014-0057-7.

Karaçam, Zekiye, Denis Walsh, and George John Bugg. 2014. "Evolving Understanding and Treatment of Labour Dystocia." European Journal of Obstetrics, Gynecology, and Reproductive Biology 182 (November): 123–27. doi:10.1016/j.ejogrb.2014.09.011.

Katoh, Masuko, and Masaru Katoh. 2009. "Transcriptional Mechanisms of WNT5A Based on NF-kappaB, Hedgehog, TGFbeta, and Notch Signaling Cascades." International Journal of Molecular Medicine 23 (6): 763–69.

Kikuchi, A, H Yamamoto, A Sato, and S Matsumoto. 2012. "Wnt5a: Its Signalling, Functions and Implication in Diseases." Acta Physiologica (Oxford, England) 204 (1): 17–33. doi:10.1111/j.1748-1716.2011.02294.x.

Kim, J, Y Ko, K Kwon, S Koo, Y Rhee, B Kang, and M Lee. 2012. "Analysis of Monocyte Subsets and Toll-like Receptor 4 Expression in Peripheral Blood Monocytes of Women in Preterm Labor." J Reprod Immunol 94 (2): 190–95. doi:S0165-0378(12)00025-3 [pii]10.1016/j.jri.2012.02.002.

Kosano, H, B Stensgard, M C Charlesworth, N McMahon, and D Toft. 1998. "The Assembly of Progesterone Receptor-hsp90 Complexes Using Purified Proteins." The Journal of Biological Chemistry 273 (49): 32973–79.

Langfelder, Peter, Rui Luo, Michael C Oldham, and Steve Horvath. 2011. "Is My Network Module Preserved and Reproducible?" PLoS Computational Biology 7 (1): e1001057. doi:10.1371/journal.pcbi.1001057.

Lappas, Martha, Michael Permezel, and Gregory E Rice. 2003. "N-Acetyl-Cysteine Inhibits Phospholipid Metabolism, Proinflammatory Cytokine Release, Protease Activity, and Nuclear Factor-kappaB Deoxyribonucleic Acid-Binding Activity in Human Fetal Membranes *in vitro*." The Journal of Clinical Endocrinology and Metabolism 88 (4): 1723–29. doi:10.1210/jc.2002-021677.

Lee, Yun, Suren R Sooranna, Vasso Terzidou, Mark Christian, Jan Brosens, Kaisa Huhtinen, Matti Poutanen, Geraint Barton, Mark R Johnson, and Phillip R Bennett. 2012. "Interactions between Inflammatory Signals and the Progesterone Receptor in Regulating Gene Expression in Pregnant Human Uterine Myocytes." Journal of Cellular and Molecular Medicine 16 (10): 2487–2503. doi:10.1111/j.1582-4934.2012.01567.x.

Lévesque, J P, and P J Simmons. 1999. "Cytoskeleton and Integrin-Mediated Adhesion Signaling in Human CD34+ Hemopoietic Progenitor Cells." Experimental Hematology 27 (4): 579–86.

Lim, H, B C Paria, S K Das, J E Dinchuk, R Langenbach, J M Trzaskos, and S K Dey. 1997. "Multiple Female Reproductive Failures in Cyclooxygenase 2-Deficient Mice." Cell 91 (2): 197–208. doi:S0092-8674(00)80402-X [pii].

Lim, Sheri, David A MacIntyre, Yun S Lee, Shirin Khanjani, Vasso Terzidou, T G Teoh, and Phillip R Bennett. 2012. "Nuclear Factor Kappa B Activation Occurs in the Amnion prior to Labour Onset and Modulates the Expression of Numerous Labour Associated Genes." PloS One 7 (4): e34707. doi:10.1371/journal.pone.0034707.

Lirussi, F, M O'Brien, M Wendremaire, F Goirand, P Sagot, M Dumas, J J Morrison, and M Bardou. 2010. "SAR150640, a Selective beta3-Adrenoceptor Agonist, Prevents Human Myometrial Remodelling and Activation of Matrix Metalloproteinase in an *in vitro* Model of Chorioamnionitis." Br J Pharmacol 159 (6): 1354–66. doi:BPH616 [pii]10.1111/j.1476-5381.2009.00616.x.

Liu, L, Y Li, N Xie, O Shynlova, J R Challis, D Slater, S Lye, and X Dong. 2013. "Proliferative Action of the Androgen Receptor in Human Uterine Myometrial Cells--a Key Regulator for Myometrium Phenotype Programming." J Clin Endocrinol Metab 98 (1): 218–27. doi:jc.2012-2451 [pii]10.1210/jc.2012-2451.

Liu, Xuejun, Marta Milo, Neil D Lawrence, and Magnus Rattray. 2005. "A Tractable Probabilistic Model for Affymetrix Probe-Level Analysis across Multiple Chips." Bioinformatics (Oxford, England) 21 (18): 3637–44. doi:10.1093/bioinformatics/bti583.

Liu, Xuejun, Marta Milo, Neil D Lawrence, and Magnus Rattray. 2006. "Probe-Level Measurement Error Improves Accuracy in Detecting Differential Gene Expression." Bioinformatics (Oxford, England) 22 (17): 2107–13. doi:10.1093/bioinformatics/btl361.

Lockwood, C J, A Ghidini, R Wein, R Lapinski, D Casal, and R L Berkowitz. 1994. "Increased Interleukin-6 Concentrations in Cervical Secretions Are Associated with Preterm Delivery." Am J Obstet Gynecol 171 (4): 1097–1102. doi:0002-9378(94)90043-4 [pii].

MacIntyre, David A, Yun S Lee, Roberta Migale, Bronwen R Herbert, Simon N Waddington, Donald Peebles, Henrik Hagberg, Mark R Johnson, and Phillip R Bennett. 2014. "Activator Protein 1 Is a Key Terminal Mediator of Inflammation-Induced Preterm Labor in Mice." FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 28 (5): 2358–68. doi:10.1096/fj.13-247783.

Makino, S, D B Zaragoza, B F Mitchell, S Robertson, and D M Olson. 2007. "Prostaglandin F2alpha and Its Receptor as Activators of Human Decidua." Semin Reprod Med 25 (1): 60–68. doi:10.1055/s-2006-956776.

Martínez-Jiménez, Francisco, and Marc A Marti-Renom. 2015. "Ligand-Target Prediction by Structural Network Biology Using nAnnoLyze." PLoS Computational Biology 11 (3): e1004157. doi:10.1371/journal.pcbi.1004157.

Masckauchán, T Néstor H, Dritan Agalliu, Marina Vorontchikhina, Audrey Ahn, Nancy L Parmalee, Chi-Ming Li, Alan Khoo, Benjamin Tycko, Anthony M C Brown, and Jan Kitajewski. 2006. "Wnt5a Signaling Induces Proliferation and Survival of Endothelial Cells *in vitro* and Expression of MMP-1 and Tie-2." Molecular Biology of the Cell 17 (12): 5163–72. doi:10.1091/mbc.E06-04-0320.

McCall, M. N., B. M. Bolstad, and R. A. Irizarry. 2010. "Frozen Robust Multiarray Analysis (fRMA)." Biostatistics 11 (2): 242–53. doi:10.1093/biostatistics/kxp059.

McCall, Matthew N, Harris A Jaffee, Susan J Zelisko, Neeraj Sinha, Guido Hooiveld, Rafael A Irizarry, and Michael J Zilliox. 2014. "The Gene Expression Barcode 3.0: Improved Data Processing and Mining Tools." Nucleic Acids Research 42 (Database issue): D938–43. doi:10.1093/nar/gkt1204.

McCall, Matthew N, Karan Uppal, Harris A Jaffee, Michael J Zilliox, and Rafael A Irizarry. 2011. "The Gene Expression Barcode: Leveraging Public Data Repositories to Begin Cataloging the Human and Murine Transcriptomes." Nucleic Acids Research 39 (Database issue): D1011–15. doi:10.1093/nar/gkq1259.

Mi, S, X Lee, X Li, G M Veldman, H Finnerty, L Racie, E LaVallie, et al. 2000. "Syncytin Is a Captive Retroviral Envelope Protein Involved in Human Placental Morphogenesis." Nature 403 (6771): 785–89. doi:10.1038/35001608.

Mittal, P, R Romero, A L Tarca, S Draghici, C L Nhan-Chang, T Chaiworapongsa, J Hotra, et al. 2011. "A Molecular Signature of an Arrest of Descent in Human Parturition." Am J Obstet Gynecol 204 (2): 177 e15–33. doi:10.1016/j.ajog.2010.09.025.

Mittal, Pooja, Roberto Romero, Adi L Tarca, Juan Gonzalez, Sorin Draghici, Yi Xu, Zhong Dong, et al. 2010. "Characterization of the Myometrial Transcriptome and Biological Pathways of Spontaneous Human Labor at Term." J Perinat Med 38 (6): 617–43. doi:10.1515/JPM.2010.097 [doi].

Montalbano, Alina P, Samuel Hawgood, and Carole R Mendelson. 2013. "Mice Deficient in Surfactant Protein A (SP-A) and SP-D or in TLR2 Manifest Delayed Parturition and Decreased Expression of Inflammatory and Contractile Genes." Endocrinology 154 (1): 483–98. doi:10.1210/en.2012-1797.

Neal, Jeremy L., Nancy K. Lowe, Mavis N. Schorn, Sharon L. Holley, Sharon L. Ryan, Margaret Buxton, and Angela M. Wilson-Liverman. 2015. "Labor Dystocia: A Common Approach to Diagnosis." Journal of Midwifery & Women's Health 60 (5): 499–509. doi:10.1111/jmwh.12360.

Nepusz, Tamás, Haiyuan Yu, and Alberto Paccanaro. 2012. "Detecting Overlapping Protein Complexes in Protein-Protein Interaction Networks." Nature Methods 9 (5): 471–72. doi:10.1038/nmeth.1938.

Norwitz, E R, P M Starkey, A Lopez Bernal, and A C Turnbull. 1991. "Identification by Flow Cytometry of the Prostaglandin-Producing Cell Populations of Term Human Decidua." J Endocrinol 131 (2): 327–34.

O'Brien, Margaret, John J Morrison, and Terry J Smith. 2008. "Upregulation of PSCDBP, TLR2, TWIST1, FLJ35382, EDNRB, and RGS12 Gene Expression in Human Myometrium at Labor." Reproductive Sciences (Thousand Oaks, Calif.) 15 (4): 382–93. doi:10.1177/1933719108316179.

Osman, Inass, Anne Young, Marie Anne Ledingham, Andrew J Thomson, Fiona Jordan, Ian A Greer, and Jane E Norman. 2003. "Leukocyte Density and pro-Inflammatory Cytokine Expression in Human Fetal Membranes, Decidua, Cervix and Myometrium before and during Labour at Term." Mol Hum Reprod 9 (1): 41–45.

Osmers, R G, J Bläser, W Kuhn, and H Tschesche. 1995. "Interleukin-8 Synthesis and the Onset of Labor." Obstetrics and Gynecology 86 (2): 223–29.

Owen, G I, J K Richer, L Tung, G Takimoto, and K B Horwitz. 1998. "Progesterone Regulates Transcription of the p21(WAF1) Cyclin- Dependent Kinase Inhibitor Gene through Sp1 and CBP/p300." The Journal of Biological Chemistry 273 (17): 10696–701.

Peng, Hsiu-Huei, Chuan-Chi Kao, Shuenn-Dyh Chang, An-Shine Chao, Yao-Lung Chang, Chao-Nin Wang, Po-Jen Cheng, Yun-Shien Lee, Tzu-Hao Wang, and Hsin-Shih Wang. 2011. "The Effects of Labor on Differential Gene Expression in Parturient Women, Placentas, and Fetuses at Term Pregnancy." The Kaohsiung Journal of Medical Sciences 27 (11): 494–502. doi:10.1016/j.kjms.2011.06.012.

Phillips, R J, H Al-Zamil, L P Hunt, M A Fortier, and A López Bernal. 2011. "Genes for Prostaglandin Synthesis, Transport and Inactivation Are Differentially Expressed in Human Uterine Tissues, and the Prostaglandin F

Synthase AKR1B1 Is Induced in Myometrial Cells by Inflammatory Cytokines." Molecular Human Reproduction 17 (1): 1–13. doi:10.1093/molehr/gaq057.

Phillips, Robert J, Michel A Fortier, and Andrés López Bernal. 2014. "Prostaglandin Pathway Gene Expression in Human Placenta, Amnion and Choriodecidua Is Differentially Affected by Preterm and Term Labour and by Uterine Inflammation." BMC Pregnancy and Childbirth 14 (1): 241. doi:10.1186/1471-2393-14-241.

Qian, Jiang, Jimmy Lin, Nicholas M Luscombe, Haiyuan Yu, and Mark Gerstein. 2003. "Prediction of Regulatory Networks: Genome-Wide Identification of Transcription Factor Targets from Gene Expression Data." Bioinformatics (Oxford, England) 19 (15): 1917–26.

Rehman, K S, S Yin, B A Mayhew, R A Word, and W E Rainey. 2003. "Human Myometrial Adaptation to Pregnancy: cDNA Microarray Gene Expression Profiling of Myometrium from Non-Pregnant and Pregnant Women." Mol Hum Reprod 9 (11): 681–700.

Renthal, N E, C C Chen, K C Williams, R D Gerard, J Prange-Kiel, and C R Mendelson. 2010. "miR-200 Family and Targets, ZEB1 and ZEB2, Modulate Uterine Quiescence and Contractility during Pregnancy and Labor." Proceedings of the National Academy of Sciences 107 (48): 20828–33. doi:10.1073/pnas.1008301107.

Riley, S C, J C Walton, J M Herlick, and J R Challis. 1991. "The Localization and Distribution of Corticotropin-Releasing Hormone in the Human Placenta and Fetal Membranes throughout Gestation." The Journal of Clinical Endocrinology and Metabolism 72 (5): 1001–7. doi:10.1210/jcem-72-5-1001.

Romero, R, M Ceska, C Avila, M Mazor, E Behnke, and I Lindley. 1991. "Neutrophil Attractant/activating Peptide-1/interleukin-8 in Term and Preterm Parturition." Am J Obstet Gynecol 165 (4 Pt 1): 813–20.

Romero, R, R Gomez, M Galasso, H Munoz, L Acosta, B H Yoon, D Svinarich, and D B Cotton. 1994. "Macrophage Inflammatory Protein-1 Alpha in Term and Preterm Parturition: Effect of Microbial Invasion of the Amniotic Cavity." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 32 (2): 108–13.

Sakurai, Takayuki, Ryo Takai, Heinrich Bürgin, Kana Ishihara, Yuichiro Sakamoto, Jun Amano, Yoshinobu Higuchi, et al. 2012. "The Effects of Interleukin-6 Signal Blockade on Fertility, Embryo-Fetal Development, and Immunization *In vivo*." Birth Defects Research Part B: Developmental and Reproductive Toxicology 95 (4): 304–17. doi:10.1002/bdrb.21019.

Salomonis, Nathan, Nathalie Cotte, Alexander C Zambon, Katherine S Pollard, Karen Vranizan, Scott W Doniger, Gregory Dolganov, and Bruce R Conklin. 2005. "Identifying Genetic Networks Underlying Myometrial Transition to Labor." Genome Biology 6 (2): R12. doi:10.1186/gb-2005-6-2-r12.

Sauvé, Karine, Julie Lepage, Mélanie Sanchez, Nikolaus Heveker, and André Tremblay. 2009. "Positive Feedback Activation of Estrogen Receptors by the CXCL12-CXCR4 Pathway." Cancer Research 69 (14): 5793–5800. doi:10.1158/0008-5472.CAN-08-4924.

Schroeder, Andreas, Odilo Mueller, Susanne Stocker, Ruediger Salowsky, Michael Leiber, Marcus Gassmann, Samar Lightfoot, Wolfram Menzel, Martin Granzow, and Thomas Ragg. 2006. "The RIN: An RNA Integrity Number for Assigning Integrity Values to RNA Measurements." BMC Molecular Biology 7 (January): 3. doi:10.1186/1471-2199-7-3.

Seal, Abhik, Yong-Yeol Ahn, and David J Wild. 2015. "Optimizing Drug-Target Interaction Prediction Based on Random Walk on Heterogeneous Networks." Journal of Cheminformatics 7 (January): 40. doi:10.1186/s13321-015-0089-z.

Seibert, K, and J L Masferrer. 1994. "Role of Inducible Cyclooxygenase (COX-2) in Inflammation." Receptor 4 (1): 17–23.

Shannon, Paul, Andrew Markiel, Owen Ozier, Nitin S Baliga, Jonathan T Wang, Daniel Ramage, Nada Amin, Benno Schwikowski, and Trey Ideker. 2003. "Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks." Genome Research 13 (11): 2498–2504. doi:10.1101/gr.1239303.

Sherwood, Victoria, Shivendra Kumar Chaurasiya, Elin J Ekström, William Guilmain, Qing Liu, Tomas Koeck, Kate Brown, et al. 2014. "WNT5A-Mediated β-Catenin-Independent Signalling Is a Novel Regulator of Cancer Cell Metabolism." Carcinogenesis 35 (4): 784–94. doi:10.1093/carcin/bgt390.

Shynlova, O, P Tsui, A Dorogin, B L Langille, and S J Lye. 2007. "Insulin-like Growth Factors and Their Binding Proteins Define Specific Phases of Myometrial Differentiation during Pregnancy in the Rat." Biol Reprod 76 (4): 571–78. doi:biolreprod.106.056929 [pii]10.1095/biolreprod.106.056929.

Shynlova, Oksana, Prudence Tsui, Shabana Jaffer, and Stephen J Lye. 2009. "Integration of Endocrine and Mechanical Signals in the Regulation of Myometrial Functions during Pregnancy and Labour." Eur J Obstet Gynecol Reprod Biol 144 Suppl (May): S2–10. doi:S0301-2115(09)00134-1 [pii]10.1016/j.ejogrb.2009.02.044.

Shynlova, O, A Dorogin, and Lye, S J. 2010a. "Stretch-Induced Uterine Myocyte Differentiation during Rat Pregnancy: Involvement of Caspase Activation." Biol Reprod 82 (6): 1248–55. doi:biolreprod.109.081158 [pii] 10.1095/biolreprod.109.081158.

Shynlova, O, R Kwong, and S J Lye. 2010b. "Mechanical Stretch Regulates Hypertrophic Phenotype of the Myometrium during Pregnancy." Reproduction 139 (1): 247–53. doi:REP-09-0260 [pii]10.1530/REP-09-0260.

Shynlova, Oksana, Tamara Nedd-Roderique, Yunqing Li, Anna Dorogin, and Stephen J Lye. 2013a. "Myometrial Immune Cells Contribute to Term Parturition, Preterm Labour and Post-Partum Involution in Mice." Journal of Cellular and Molecular Medicine 17 (1): 90–102. doi:10.1111/j.1582-4934.2012.01650.x.

Shynlova, Oksana, Tamara Nedd-Roderique, Yunqing Li, Anna Dorogin, Tina Nguyen, and Stephen J. Lye. 2013b. "Infiltration of Myeloid Cells into Decidua Is a Critical Early Event in the Labour Cascade and Post-Partum Uterine Remodelling." Journal of Cellular and Molecular Medicine 17 (2): 311–24. doi:10.1111/jcmm.12012.

Shynlova, Oksana, Anna Dorogin, Yunqing Li, and Stephen Lye. 2014. "Inhibition of Infection-Mediated Preterm Birth by Administration of Broad Spectrum Chemokine Inhibitor in Mice." Journal of Cellular and Molecular Medicine 18 (9): 1816–29. doi:10.1111/jcmm.12307.

Smoot, Michael E, Keiichiro Ono, Johannes Ruscheinski, Peng-Liang Wang, and Trey Ideker. 2011. "Cytoscape 2.8: New Features for Data Integration and Network Visualization." Bioinformatics (Oxford, England) 27 (3): 431–32. doi:10.1093/bioinformatics/btq675.

Spiro, Zoltan, Istvan A Kovacs, and Peter Csermely. 2008. "Drug-Therapy Networks and the Prediction of Novel Drug Targets." Journal of Biology 7 (6): 20. doi:10.1186/jbiol81.

Stephen, Gillian L, Sylvia Lui, Sarah A Hamilton, Clare L Tower, Lynda K Harris, Adam Stevens, and Rebecca L Jones. 2014. "Transcriptomic Profiling of Human Choriodecidua During Term Labor: Inflammation as a Key Driver of Labor." American Journal of Reproductive Immunology (New York, N.Y. : 1989), October. doi:10.1111/aji.12328.

Sugimoto, Y, A Yamasaki, E Segi, K Tsuboi, Y Aze, T Nishimura, H Oida, et al. 1997. "Failure of Parturition in Mice Lacking the Prostaglandin F Receptor." Science 277 (5326): 681–83.

Szalay-Beko, Máté, Robin Palotai, Balázs Szappanos, István a Kovács, Balázs Papp, and Péter Csermely. 2012. "ModuLand Plug-in for Cytoscape: Determination of Hierarchical Layers of Overlapping Network Modules and Community Centrality." Bioinformatics (Oxford, England) 28 (16): 2202–4. doi:10.1093/bioinformatics/bts352.

Takayanagi, Y., M. Yoshida, I. F. Bielsky, H. E. Ross, M. Kawamata, T. Onaka, T. Yanagisawa, et al. 2005. "Pervasive Social Deficits, but Normal Parturition, in Oxytocin Receptor-Deficient Mice." Proceedings of the National Academy of Sciences 102 (44): 16096–101. doi:10.1073/pnas.0505312102.

Tang, Jing, Leena Karhinen, Tao Xu, Agnieszka Szwajda, Bhagwan Yadav, Krister Wennerberg, and Tero Aittokallio. 2013. "Target Inhibition Networks: Predicting Selective Combinations of Druggable Targets to Block Cancer Survival Pathways." PLoS Computational Biology 9 (9): e1003226. doi:10.1371/journal.pcbi.1003226.

Terzidou, V, A M Blanks, S H Kim, S Thornton, and P R Bennett. 2011. "Labor and Inflammation Increase the Expression of Oxytocin Receptor in Human Amnion." Biol Reprod 84 (3): 546–52. doi:biolreprod.110.086785 [pii]10.1095/biolreprod.110.086785 [doi].

Thomson, A J, J F Telfer, A Young, S Campbell, C J Stewart, I T Cameron, I A Greer, and J E Norman. 1999. "Leukocytes Infiltrate the Myometrium during Human Parturition: Further Evidence That Labour Is an Inflammatory Process." Hum Reprod 14 (1): 229–36.

Tolosa, J M, J E Schjenken, V L Clifton, A Vargas, B Barbeau, P Lowry, K Maiti, and R Smith. 2012. "The Endogenous Retroviral Envelope Protein Syncytin-1 Inhibits LPS/PHA-Stimulated Cytokine Responses in Human Blood and Is Sorted into Placental Exosomes." Placenta 33 (11): 933–41. doi:10.1016/j.placenta.2012.08.004.

van Kooyk, Y, M Lub, and C G Figdor. 1998. "Adhesion and Signaling Mediated by the Cytoplasmic Tails of Leucocyte Integrins." Cell Adhesion and Communication 6 (2-3): 247–54.

Wahid, Hanan H., Camilla L. Dorian, Peck Yin Chin, Mark R. Hutchinson, Kenner C. Rice, David M. Olson, Lachlan M. Moldenhauer, and Sarah A. Robertson. 2015. "Toll-Like Receptor 4 Is an Essential Upstream Regulator of On-Time Parturition and Perinatal Viability in Mice." Endocrinology 156 (10): 3828–41. doi:10.1210/en.2015-1089.

Weiner, C P, C W Mason, Y Dong, I A Buhimschi, P W Swaan, and C S Buhimschi. 2010. "Human Effector/initiator Gene Sets That Regulate Myometrial Contractility during Term and Preterm Labor." Am J Obstet Gynecol 202 (5): 474 e1–20. doi:S0002-9378(10)00247-4 [pii]10.1016/j.ajog.2010.02.034.

Williams, Koriand'r C, Nora E Renthal, Robert D Gerard, and Carole R Mendelson. 2012. "The microRNA (miR)-199a/214 Cluster Mediates Opposing Effects of Progesterone and Estrogen on Uterine Contractility during Pregnancy and Labor." Molecular Endocrinology (Baltimore, Md.) 26 (11): 1857–67. doi:10.1210/me.2012-1199.

Yellon, S M, A E Burns, J L See, T J Lechuga, and M A Kirby. 2009. "Progesterone Withdrawal Promotes Remodeling Processes in the Nonpregnant Mouse Cervix." Biol Reprod 81 (1): 1–6. doi:biolreprod.108.074997 [pii] 10.1095/biolreprod.108.074997.

Yuan, M, F Jordan, I B McInnes, M M Harnett, and J E Norman. 2009. "Leukocytes Are Primed in Peripheral Blood for Activation during Term and Preterm Labour." Mol Hum Reprod 15 (11): 713–24. doi:gap054 [pii]10.1093/molehr/gap054.

Yuan, Subo, Yuqiang Shi, and Shao-Jun Tang. 2012. "Wnt Signaling in the Pathogenesis of Multiple Sclerosis-Associated Chronic Pain." Journal of Neuroimmune Pharmacology : The Official Journal of the Society on NeuroImmune Pharmacology 7 (4): 904–13. doi:10.1007/s11481-012-9370-3.

Zhao, Baohui, Deanna Koon, Allyson L Curtis, Jessica Soper, and Kathleen E Bethin. 2007. "Identification of 9 Uterine Genes That Are Regulated during Mouse Pregnancy and Exhibit Abnormal Levels in the Cyclooxygenase-1 Knockout Mouse." Reprod Biol Endocrinol 5 (January): 28. doi:10.1186/1477-7827-5-28.

Zilliox, Michael J, and Rafael A Irizarry. 2007. "A Gene Expression Bar Code for Microarray Data." Nature Methods 4 (11): 911–13. doi:10.1038/nmeth1102.

Figures and tables

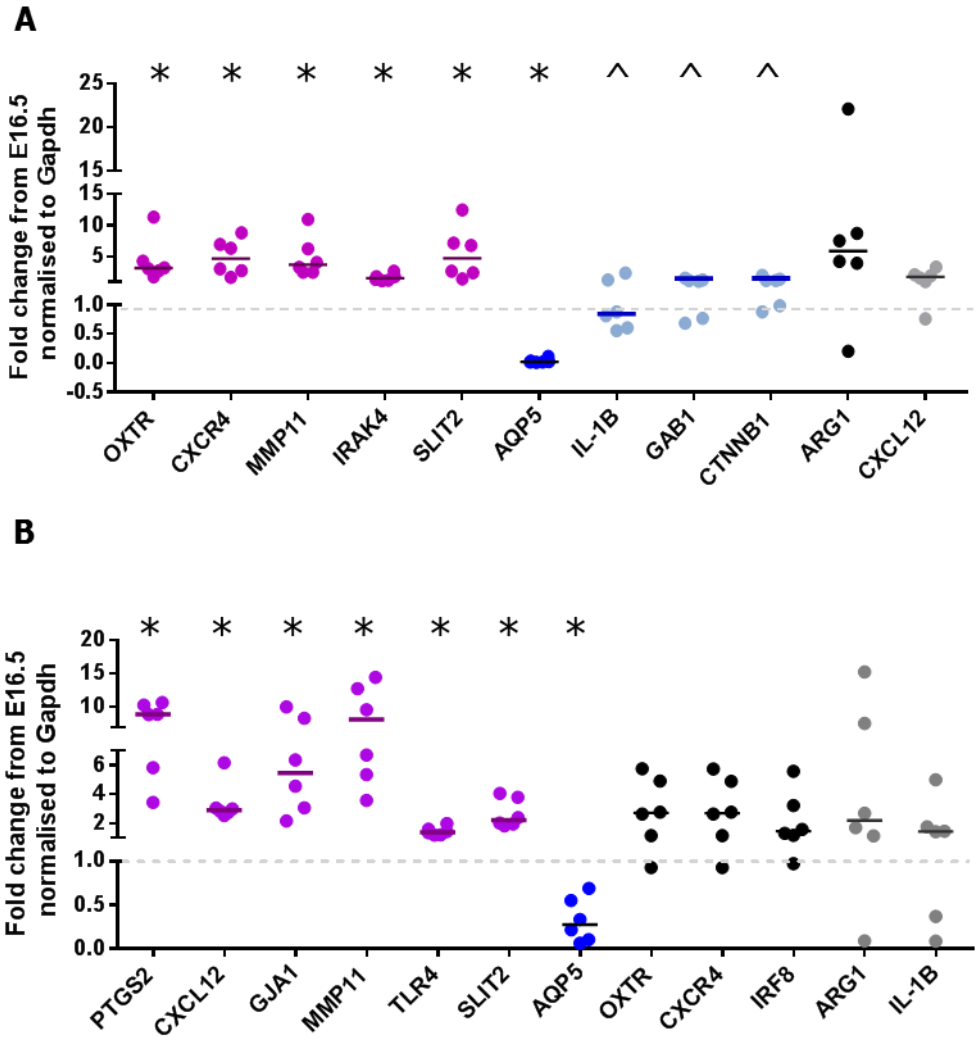


Figure 1. Microarray validation of gene expression at E19.0 using qRT-PCR.
Genes were amplified in individual samples, and analysed by fold change of expression at E19.0 compared to E16.5 in (A) decidua and (B) myometrium. Light grey line indicates normalised expression on E16.5. Purple points indicate validated upregulation, dark blue validated down-regulation, light blue indicates validation for predicted no change in expression. Black points indicate differential expression that did not reach statistical significance (p=0.06). Grey points indicate no change in expression (p>0.05). * Statistical significance p<0.05 Wilcoxon signed rank test. ^ no change in expression was validated (A only).

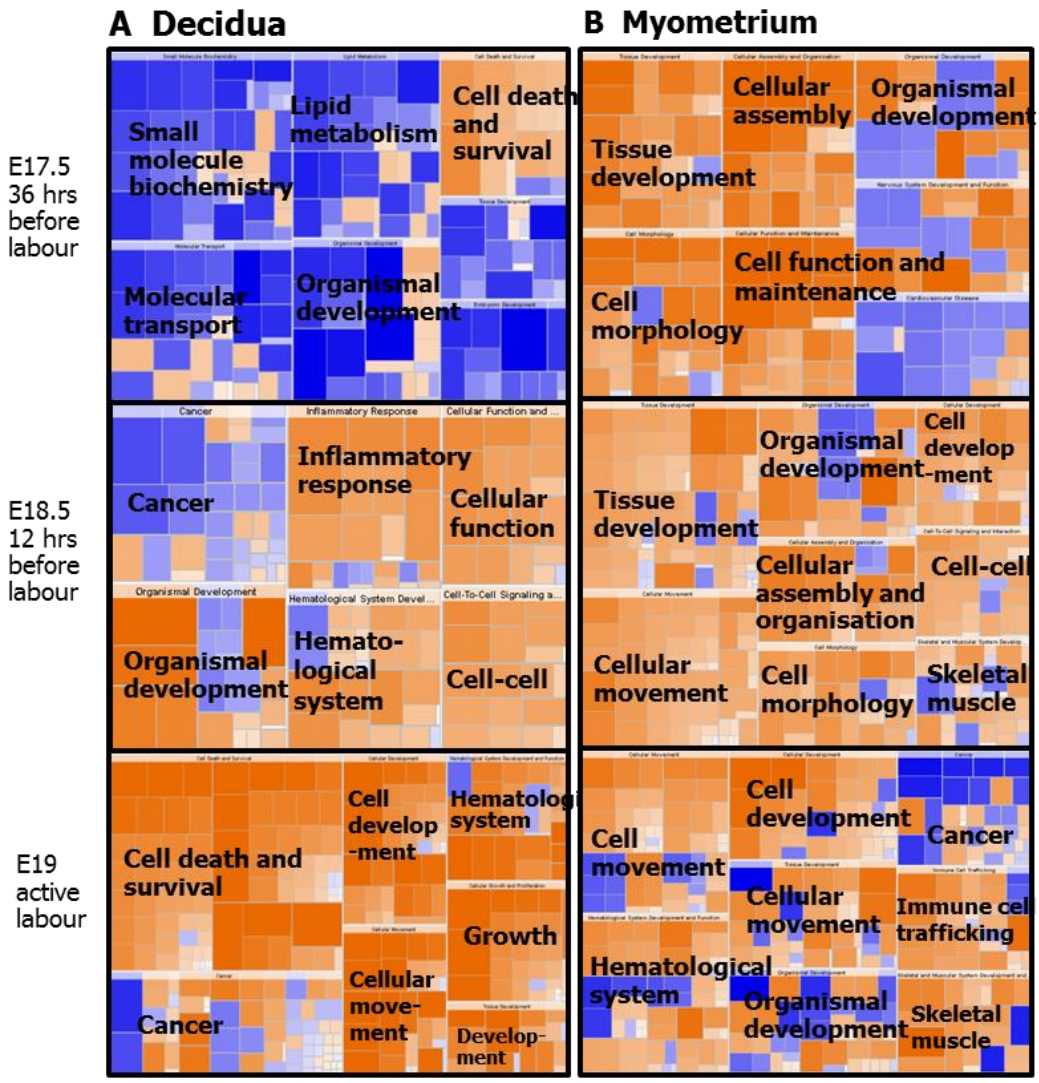


Figure 2. Heat map showing the pattern of gene expression relative to E16.5 and the top biological pathways during late pregnancy and active labour

(A) Decidua and (B) Myometrium. Embryonic day (E) 17.5, E18.5 and E19. Each box represents differentially expressed genes that cluster in the indicated function. The intensity of colour specifies the degree of change. Orange = upregulation, Blue= down-regulation, white= significant but low level of change.

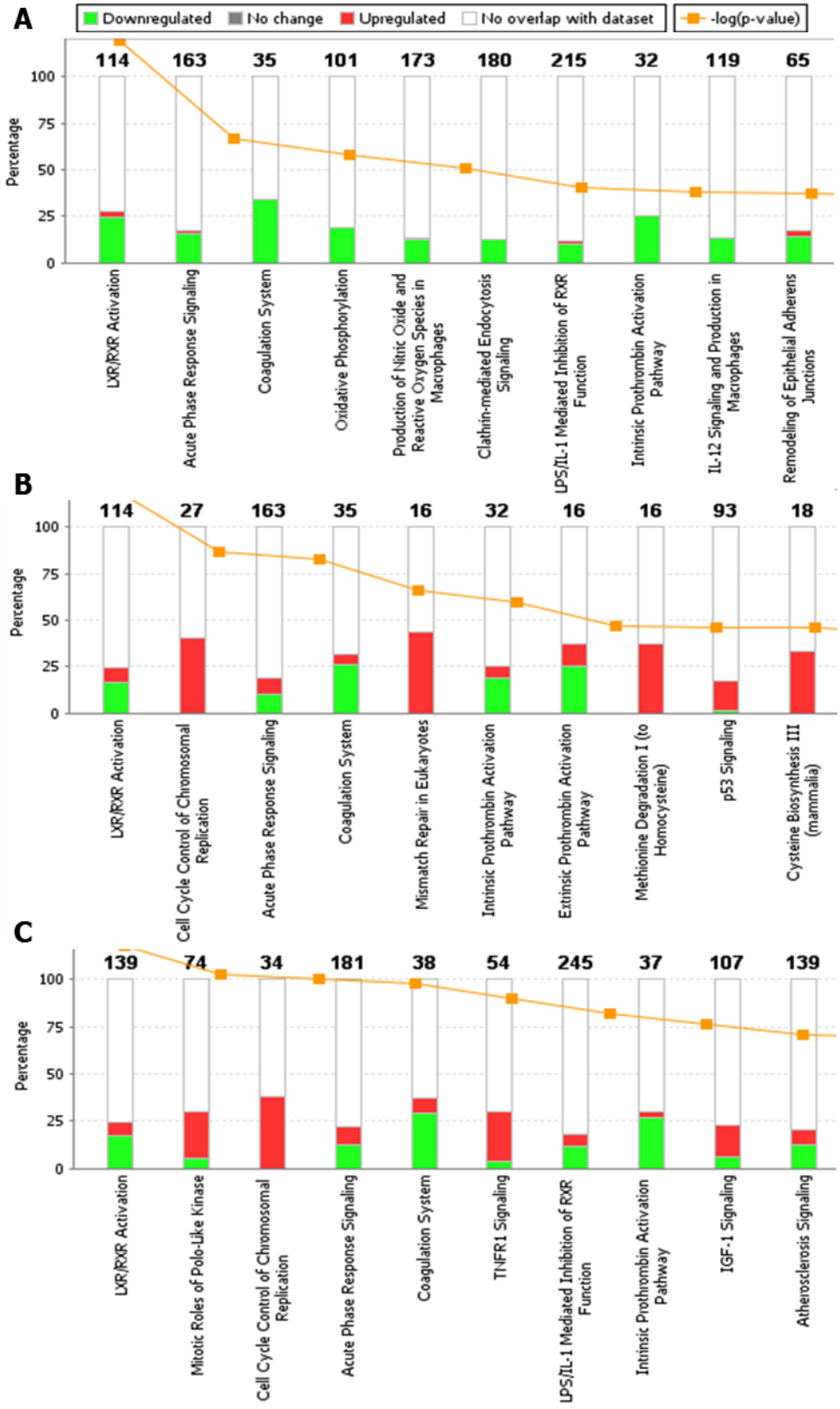


Figure 3. Canonical pathways in the decidua preceding and during active labour.

The differentially expressed genes and their top 10 associated canonical pathways at embryonic day (E): (A) E17.5, (B) E18.5, (C) E19.0 compared to E16.5. The y-axis indicates the percentage of the differentially expressed genes in the cellular pathway. The numbers at the top of the graph indicate the number of known genes within the pathway. The yellow line indicates the calculated pattern of gene activity (-log p-value) in each pathway. Red bar = upregulation, green bar = down-regulation.

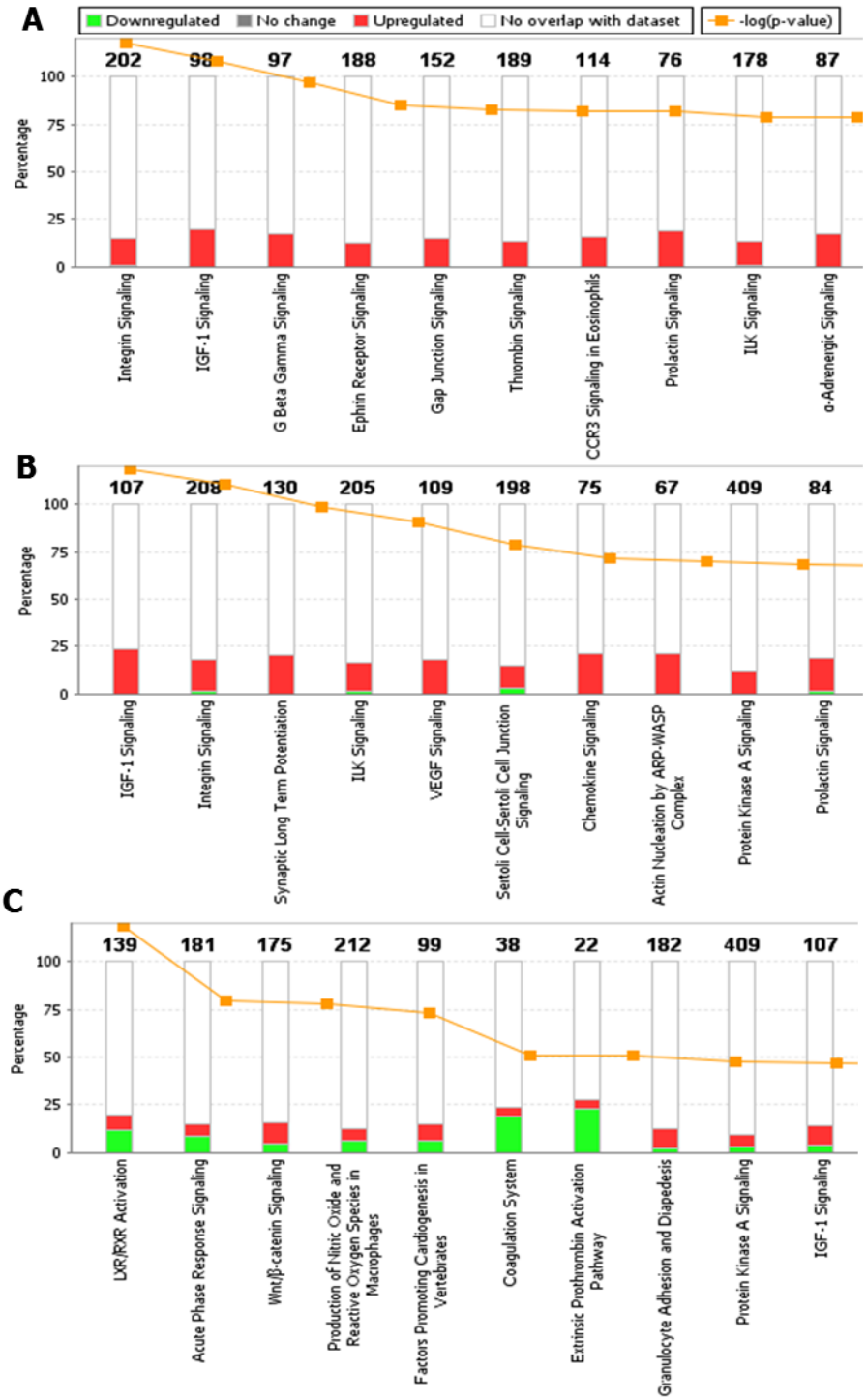


Figure 4. Canonical pathways in the myometrium preceding and during active labour. The differentially expressed genes and their top 10 associated canonical pathways at embryonic day (E): (A) E17.5, (B) E18.5, (C) E19.0 compared to E16.5. The y-axis indicates the percentage of the differentially expressed genes in the cellular pathway. The numbers at the top of the graph indicates the number of known genes within the pathway. The yellow line indicate the calculated pattern of gene activity (-log p-value) in each pathway. Red bar = upregulation, green bar = down-regulation.

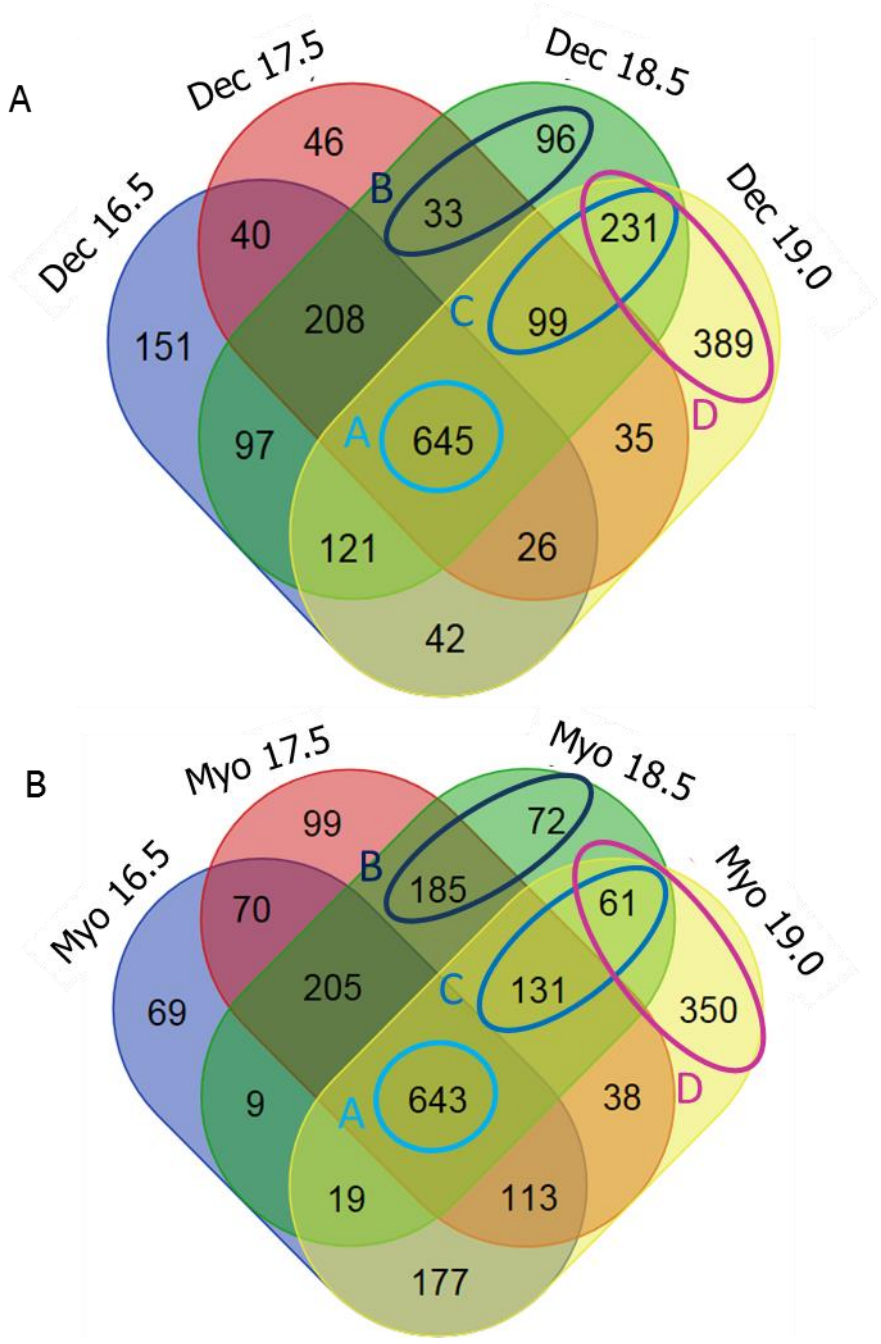


Figure 5. Comparative analysis of the absolute gene expression pattern between the stages of pregnancy leading up to labour.

This Venn diagram identifies the distinguishing pattern of shared and discrete genes between the stages of pregnancy leading up to active labour. A. all shared genes E16.5 to E19, B. pattern of genes between E17.5 and E18.5, C. gene activity between E17.5, E18.5 and E19, D. genes at active labour E19.0. Dec= decidua, Myo= myometrium.

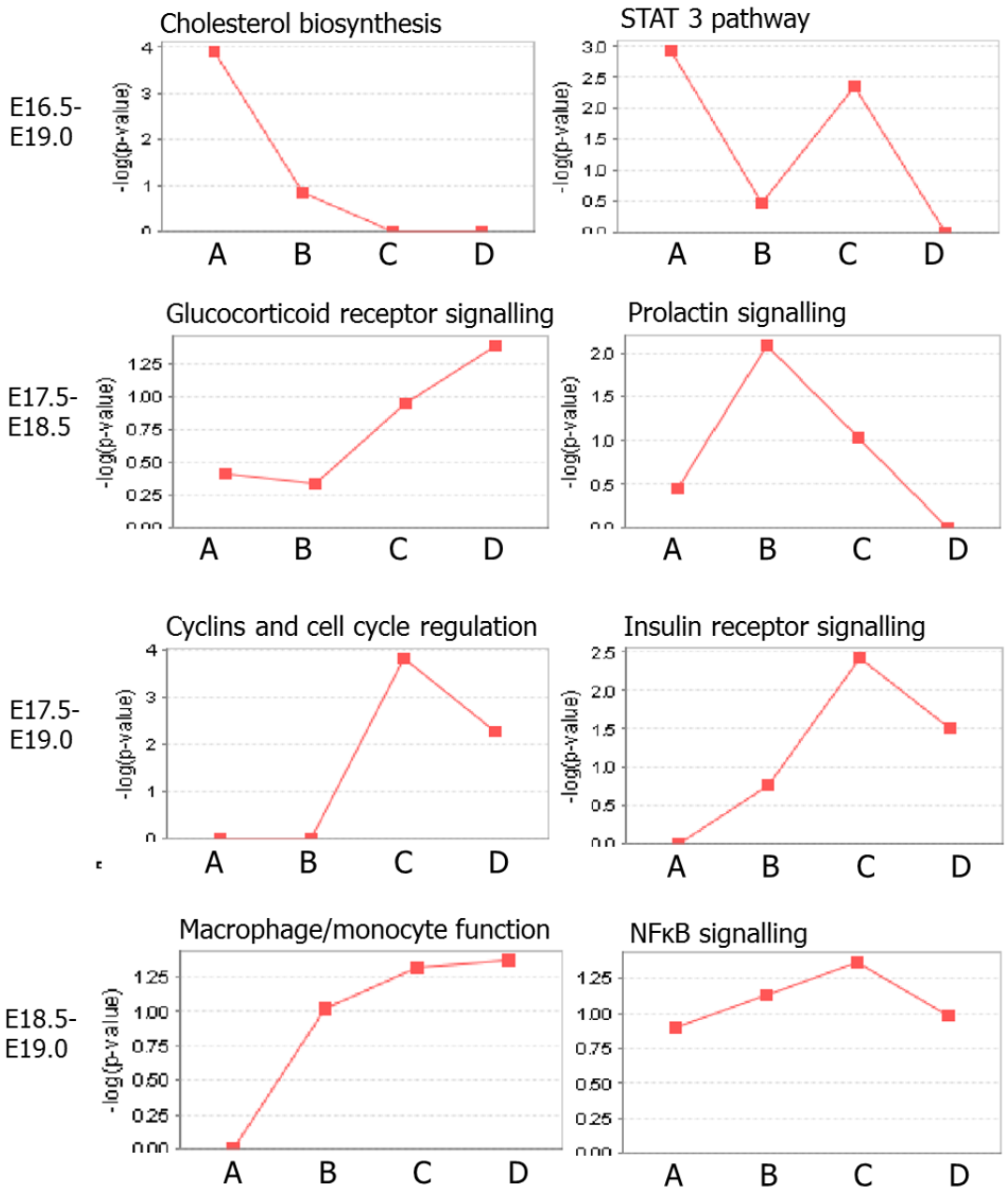


Figure 6. The temporal significance of signalling pathways across gestational stages and active labour in the decidua.

A. Gene set consistently expressed (E16.5 to E19). B. Gene set transiently expressed 12-36 hours before labour (E17.5 and E18.5). C. Gene set expressed 12-36 hours before and during labour (E17.5, E18.5 and E19). D. Gene set expressed during active labour (E18.5 and E19).

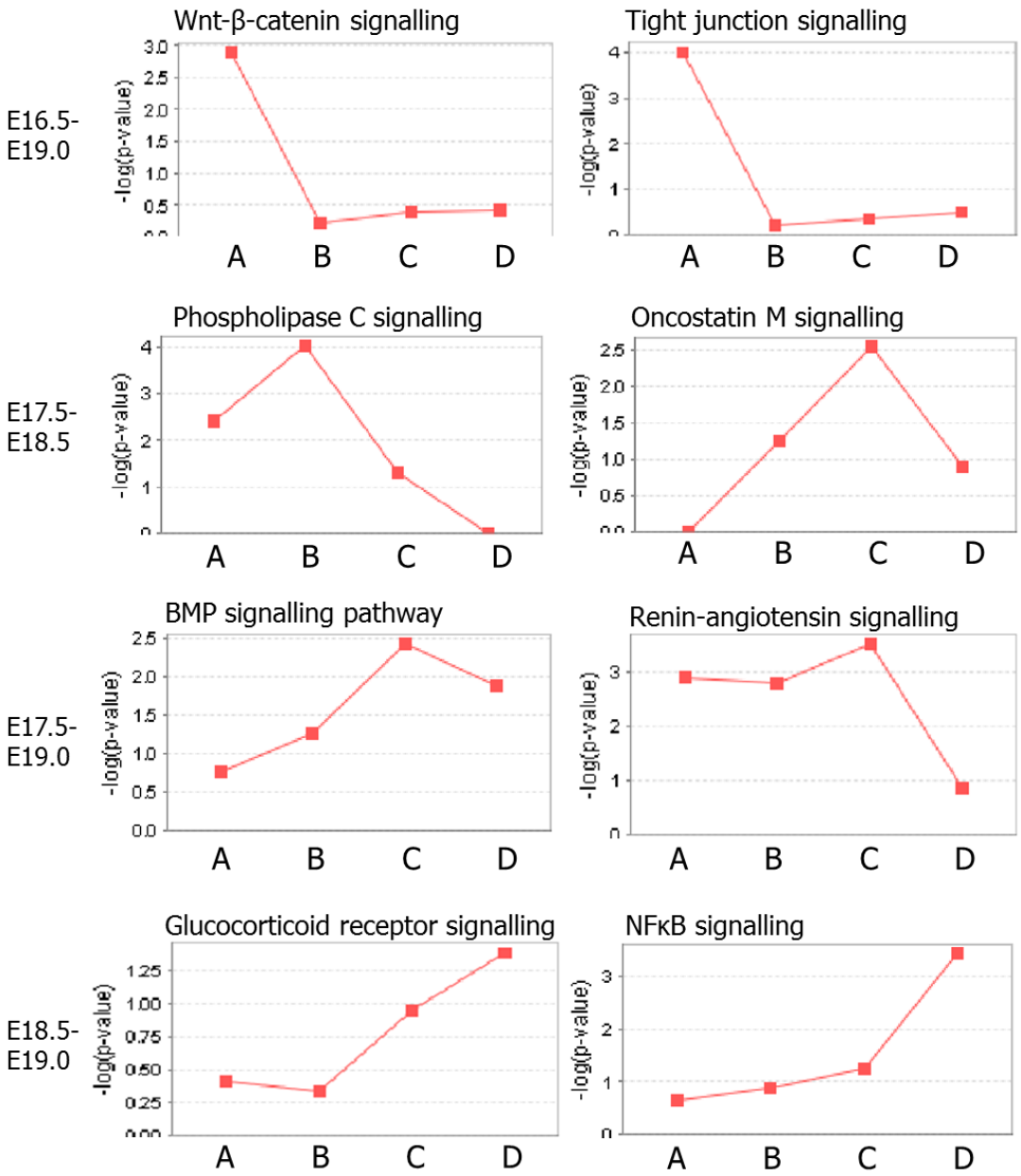


Figure 7. The temporal significance of signalling pathways across gestational stages and active labour in the myometrium.

A. Gene set consistently expressed (E16.5 to E19). B. Gene set transiently expressed 12-36 hours before labour (E17.5 and E18.5). C. Gene set expressed 12-36 hours before and during labour (E17.5, E18.5 and E19). D. Gene set expressed during active labour (E18.5 and E19).

A

Level	Nodes	Interactions
0	2870	4250
1	69	1882

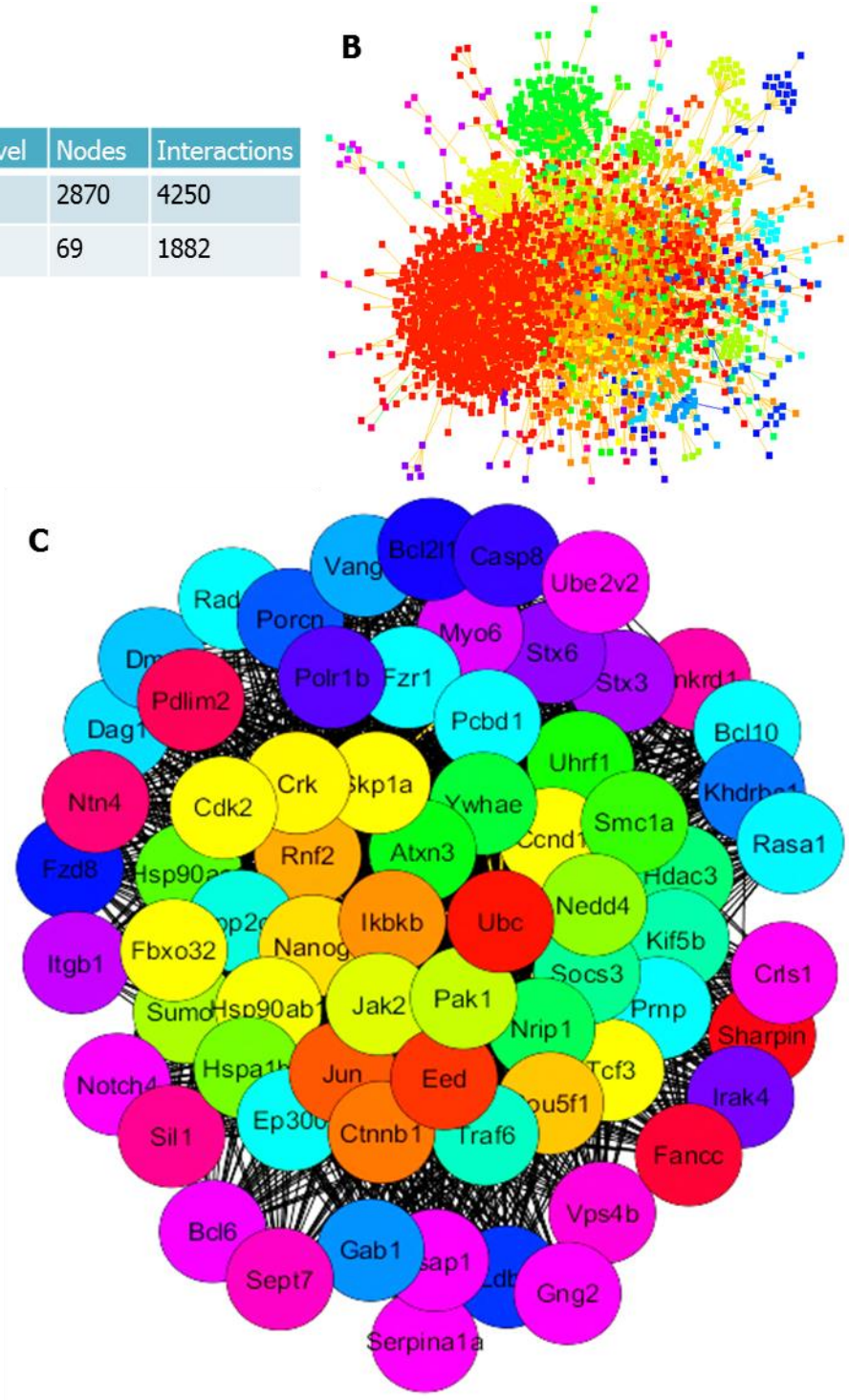


Figure 8. ModuLand hierarchical network analysis of decidual genes differentially expressed at E19 compared to E16.5.

A. Summary of network analysis showing the number of nodes and interactions formed, B. the network system at level 0, and C. the network at the next hierarchical level 1. The nodes at this level represent all the lower level interactions at level 0.

A

Level	Nodes	Interactions
0	2217	2700
1	34	408

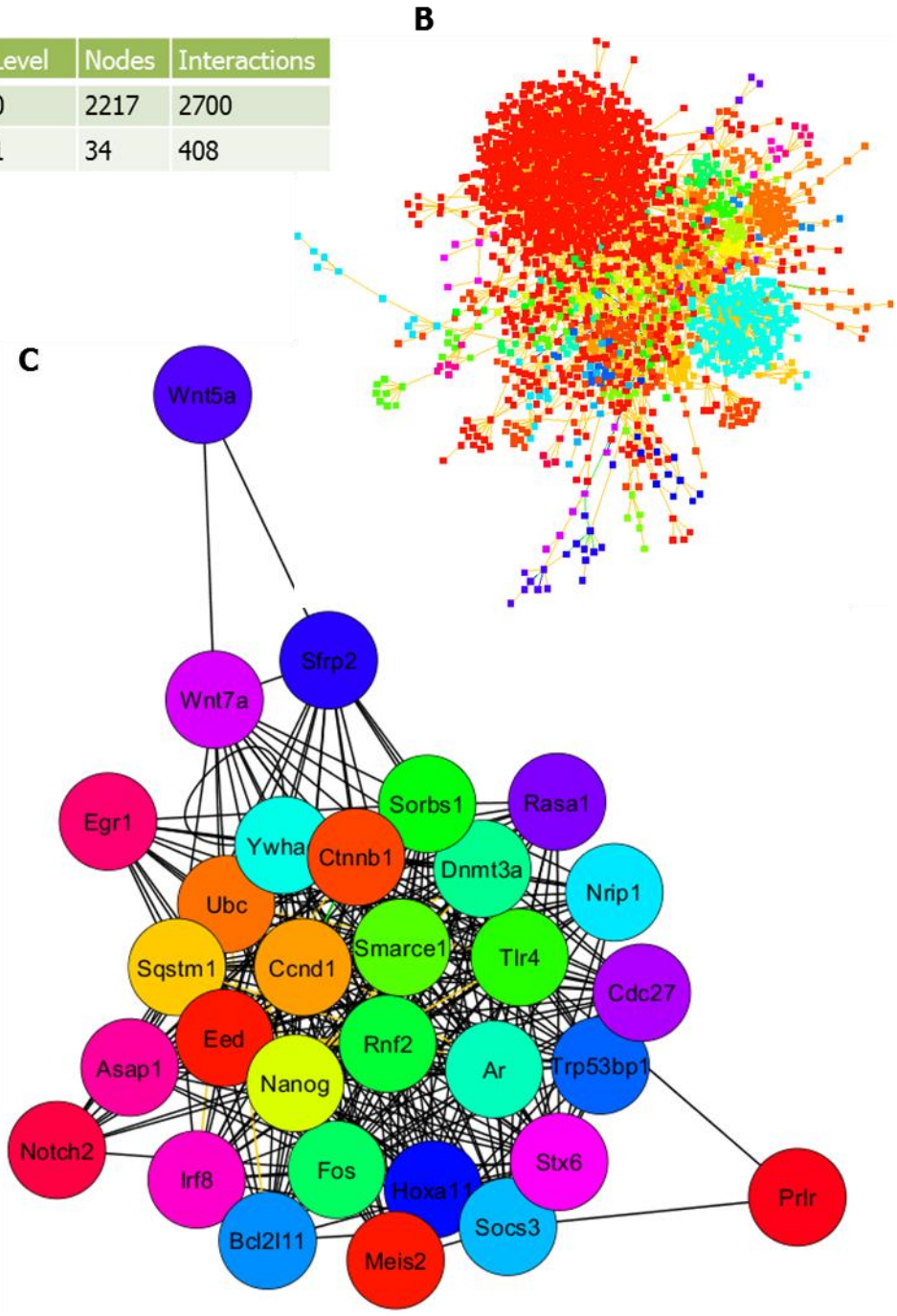


Figure 9. ModuLand hierarchical network analysis of myometrial genes differentially expressed at E19 compared to E16.5.

A. Summary of network analysis showing the number of nodes and interactions formed, B. the network system at level 0, and C. the network at the next hierarchical level 1. The nodes at this level represent all the lower level interactions at level 0.

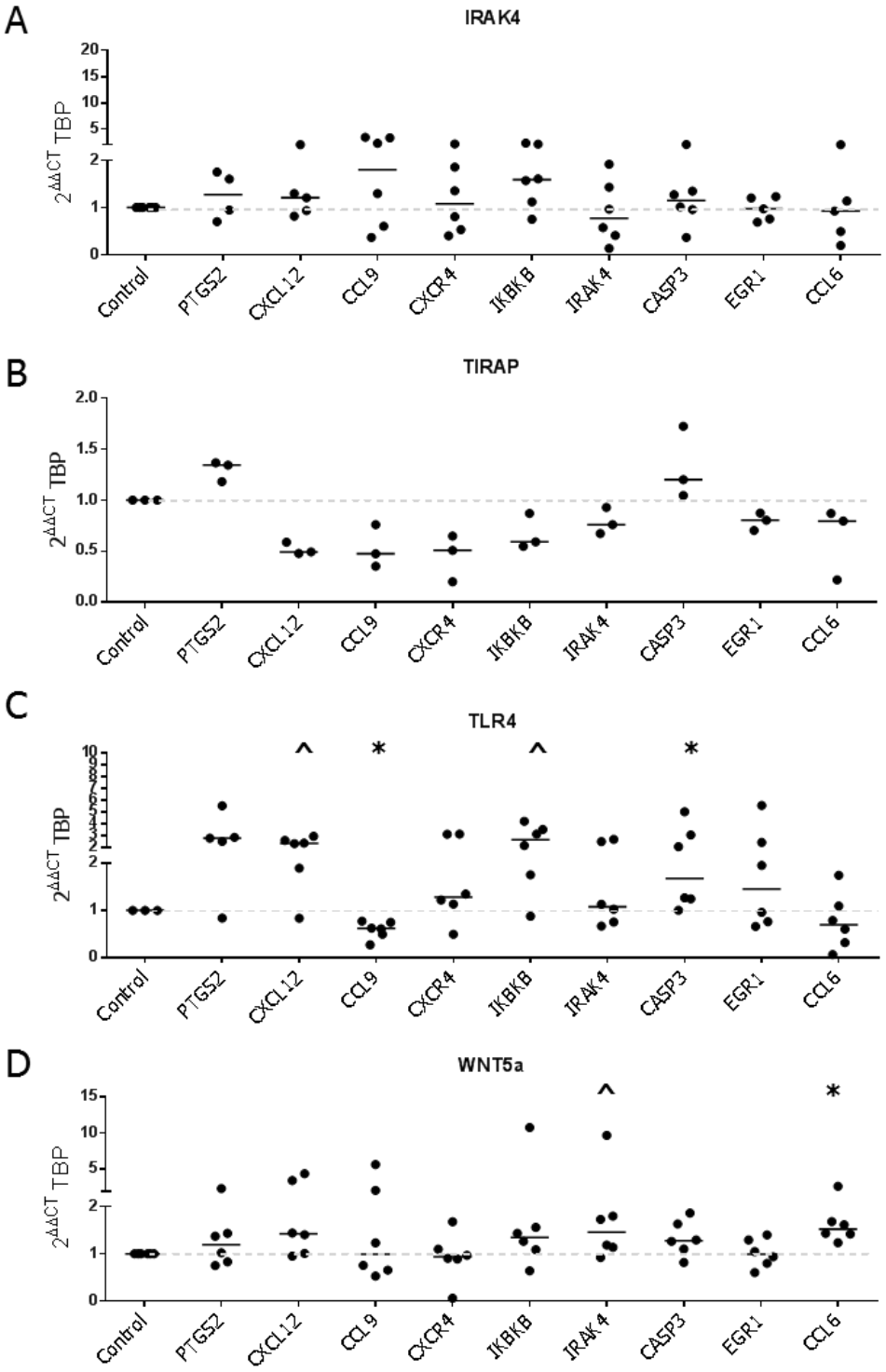


Figure 10. Effect of chemical inhibition of master regulators on downstream gene expression in mouse MOVA cells after 24h.

qRT-PCR assessment of inhibition of: (A) IRAK4, (B) TIRAP, (C) TLR4, and (D) WNT5a (n=3-6), on mRNA expression of on selected downstream genes predicted from the networks. Data presented as relative expression 2-ΔΔCT to vehicle control with normalisation to reference gene TBP. * Significance at $p<0.05$ Wilcoxon signed rank test. ^$p=0.06$.

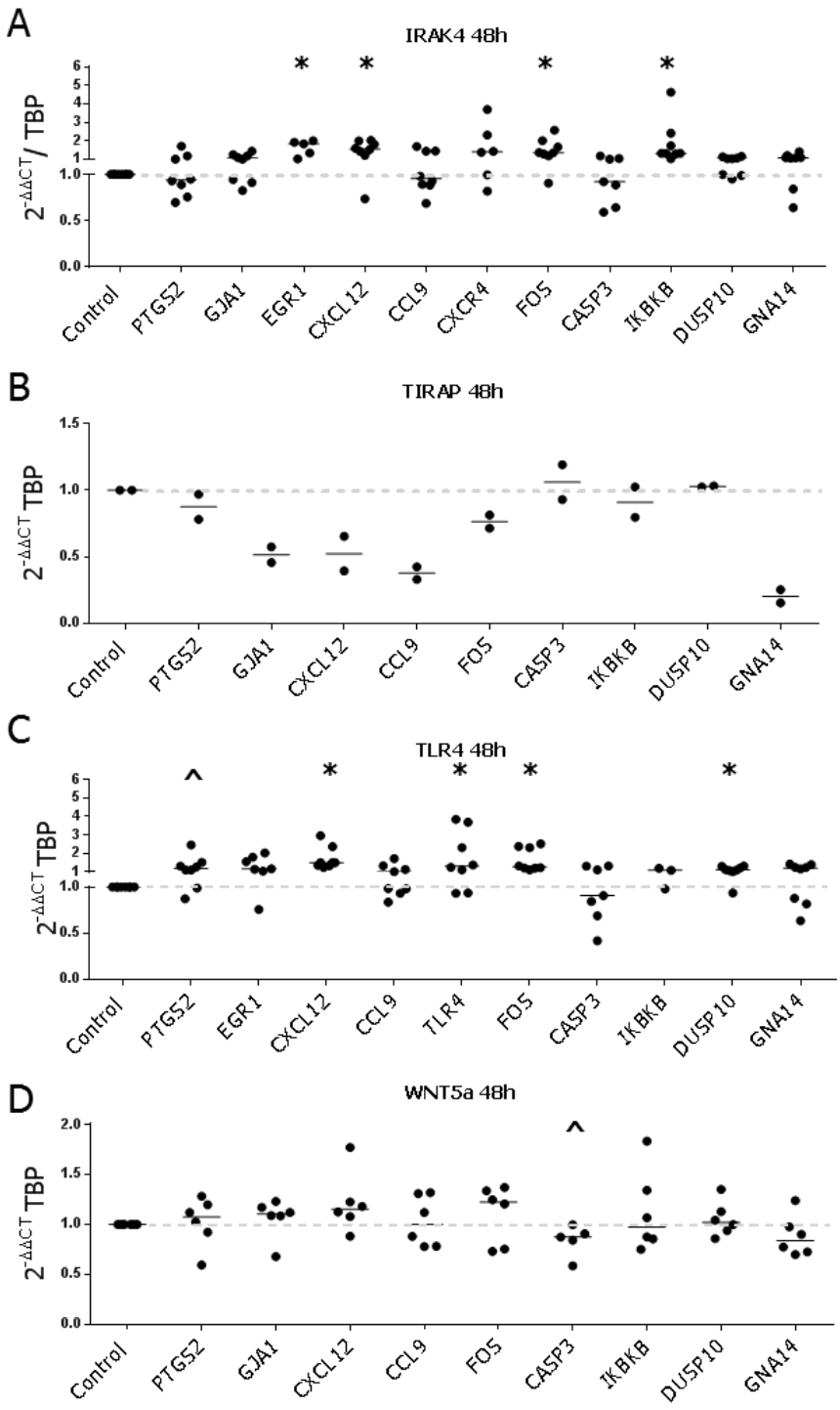


Figure 11. Effect of chemical inhibition of master regulators on downstream gene expression in mouse MOVA cells after 48h.

qRT-PCR assessment of inhibition of: (A) IRAK4, (B) TIRAP, (C) TLR4, and (D) WNT5a (n=2-8), on mRNA expression of selected downstream genes predicted from the networks. Data presented as relative expression $2^{-\Delta\Delta CT}$ to vehicle control with normalisation to reference gene TBP. * Significance at $p<0.05$ Wilcoxon signed rank test. ^$p=0.06$.

Table 1. Summary of mouse primers used. A) reference genes used and B) genes of interest.

A

ID	Forward 5'	Reverse 3'	Accession number
TBP	CACAGGAGCCAAGAGTGAAGA	CACAAGGCCTTCCAGCCTTA	NM_013684.3
YWHAZ	TGAGCTGTCGAATGAGGAGAG	CCTCCACGATGACCTACGG	NM_011740.3
GAPDH	Sequence not provided (Qiagen, UK)		
MBP	TCACAGCGATCCAAGTACCTG	CCCCTGTCACCGCTAAAGAA	NM_001025251

B

ID	Frward 5'	Reverse 3'	Accession number
AQP5	TCTTGTGGGGATCTACTTCACC	TGAGAGGGGCTGAACCGAT	NM_009701
ARG1	Sequence not provided (Qiagen, UK)		
CASP3	GGGGAGCTTGGAACGCTAAG	GAGTCCACTGACTTGCTCCC	NM_009810.3
CCL6	AGGCTGGCCTCATACAAGAAAT	ACATGGGATCTGTGTGGCAT	NM_009139
CCL9	TCACACATGCAACAGAGACA	TGTAGGTCCGTGGTTGTGAG	NM_011338
CTNNB1	CCCAGTCCTTCACGCAAGAG	CATCTAGCGTCTCAGGGAACA	NM_007614
CXCL12	AGATTGTTGCACGGCTGAAGA	CCTTTGGGCTGTTGTGCTTAC	NM_021704
CXCL16	AACTCTGCAGGTTTGCAGCTC	TCACTGATGGAGACGAGCCT	NM_023158
CXCL17	TGCAAAGATTGGTTCCTGCAA	TCCTGTGGTGCTTTTGGTGT	NM_153576
CXCR4	GACTGGCATAGTCGGCAATG	AGAAGGGGAGTGTGATGACAAA	NM_009911
DUSP10	TGGGGATCAATGAAGCTGAGTG	TGAGATCCTGAGGTCGGACA	NM_022019.5
EGR1	TATGAGCACCTGACCACAGAG	GCTGGGATAACTCGTCTCCA	NM_007913
FOS	CCTGGGACAGAAGACCACTC	TGAGATCTCCCGGACATGGT	NM_010216.1
GAB1	GAAGTTGAAGCGTTATGCGTG	TCCAGGACATCCGGGTCTC	NM_021356
GJA1	CAGGTCTGAGAGCCCGAACT	TCTGGGCACCTCTCTTTCACTTA	NM_010288
GNA14	AGCGATCTGAACGACGGAAA	TCCTCCATGCGGTTCTCATTG	NM_008137
IKBKB	TTTCCAGCAAGCTTGAGTCTAC	TGTGAGCATCTCTTCGCTAGT	NM_026166.2
IL-1β	Sequence not provided (Qiagen, UK)		
IRAK4	CATACGCAACCTTAATGTGGGG	GGAACTGATTGTATCTGTCGTCG	NM_029926
IRF8	AGACCATGTTCCGTATCCCCT	CACAGCGTAACCTCGTCTTCC	NM_008320
MMP11	CCGGAGAGTCACCGTCATC	GCAGGACTAGGGACCCAATG	NM_008606
OXTR	GATCACGCTCGCCGTCTAC	CCGTCTTGAGTCGCAGATTC	NM_001081147
PTGS2	GCTCAGCCAGGCAGCAAATC	ATCCAGTCCGGGTACAGTCA	NM_011198
SLIT2	GGCAGACACTGTCCCTATCG	GTGTTGCGGGGGATATTCCT	NM_178804
TLR4	ATGGCATGGCTTACACCACC	GAGGCCAATTTTGTCTCCACA	NM_021297

Table 2. Summary of inhibitors used for *in vitro* experiments

Candidate	Inhibitor	Target	Conc	Source	Reference
IRAK4	Aminobenzi-midazole	IRAK1/4	150nM	Sigma, UK	Powers et al. 2006
TIRAP	Peptide inhibitor	RQIKIWFQNRRMKWKK at the NH_2-terminal of LQLRDAAPGGAIVS	25μM	Insight Bio, UK	Horng et al. 2001
TLR4	Antibody	TAK-242 (tlrl-cli95)	2μM	Invivogen, FR	Zhang et al. 2014
WNT5a	Peptide inhibitor (BOX5)	On the Wnt5a N-terminal group; t-boc-Met-Asp-Gly-Cys-Glu-Leu	200μM	Insight Bio, UK	Jenei et al. 2009

Table 3. The top pathways, key genes and the highest causal regulators of the absolute gene expression of pregnancy in the lead up to labour of the decidua.

A. Gene set consistently expressed (E16.5 to E19). B. Gene set transiently expressed 12-36 hours before labour (E17.5 and E18.5). C. Gene set expressed 12-36 hours before and during labour (E17.5, E18.5 and E19). D. Gene set expressed during active labour (E18.5 and E19).

Decidual Gene Set	Top biological pathways	Key genes within biological pathways	Major upstream regulators
A: Genes consistently expressed	Tight junctions, cholesterol biosynthesis, prothrombin, cell junction signalling, STAT3 signalling	Claudins, occludens, Cytochrome P450 51A, Dehydrocholesterol reductase, sterol c5 desaturase, NADP dependent steroid dehydrogenase like, thrombin, fibrinogen, kallikrein-related peptidase, insulin receptor, BMP Receptor 1B	Progesterone, GRIP1, TNFα, PPARGC1B, LAMPTOR2, SMAD2 ($p=2.14x10^{-16}$ – $7.24x10^{-20}$)
B: Genes transiently expressed 12-36 hours before labour	Calcium transport, acute phase response, prolactin signalling, TLR signalling, nucleotide synthesis	Plasma membrane calcium ATPase 1 and 2, Glucocorticoid receptor (NR3C1), SOCS4, IL-33, TLR1, MAPK3k1	TRAF2, 4 and 6, ZEB1, PLC β, IL-10 receptor ($p=1.10x10^{-6}$ – $3.11x10^{-8}$)
C: Genes expressed 12-36 hours before and during labour	Cell cycle regulation, molecular mechanisms of cancer, PTEN, macrophage function, growth hormone and insulin receptor signalling	CDK7, cyclins A2, B1, B2, Cyclin-dependent kinase inhibitor (CDKN) 1a,2c, 3b, mini-chromosome maintenance proteins (MCM) 2-5, CASP3, BCL2-like 11, FOX01, IGF-R1, PTEN, BMP2, PDGFC, PDGF receptor A, SOCS3, IL-18R1, IL-1RL2, TLR3, VCAM1,	TRIM65, PURA, CDX1, CDKN2B, β-estradiol ($p=1.07x10^{-10}$ – $1.07x10^{-14}$)
D: Genes expressed during active labour	Cell cycle regulation, cancer, DNA repair, Wnt signalling, macrophage functions	Cyclins, CDKNs, Bcl2, BRCA1, Rad51, Ctnnb1, endothelin1, PTEN, Wnt7a, PDGFC, Frizzled receptors 2b ad 7, dickkopf-related protein 3, IRAK4, TLR2 and 3, TRAF3, PLC β3 and γ1	NFATC2, ZEB1, SMAD1, IkB complex misoprostol (PGE_1), SYN1 ($p=2.73x10^{-19}$ – $6.38x10^{-23}$)

Table 4. The top pathways, governing genes and top causal regulators of the absolute gene expression during pregnancy in the lead up to labour of the myometrium.

A. Gene set consistently expressed (E16.5 to E19). B. Gene set transiently expressed 12-36 hours before labour (E17.5 and E18.5). C. Gene set expressed 12-36 hours before and during labour (E17.5, E18.5 and E19). D. Gene set expressed during active labour (E18.5 and E19).

Myometrial Gene Set	Top biological pathways	Key genes within biological pathways	Major upstream regulators
A: Genes consistently expressed	Integrin signalling, regulation of smooth muscle cell function, eNOS signalling, tight junctions, gap junctions	Myosin heavy chain, actinA1 and 2, cofilin, fibronectin, PDGFC, CASP3, PTEN, endothelin, AKT3, Bax, BCl2, angiotensin R1a, TIMP2, TGFβ3	Wnt1, SMAD6, HIF1A, PTGS2, TNFα, DNMT1 ($p=1.12x10^{-20}$ - $4.66x10^{-24}$)
B: Genes transiently expressed 12-36 hours before labour	NFAT, PPARα/RXR signalling, calcium signalling, molecular mechanism of cancer, growth factor signalling, phospholipase signalling	Myocyte enhancer factor 2A and C, PLCb and g, calcium/calmodulin-dependent PK1, SHC1, adiponectin, CD36, Calcium transporters ATP2a2 and B1, SCL8a1 (sodium/calcium transportation), integrins a5 and b1, PDGFA, PLCs, BMP1, cyclins D1 and 2	Cytokine receptor-like factor-1 (CRLF1), JAK1/2, IFNgR2, Oncostatin M, HGF, HSPA8 ($p=1.01x10^{-8}$ – $8.4x10^{-8}$)
C: Genes expressed 12-36 hours before and during labour	Molecular mechanism of cancer, IGF signalling, Renin-Angiotensin signalling, HGF signalling	FOXO1, Raf1, RB1, Hras, PI3kca, PKC, BMP4, VEGFC, SOCS4, smoothened (SMO) frizzled receptor	SCD, Interferon regulatory factor 2 (IRF2), HSP-A1A & A1B and 70 MAPK12, TNFRSF9, SMAD1 ($p=3.1x10^{-8}$ – $1.33x10^{-9}$)
D: Genes expressed during active labour	TGFβ signalling, NFkB, STAT3, macrophage and granulocyte function, cancer, IL-6 signalling, ErbB signalling, IFN signalling, TLR signalling	BMP-2, Smad 3 + 6, MAPK3 and 13, Raf1, IL-36, Ctnnb1, MMP3 +10, TNFSF11b, IFNL 1 + 9, TLR2, BCL10, INSR, SOCS3, HBEGF, CCl2 + 7 + 11, CXCL14	CDK1, CTSB, SMAD1, PTX3, TNF, BCL3, CD11c ($p=1.85x10^{14}$ – $1.86x10^{17}$)

Table 5. Candidate master regulators of active labour in the mouse decidua.

(A) Characteristics of candidates from the microarray, ClusterONE and IPA causal analysis after prioritisation through the ModuLand plugin, and (B) final master regulators after detailed analysis for downstream labour-associated genes. In the causal analysis, the duplicates indicate the master regulator formed more than one network.

A

Decidua	Microarray		Cluster ONE	Causal Analysis		
Master regulator candidate	**Fold change**	**PPLR**	**p-value**	**Activation z-score**	**p-value**	**Downstream genes (regulators)**
Downregulated system activity						
NFE2L2	1.4	0.9999	0.02	-2.744	0.0001	306(20), 109(7)
CDH1	1.5	0.9998	$9.8x10^{-5}$	-2.258	0.008	143(25)
HSPA1B	1.9	1	0.0009	-2.121	0.0135	32(5), 163(18)
Upregulated system activity						
IRAK4	1.5	0.9979	0.04	3.816	0.0025	327(43), 68(11)
CCND1	1.6	0.9997	0.04	2.982	0.0001	19(1), 193(22), 79(5)
IL6ST	-2.0	$6x10^{-5}$	0.004	2.82	0.0223	136(17)
TIRAP	1.5	0.9999	0.04	2.189	0.0041	368(53)
APP	1.7	0.9999	$8.0x10^{-4}$	1.923	0.0004	143(5)
CTNNB1	2.8	1	$9.9x10^{-5}$	1.665	0.014	159(9)

B

Decidua	Microarray		ClusterONE	Causal Analysis		
Master regulator	**Fold change**	**PPLR**	**p-value**	**Activation z-score**	**p-value**	**Downstream genes (regulators)**
IRAK4	1.5	0.9979	0.04	3.816	0.0025	327(43) or 68(11)
TIRAP	1.5	0.9999	0.04	2.189	0.0041	368 (53)

Table 6. Candidate master regulators of active labour in the mouse myometrium.
(A) Characteristics of candidates from the microarray, ClusterONE and IPA causal analysis after prioritisation through the ModuLand plugin, and (B) final master regulators after detailed analysis for downstream labour-associated genes.

A

Myometrium	Microarray		Cluster ONE	Causal Analysis		
Master regulator candidate	**Fold change**	**PPLR**	**p-value**	**Activation z-score**	**p-value**	**Downstream genes (regulators)**
Downregulated system activity						
EGR1	1.8	0.99998	0.0065	-1.429	2.57×10^{-2}	96 (20)
WNT7A	3.7	0.99899	0.0095	-1.414	1.78×10^{-2}	2 (1)
FOS	2.3	0.99998	5.33×10^{-5}	-0.816	2.36×10^{-2}	54(12)
AR	-2.2	5.78×10^{-6}	0.0021	-0.557	3.26×10^{-2}	29 (4)
Upregulated system activity						
SOCS3	2.5	0.99735	0.0102	0.458	2.08×10^{-2}	119 (26)
SQSTM1	1.4	0.99994	5.01×10^{-7}	0.822	2.31×10^{-2}	148 (22)
TLR2/4	1.5	0.99984	1.08×10^{-5}	1.285	2.00×10^{-4}	175 (29)
IRF8	-1.6	1.68×10^{-8}	0.0144	1.896	2.00×10^{-4}	188 (31)
WNT5A	1.5	0.99941	0.0032	2.309	4.00×10^{-2}	108 (19)

B

Myometrium	Microarray		Cluster ONE	Causal Analysis		
Master regulator	**Fold change**	**PPLR**	**p-value**	**Activation z-score**	**p-value**	**Downstream genes (regulators)**
TLR2/4	1.5	0.9998	1.08×10^{-5}	1.285	2×10^{-4}	175 (29)
WNT5A	1.5	0.9994	0.00323	2.309	4×10^{-2}	108 (19)

Table 7. Summary of master regulators mapped with absolute gene expression and network analysis.

Candidates identified individually from the decidua and myometrium were mapped across both tissue types and time points leading up to labour. (A) decidua master regulators. (B) myometrial master regulators. Blue text=decidua candidate mapped to decidua, red=myometrial candidate mapped to myometrium, black=mapped to the other tissue type. Time periods relate to figure 9 (A= E16.5 to E19, B= 17.5 and E18.5, C= E17.5, E18.5 and E19, D= E18.5 and E19).

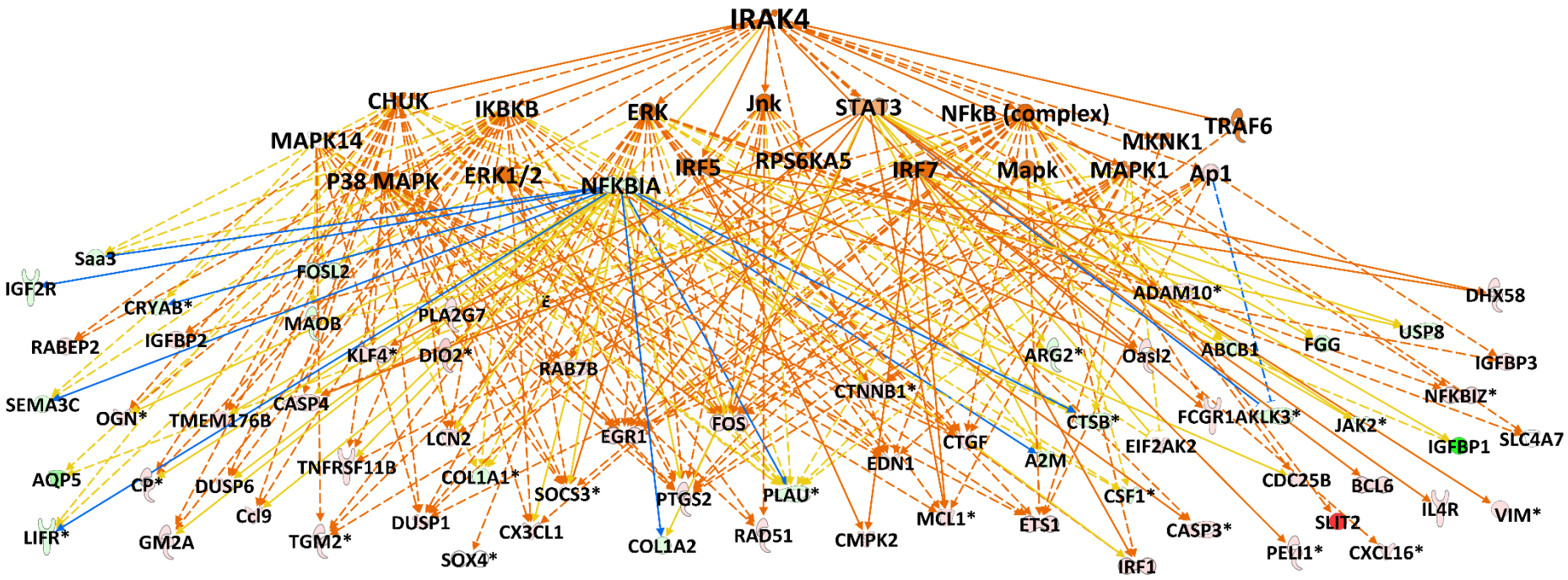
Overlap of the candidate into different tissue network maps | Location of the candidate in the overlap network map

A

DECIDUA				
Candidate	**Tissue & Time Period**	**Absolute expression meta-node**	**Meta-node hierarchy**	**Meta-node size**
IRAK4	*DEC A*	TIRAP	40	18
	DEC D	IRAK4	47	15
	MYO A	PELI2	80	7
	MYO D	PELI1	39	9
TIRAP	*DEC A*	MAP3K5	12	165
	"	TIRAP	40	18
	DEC D	IRAK4	47	15
	MYO D	TLR2	86	8

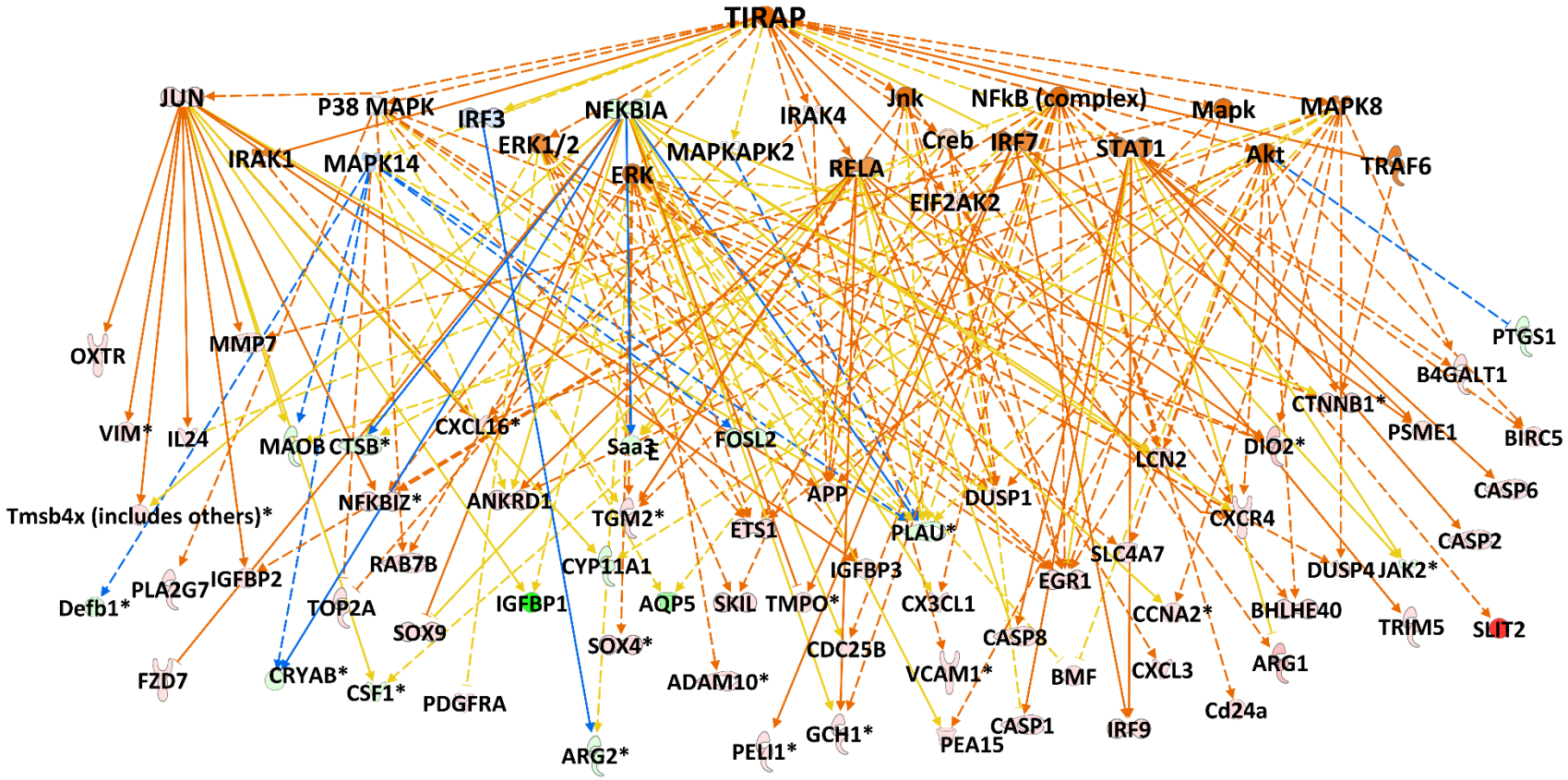
B

MYOMETRIUM				
Candidate	**Tissue & Time Period**	**Absolute expression meta-node**	**Meta-node hierarchy**	**Meta-node size**
TLR2	*MYO D*	PELI1	39	9
	"	TLR2	86	8
	DEC A	TIRAP	40	18
	DEC D	IRAK4	47	15
TLR4	*DEC A*	TIRAP	40	18
	DEC C	TLR1	41	3



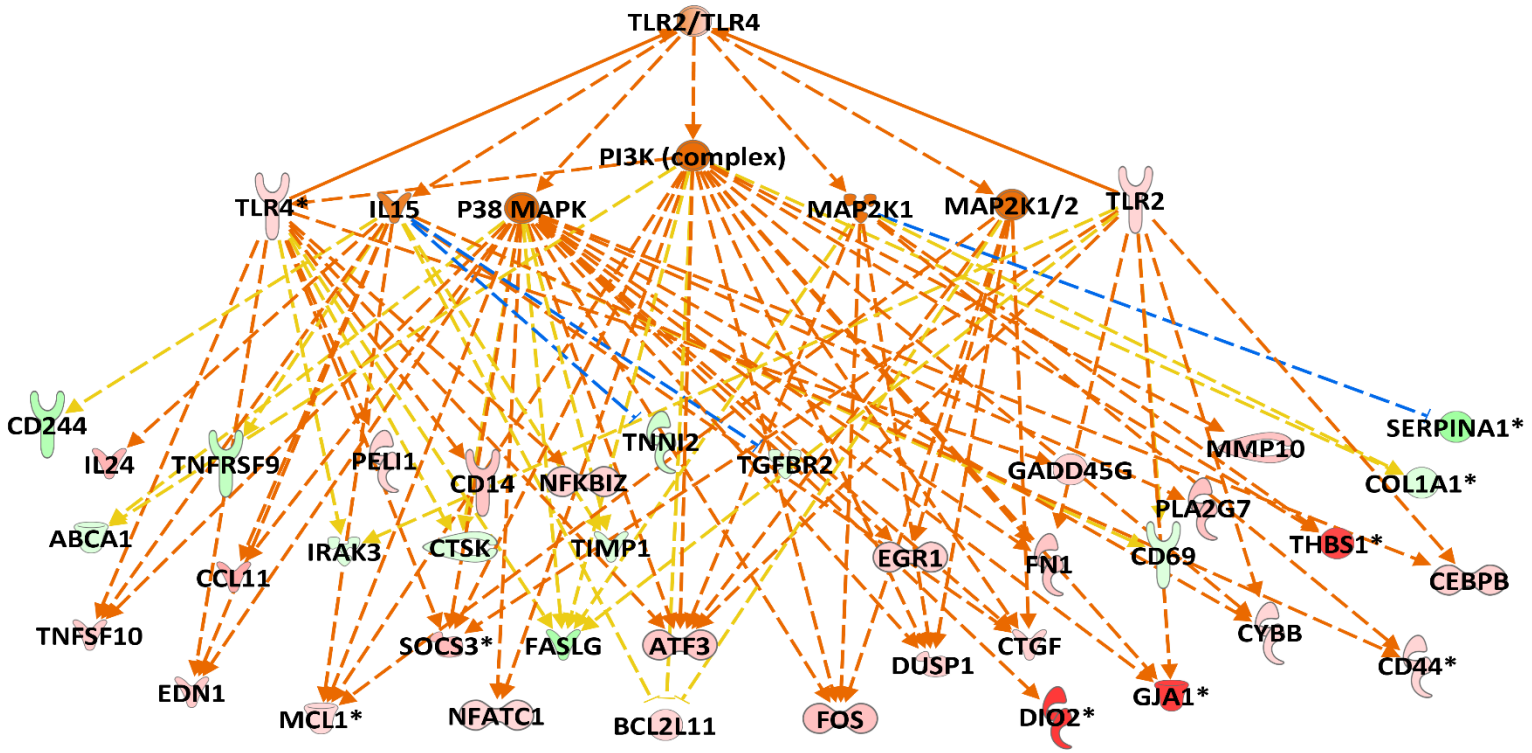
Supplemental figure 1. Causal analysis of the decidual master regulator IRAK4.

The network shows the relationships with the downstream genes in the system. IRAK4 is the master regulator. At the next level are 42 regulators with 327 downstream genes in the full network. This diagram shows a summary of genes with: i) at least two network connections (dashed line) or ii) have multiple associated functions from a single regulator (solid line). Upregulated regulator= orange; down-regulated regulator= blue; upregulated gene=red to pink, down-regulated gene = green; The intensity of colour indicates the scale of change.



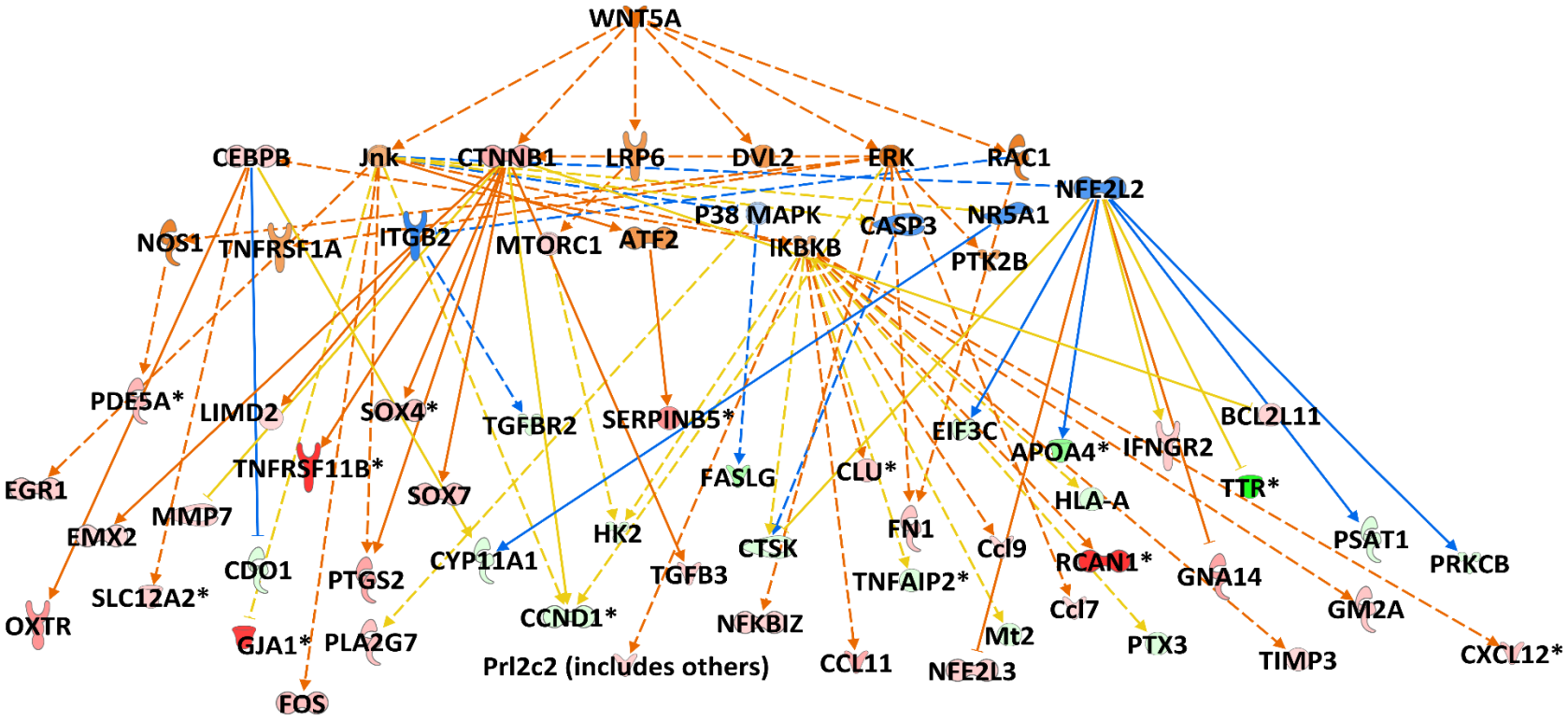
Supplemental figure 2. Causal analysis of the decidual master regulator TIRAP.

The network shows the relationships with the downstream genes in the system. TIRAP is the master regulator with 52 regulators with 368 downstream genes in the full network. This diagram shows a summary of genes with i) at least two network connections (dashed line) or ii) have multiple associated functions from a single regulator (solid line). Upregulated regulator= orange; down-regulated regulator= blue; upregulated gene=red to pink, down-regulated gene = green; The intensity of colour indicates the scale of change.



Supplemental figure 3. Causal analysis of the myometrium identified master regulator TLR2/4 complex.

The network shows the relationships with the downstream genes associated in the system. TLR2/4 are the master regulator with 29 regulators and 175 downstream genes in the full network. This diagram shows a summary i) at least two network connections (dashed line) or ii) have multiple associated functions from a single regulator (solid line). Upregulated regulator= orange; down-regulated regulator= blue; upregulated gene=red to pink, down-regulated gene = green; The intensity of colour indicates the scale of change.



Supplemental figure 4. Causal analysis of the myometrium identified master regulator WNT5a.

The network shows the relationships with the downstream genes associated in the system. WNT5a is the master regulator with 19 regulators and 108 downstream genes in the full network. This diagram shows a summary i) at least two network connections (dashed line) or ii) have multiple associated functions from a single regulator (solid line). Upregulated regulator= orange; down-regulated regulator= blue; upregulated gene=red to pink, down-regulated gene = green; The intensity of colour indicates the scale of change.

6.0 General discussion

Labour is a complex process requiring interconnectivity and coordination of signals from multiple sources. The most recognisable physiological process of labour, myometrial contraction, is currently the only target for therapeutic intervention for labour complications and has been the focus of the majority of studies; however, the regulation and interaction of the molecular events occurring during labour, in particular the non-contractile-associated processes, have not been clearly defined. This study sought to profile and map the events and processes occurring in the uterine tissues prior to and during labour. The major findings include that there are a multitude of integrated active processes accompanied by widespread alterations in gene expression in the decidua and myometrium in preparation for, and during, active labour. These have overlapping upstream regulators indicating redundancy in the system, but in accordance with their critical role in the culmination of pregnancy and delivery of the infant. As genes are not expressed individually, we used network analysis methods to identify significant genes that possess a number of functional relationships and significant interconnectivity in the network, and thus high centrality during labour. These findings reinforce that targeting a single factor, or a late stage process such as myometrial contractions, are unlikely to be successful in stopping preterm labour (PTL).

The involvement of the decidua in labour

This project firstly aimed to explore the physiological and biochemical involvement of the human choriodecidua in the labouring process, as emerging data suggests that labour may involve multiple signalling pathways in the uterine tissues prior to activation of contractions (Marvin et al. 2002; Haddad et al. 2006; Hamilton et al. 2012; Hamilton et al. 2013; Shynlova, et al. 2013c; Castillo-Castrejon et al. 2014). The global transcriptomic approach undertaken in this study has shown that in humans, the choriodecidua is highly active during normal term labour (TL). This was the first study to characterise the functions, pathways and regulators in detail in the choriodecidua. Instead of using a high fold change threshold to select differentially expressed genes, we performed pathway and network analysis on all genes that were significantly altered with labour using a stringent statistical threshold. Inclusion of all gene changes created gene networks that better represented the functional events taking place in the labouring system, and identified enrichment of altered genes in common pathways and networks. Even modest changes in the expression levels of multiple genes in the same biological pathway indicate its importance in the process of labour.

The prominent gene functions and cellular pathways that characterised the decidual labouring system were inflammatory related, as we had previously hypothesised, with multiple upstream components that have emerging roles in regulation of labour, such as the mir-200 family that increase contractile associated signalling through ZEB1/2 in the myometrium in TL and PTL (Renthal et al. 2010; Williams et al. 2012). The transcription factors identified as putative upstream regulators of the labour transcriptome events were also inflammatory-related in the choriodecidua. These included those with established associations in the labour cascade, such as NFkB

components (Khanjani et al. 2011; Sharp et al. 2013), and those previously identified in uterine tissues, including HMGB1 (Park et al. 2006; Dubicke et al. 2010b; Khanjani et al. 2011), STAT1 and 3 (Han et al. 2008; Mittal et al. 2010) and the glucocorticoid receptor NR3C1 (Li et al. 2011). This study tentatively suggests that inflammation and other immune-related activity pathways are upstream regulators of labour and we hypothesize they may mediate critical preparatory events. The microRNAs miR-21 and miR-101 were also identified as putative upstream regulators in this study. Previous studies have demonstrated that miR-101 is regulated by PTGS2 expression in the uterus (Chakrabarty et al. 2007) and miR-21 is expressed in the uterus during embryo development (Hu et al. 2008). With many roles in implantation and pregnancy, with immune cell infiltration and spiral artery remodelling, decidualisation at implantation, and shedding and repair during menstruation, the decidua is a highly dynamic tissue that is also likely to contribute to active labour. Thus, this study has furthered our knowledge of the reproductive roles of the decidua by defining related functions during labour.

Network analysis of the labour transcriptome in human decidua and myometrium and discovery of master regulators

We are in the infancy of understanding the labour transcriptome, and, especially in translating these data into therapeutic benefit. Microarrays generate vast amounts of linear data; understanding how the genes involved in labour interrelate, and identifying key molecules and pathways is essential for a better understanding of the processes involved. Using an unbiased systems biology approach, signalling events in the decidua and myometrium - two intimately associated tissues - were investigated in detail to generate network maps to define interconnections and master regulators of the labour process in the two uterine tissues.

Using *in silico* prediction and *in vitro* techniques, this project has identified genes and pathways previously not recognised as crucial in labour in the human choriodecidua and myometrium. Detecting these interactions allowed categorisation of potential regulators amongst all the differentially expressed genes of labour. Some of the master regulators identified already have an established role in pregnancy, such as the TLR family and NFκB, but this study characterised their regulatory networks during labour and identified their downstream target genes. We have also identified strong regulatory roles for these TLR4 in the choriodecidua, which was previously undefined. Other regulatory genes detected had not previously been described in detail in relation to labour, including the intermediate filament vimentin and TNFSF13B. Vimentin has roles in leukocyte translocation and intracellular communication (Nieminen et al. 2006; Ivaska et al. 2007). The identification of vimentin in the decidua suggests it plays an extensive role in regulating immune cell infiltration, which we have identified is a key preparatory signalling pathway in the stages preceding labour and during active labour. TNFSF13B has a role in maintaining B and T cell functions, with elevated expression in autoimmune diseases (Mackay & Schneider 2009). Its wide-ranging immune-related functions implies that in the decidua it is potentially contributing to maternal/fetal immunoregulation, as variant expression is associated with pregnancy complications (Hever et al. 2007; Guo et al. 2008; Fenstad et al. 2010).

In the human myometrium, TLR2 and RelB were identified as master regulators. TLR2 has established roles in labour in uterine tissues promoting contractile processes (O'Brien et al. 2008; Re & Strominger 2001; Montalbano et al. 2013; Lim et al. 2014). This study supports its key role in labour and demonstrates its relationships as a regulatory molecule, with high centrality in a network of the myometrial labour transcriptome. Although the role of NFκB in labour has been documented previously, the classical component of NFκB in associated with labour is through activation by RelA dimers (Kalkhoven et al. 1996; Zaragoza et al. 2006; Cookson & Chapman 2010; De Silva et al. 2010; Zhang et al. 2011b). The role of RelB in uterine tissues during labour is still unclear (Cookson & Chapman 2010). This is the first study to indicate that RelB has a regulatory role during labour, associated with a number of downstream genes that are selectively expressed during labour. It is interesting to note that RelB dimerization can be induced by TNFSF13B (Senftleben et al. 2001), a predicted regulator of labour in the choriodecidua, and NFκB signalling in human decidual endothelial cells is predominately through the non-canonical RelB (Masat et al. 2015). These data support the idea of cross-talk and mediation of labouring signals between the decidua and myometrium. RelB as a master regulator of labour is also supported by recent studies specifically identifying it as the NFκB component that promotes CRH production in the placenta through glucocorticoid action (Di Stefano et al. 2015).

Preliminary *in vitro* experiments indicate that the predicted master regulators can alter the expression of network predicted downstream genes in an unstimulated cell model, although some inhibitors were more effective than others. Experimental inhibition of TLR2 in the myometrium, and vimentin and TLR4 in choriodecidua was limited in their *in vitro* regulatory effects on downstream genes predicted by the network analyses. The possible reasons for this will be discussed in the limitations section. The most promising results were associated with metallotheionein 2 (MT2), an intracellular ion-regulator involved in cellular homeostasis maintenance (Vašák & Meloni 2011). We identified MT2 as a major upstream causal regulator, suppressing the mRNA expression of established labour associated pro-inflammatory genes (IL-1α, -1β, -6, -8), as well as network predicted genes PTGER4 (EP4) and vimentin. In addition, as an individual gene, MT2 was downstream of all three myometrial regulator candidates MT2, RelB and TLR2, indicating multiple biological roles of metallothionein within labour. It is induced during inflammation (Min et al. 1992), and is associated with hormonal signalling (Slater et al. 1988; Cano-Gauci & Sarkar 1996; Harris et al. 2001). We suggest that MT2 may be regulating inflammatory processes during labour, and in particular during cellular stress, via alteration of intracellular heavy metal homeostasis.

Phenotyping the decidual and myometrial events that lead up to labour in a mouse model

One of the limitations of using human tissues is the ability to investigate samples from women in active labour or in the days leading up to labour. This means it is impossible to study the preparatory processes, or the mediators involved in driving labour, without the confounding effects of the labour process itself on the tissue. To be precise, the human tissue samples are obtained immediately postpartum, and therefore some of the gene changes observed may be as a

consequence of labour. Samples can be collected during emergency caesarean following the onset of labour, but this introduces other confounding factors, especially inflammatory responses, from maternal and fetal distress. Although there are anatomical, physiological and biochemical differences underlying pregnancy and labour in humans and mice, many similarities lie in the functional processes. Animal models provide an accessible experimental model to investigate the normal changes that precede, and activate, successful labour.

Using a mouse model to examine the stages leading up to active labour from mid-late pregnancy (E16.5-E19), this study demonstrated widespread changes occur in both tissues in the days preceding labour. This was expected as priming of the myometrium for labouring activity is an established phenomenon, including upregulation of contractile associated signalling such as GJA1 mRNA and protein expression, and gap junction formation (McNutt et al. 1994; Kilarski et al. 1996; McKillen et al. 1999; Doring et al. 2006), oxytocin (Kilarski et al. 1996; McKillen et al. 1999; Arthur et al. 2007) and prostaglandin synthesis, receptors and transporters (Manabe et al. 1983; O'Brien 1995; Cook et al. 2000; Palliser et al. 2004; Arthur et al. 2008; Phillips et al. 2011; Phillips et al. 2014). However, there were marked differences in the timing, scale and type of preparatory events in the two uterine tissues. These studies indicate that the decidual genomics of labour are not a swift, sudden event occurring at the end of pregnancy. A clear decline in metabolic and biochemical pathways, including cholesterol biosynthesis and metabolism, maintenance of cell-to-cell junctions occurred 36 hours prior to labour, followed 24 hours later by inflammatory and immune responses, together with altered expression of pathways regulating cell death and survival. These functional pathways 12 hours before labour resemble those during active labour, providing support for their involvement in initiating and driving active labour. In contrast, there was a more dramatic alteration in the myometrial transcriptome during active labour, with pathways in tissue development and remodelling preceding labour. Importantly, inflammatory pathways were only prominent in the myometrium during active labour. Our analyses were performed with two methods, with relative expression of differentially expressed genes from E16.5, and absolute gene expression with analysis across gestational stages leading up to labour, both identifying very similar pathways and the latter identifying key genes and causal regulators for each transitional stage.

Network analysis of the labour transcriptome in mouse decidua and myometrium and discovery of master regulators of labour

An identical analysis approach was performed to characterise the transcriptome, biological pathways and identify master regulators during at active labour in the mouse. In the mouse decidua, IRAK4 and TIRAP, and in the myometrium TLR2/4 and Wnt5a were identified as master regulators. The master regulators identified in the mouse analyses are comparable to those found in the human network, as IRAK4 and TIRAP are key components of TLR activation and contribute to the specific activity of TLR4 and TLR2 in the induction of NFkB (Horng et al. 2001; Suzuki et al. 2002). As a target of interest for inflammatory diseases, IRAK1/4 inhibition has been shown to inhibit NF-κB signalling in haemopoietic cancer cells (Rhyasen et al. 2013). This suggests a

conserved role of inflammatory components which are regulators of labour. We found that there were several pathways in common between the human and mouse transcriptome. These included LXR/RXR signalling pathway, inflammatory response, Wnt/β-catenin and cell death and survival pathways that fluctuated in functional significance across the gestational stages towards labour. Our data supports that there are functional and also genomic regulator similarities, rather than similarities in gene expression, during labour in the decidua and myometrium between the human and mouse. Signalling pathway similarity in the myometrium at labour has been previously observed in mouse and human myometrium, where the majority of genes were dissimilar between the two species, but they were involved in common pathways involved in smooth muscle function and inflammation (Bethin et al. 2003). In our mouse model, the decidual regulators have shown prominence as preparatory signals for labour. Our data indicates the decidua and myometrium have, in both species, a uniquely active inflammatory role in active labour.

In the mouse model, *in vitro* inhibition of IRAK4 resulted in no significant effects on downstream genes, whereas inhibition of TLR4 and Wnt5a generated modest suppression. TIRAP indicated reduction in many of the network predicted downstream genes, including FOS, CXCR4, CXCL12, IKBKB, IRAK4, MMP11 and EGR1, but with low experimental numbers further exploration of inhibiting TIRAP is required for statistical significance. In a study using a similar approach integrating *in silico* and *in vitro* analyses, a key microRNA miR-301/627 was identified in maintaining pluripotency of embryonic stem cells by down-regulating hierarchically-high key BMP inhibitors TOB2, DAZAP2 and SLAIN1 (Lipchina et al. 2011). These candidates were three of 11 screened from the network analysis, where siRNA inhibition of individual genes produced no effect, but the inhibition of all three together maintained stem cell pluripotency. The study demonstrated that master regulators and downstream targets can be predicted, but characterisation of biological inhibition requires detailed analysis.

Important candidates of labour have been identified with high regulatory potential among all the genes differentially expressed in the system of normal labour. Using an unbiased approach has allowed identification of seemingly non-descript genes as high level regulators, and opens the possibility of exploiting overlooked pathways of labour for therapeutic benefit. In general, the candidate regulators formed networks that extended beyond the known contractile processes and identified regulatory networks in the decidua and myometrium. These data also lends support to the idea that targeting multiple processes in different tissues, rather than a single pathway in an individual tissue, may generate better efficacy in blocking the labour cascade during PTL.

The master regulators in both species were selected from a longer list of regulators due to their downstream regulatory actions including those genes with a strong association with labour. The literature has identified numerous genes with significant expression at labour. However, these genes may be downstream mediators and they may not be suitable as effective targets in halting cellular processes. This was demonstrated in previous antagonist studies that targeted IL-1R, cytokines, or chemokines (Fidel Jr. et al. 1997; Yoshimura & Hirsch 2005; Leitner et al. 2014; Shynlova et al. 2014) but found little effect on preterm delivery. A small non-competitive

antagonist associated with IL-1R, however, has shown a promising reduction in IL-1β induced preterm delivery (12%) compared to IL-1β induction alone (56%). This non-competitive inhibitor was shown to be mediated through AP-1 rather than classical NFκB activity traditionally activated by IL-1 (Nadeau-Vallée et al. 2015) or LPS (MacIntyre et al. 2014). AP-1 plays a role in the expression of the non-canonical NFkB subunit RelB (Wang & Sonenshein 2005), suggesting a potential involvement of NFκB signalling via the non-classical pathway. The complexity of interactions demonstrates the importance of identifying and targeting significant upstream components for effective therapeutic use. Understanding the pathways of action is crucial in delineating the regulators, over the prominently expressed components, of a biological system.

6.1 Limitations

This work was carried out to phenotype normal labour and identify potential master regulators to provide insight into therapeutic potentials for PTL. The decidua was identified as having major functionality and regulatory action during normal TL. However, this study used the human choriodecidua obtained from the fetal membranes, rather than isolated decidual tissue. This may be problematic as it has been shown in previous studies that the chorion and decidua distinctly express CRH receptors (Florio et al. 2000), MMPs (Riley et al. 1999), prostaglandin enzymes (Phillips et al. 2014) and cytokines (Young et al. 2002). The two layers can contribute to the genetic input in the identification of the master regulators in this study, and delineation of the contribution of the two layers is difficult without cell isolation or dissection, both of which introduce their own potential confounding influences. Our primary interest was in the decidua, due to its intimate contact with the myometrium which indicates the potential for regulatory crosstalk. Although we demonstrated that TLR4 was expressed by both the chorion and decidua (Stephen et al. 2014), vimentin was a product of decidual stromal cells with no reported expression in chorion trophoblast cells, which are epithelial in origin (unpublished observations). This provides reassurance that the master regulators identified in the choriodecidual are exerting actions in the decidua.

A mouse model was utilised due to the ease of obtaining tissues at multiple time points in gestation, the functional similarities with human tissues during labour (Bethin et al. 2003; Williams et al. 2012), and the available genetic information of the mouse genome. However, systemic progesterone withdrawal drives labouring events in mice, and potentially, the use of animals with similar functional progesterone withdrawal as humans, such as the guinea pig, may provide a more accurate picture of events (Evans et al. 1982). However, mouse models are frequently utilised as any potential therapies are likely to be tested in well-characterised rodent models prior to clinical trials. The similarities in function and regulators identified in this study between human and mice also indicate that the mouse model is an appropriate animal model for labour studies. Understanding the differences, and similarities, between mouse and human uterine signalling helps to predict whether a mouse model is appropriate for testing specific therapies or whether a different animal model (with labour signalling more similar to that of humans) is required.

Microarray analysis was performed on pooled samples with two technical replicates, as pooling of a low number of replicates can provide an equivalent detection power as individual arrays and account for inherent biological variation between samples (Peng et al. 2003; Kendziorski et al. 2005). From the literature we found that pooling samples was statistically acceptable, and were also chosen as our experimental method due to financial budget constraints. This study validated the microarray-identified gene changes using qRT-PCR, but pooling with inclusion of biological replicates would provide greater confidence in the data. Previous microarray studies have used inclusion criteria of ≥2 fold changes in differential expression analysis (Muhle et al. 2001; Bethin et al. 2003; Rehman et al. 2003; Haddad et al. 2006; Mittal et al. 2010; Khanjani et al. 2011; Heng et al. 2014). Several of these demonstrated no differential expression or enrichment in functions or cellular pathways between term and TL with these fold change criteria (Haddad et al. 2006; Sitras et al. 2008). In this study, a lower fold change with a high statistical restriction was used for the human and mice studies, as a large fold change in expression is not always biologically significant in molecular analyses. The statistical software package PUMA was used to provide a Bayesian approach to the statistical assessment of expression, which is particularly suited for small sample numbers (Liu et al. 2006; Pearson et al. 2009). To clarify the genomic changes across the gestational stages in the mouse data, this study refined the analysis to include gene barcoding to include absolute gene expression, to support the relative expression data. Relative expression analysed the data to a presumed non-labouring state. These master regulators were also refined with a fold change threshold. Gene barcoding reinforces the significance of the data and allows inclusion of all arrays for analyses, thus allowing the investigation of transitional expression of genes over several time points with no fold change inclusion but similar high statistical restriction.

Our network analysis in the mouse identified numerous genomic changes involved over time in the lead up to labour and further analyses are required to identify key regulators that are not only present at active labour but play key roles in the lead up to labour. This would include more extensive network analysis at each stage of gestation in the mouse model and also, detailed investigation of potential crosstalk between the decidua and myometrium at each stage to understand mediating factors that act prior to onset of labour.

Our *in vitro* studies investigating the effects of inhibiting the master regulators on downstream genes predicted by the network analysis used unstimulated cell lines. This is potentially problematic as the basal condition of smooth muscle cells does not represent the highly activated labouring myometrium and genes associated with labour may not be expressed to the same magnitude or, if expressed may not behave in the same way with inhibition. Our attempts to use a more physiological *in vitro* model of choriodecidual explants were largely unsuccessful. This may be due to the variability between different samples of choriodecidua in terms of the relative thickness of decidua and chorion, the extent of preparatory events for labour and the abundance of immune cells. In addition, the pharmacokinetics and penetrance of chemical inhibitors and neutralising antibodies is more complex in intact tissues. Stimulation and inclusion of a decidual cell model may be better in representing the effects of the tissue selective master regulators.

There are also potential issues with the inhibitors selected. The chemical inhibitor Gö6983 was selected from literature due to its known inhibitory effects on the master regulator, vimentin (Mor-Vaknin et al. 2003) and MT2 (Yu et al. 1997). The selectivity of the inhibitory response was due to its use at different concentrations of which block various responses downstream of PKC. The PKC inhibitor used in this study was specific for α, β, δ and ζ, however, the PKC kinase isoforms mediate a number of functions such as cell growth, proliferation, death and differentiation across many tissue types. NFκB transcription is promoted by specific isoforms of PKC in monocyte and macrophages (Wang et al. 2011) and are involved in cytokine signalling during LPS activation of mouse macrophages (St-Denis et al. 1998). Investigating the effects of PKC in the labouring network would better elucidate the target mechanisms, but for our study the use of a more specific inhibitor for the master regulators would be more effective and prevent off target effects. Our inhibitors for TLR2 and 4 were antibodies which may have limited tissue/ cell surface access due to their large size. In tissue explant models, there are also additional complications with the presence of the extracellular matrix. In *in vitro* models, the interaction of antibodies to the surface receptors may activate complement factors depending on concentration and antibody affinity (Chames et al. 2009). A major limitation to the promising results of the TIRAP inhibition was due to the cost and access of the peptide inhibitor. The peptide was commercially purchased, but custom made from reported sequences. The molecular size and the concentrations required to generate inhibition limited experimental numbers.

Our final master regulators candidates have multiple downstream functions, an experimental difficulty when attempting to pharmacologically inhibit the candidate individually. A multi- targeted approach may be required. It has been previously shown that the inhibition of the key upstream components of the TLR pathway (IRAK1 and 4) inhibits cell progression in breast cancer cells, but inhibition of another key component, BCL, significantly heightened the efficacy of the inhibition (Rhyasen et al. 2013). We have also shown downstream genes of master regulators overlap between the decidua and myometrium (Lui et al, 2015 manuscript in preparation, Chapter 4). During labour it has been shown that depletion of natural killer cells significantly contribute to labour, but ablation does not do not effect timing of labour (Li et al. 2012). The redundancy in the inflammatory system has also been shown in other models, where the roles of the inflammatory products IL-1 and -6 are established in labour, but timing of labour was not affected in mice deficient in these cytokines (Hirsch et al. 2006; Sakurai et al. 2012). Ideally, targeting multiple regulators simultaneously or multiple levels of a regulator network, would more effectively supress the network, and overcome the redundancy found in major biological systems such as the inflammatory response. A potential method to target multiple regulators is the use of miRNAs, which we have shown to be upstream of pathways associated with labour, although thorough investigation of the all downstream targets of specific miRNAs would be required.

Our project was to characterise the decidua in human and mouse models and identify master regulators in the decidua and myometrium of TL. As a primary limitation, our studies have primarily focused on normal TL and we have extrapolated potential use of our master targets for PTL. We have shown that TLR4 (Stephen et al. 2014) and vimentin (Lui et al, 2015 manuscript in

preparation) are expressed in the decidua in TL and in PTL samples. However, further work on the effects of our master regulators in PTL samples would be required to investigate whether the same gene networks and pathways are relevant.

Summary

Understanding the processes involved in the regulation and development of labour is of utmost importance if we are to develop more effective therapeutics for PTL. This study have identified that the decidua contributes significantly towards the processes of normal TL in the human and mouse. Inflammatory processes are prominent in the labour transcriptome, and in the mouse, these and related processes are initiated prior to the onset of labour, suggesting a causative rather than consequential role. Other preparatory events were evident in the decidua during the final days of pregnancy, whereas in the myometrium, activation of key functions were detected nearer to the onset of labour, supporting the hypothesis that decidual activation precedes myometrial activation for labour, at least in terms of immunological or inflammatory responses. Network analyses have identified key master regulators of the labour system in both human and mice models, and these key candidates included several with previously unknown roles in pregnancy. Some candidates with established roles in labour were shown for the first time as master regulators of labouring pathways; others were shown to have lower level functions within uterine tissues and therefore not be suitable therapeutic targets. The therapeutic potential of these master regulators requires further investigation, but initial results are encouraging. The complexity in identifying networks and the differences between the two uterine layers in normal labour strongly suggest a multiple-target approach may be essential in preventing idiopathic PTL. Understanding normal TL and investigating the regulation of the genome to identify potential therapeutic targets may also have wider implications in cases such as term obstructed labour, where failure to of labour progress places great risk to both fetal and maternal mortality.

7.0 Future directions

This study has contributed to our understanding of the mechanisms of labour in the decidua and myometrium, and between the human and mouse, but there are many more questions to be answered in order to elucidate biological importance and generate therapeutic usefulness. In particular, the transcriptomics and *in silico* analyses this study has performed have generated hypotheses and new avenues for future research. Some of these are discussed below.

Does TIRAP inhibition actually suppress the network predicted downstream genes as the preliminary data indicates?

The most immediate short term goal, in order to strengthen the data, would be to increase the experimental number of treating the mouse smooth muscle (MOVA) cells with the TIRAP inhibitor and assessing the cell mRNA expression of downstream mediators.

Can we chemically inhibit human master regulators in a stimulated cell model?

The next immediate aim would be to investigate the inhibition of master regulators in activated cells and/or primary cells from labour. We have recently successfully isolated human choriodecidual cells (Duval et al, unpublished observations). Stimulating the cells with IL-1β or isolating cells from women who have undergone labour, would help identify the effectiveness of using the chemical inhibitors on the expression of downstream labour associated genes.

Is the multiple use of inhibitors a better approach to target multiple pathways?

As each master regulator forms a causal network that consists of several associated downstream regulators, the targeting of multiple regulatory components within a system may produce more effective suppression of the labour-associated pathways. As an example, ERK1/2 signalling was identified in the network as a related (lower level) regulator in all the final mouse regulators (IRAK4, TIRAP, TLR2/4 and WNT5a), and in most of the final human regulators (VIM, TLR4, TNFSF13B, TLR4 and RelB). As part of cell growth and survival pathways, which were detected in as crucial signalling pathways prior to labour in the mouse, the ERK regulator is a promising candidate to include in multiple targeting investigations. ERK inhibitors are commercially available as they are a target of interest in cancer targeting therapeutics (Samatar & Poulikakos 2014).

Should we be targeting other key regulators?

There were numerous upstream causal regulators identified from the network analysis. A combination of these master regulators that govern inflammatory and non-inflammatory, especially those that may be preparatory for labour, i.e. were involved in the transcriptome prior to labour. These may target a wider range of inhibition of pathways, and thus be a more effective approach. In the decidua, ZEB1 was identified as a major regulator transiently appearing at 12-36 hours before labour, before reappearing with active labour in the mouse studies. We have also predicted upstream microRNA regulators of ZEB1/2 in the labouring human choriodecidua. Upregulated by progesterone, this family of zinc finger homeo box genes, negatively regulates contractile

associated signalling (Renthal et al. 2010) making it a promising future target of the labouring decidua with potential effects over the myometrium. Another potential candidate predicted from the mouse myometrial networks was BCL3, with roles in major pathways found at 12-36 hours before labour. Involved in cell death and survival, it has been previously used as a target in cancer cell studies in reducing tumour growth. In other studies, the simultaneous targeting of multiple pathways such as combining BCL3 and IRAK1 increased efficiency of tumour growth suppression (Rhyasen et al. 2013).

Would the use of drug-drug and drug-target network mapping identify reagents with higher specificity for the master regulators?

Future directions in the long term would include the use of drug-target mapping, which would assist in selecting reagents that are more likely to be specific to our master regulator candidates. A system biology approach in predicting the interactions between chemical and the activated pathways would also identify inhibitors and any known off-target effects. There are multiple free to access repositories available. For example, chemical-drug-target interaction databases include DrugBank (Knox et al. 2011) and Supertarget (Hecker et al. 2012). Databases with chemical properties, and pharmacology to biological interactions include BioAssay Database (Wang et al. 2014) and ChEMBL (Gaulton et al. 2012).

Are there similar activating pathways and regulators in preterm labour?

We would perform analysis of the transcriptome of human PTL in the decidua and myometrium using microarray and network analysis to investigate the similarities between TL and PTL. We would hypothesize that there would be strong similarities, based on previous studies showing there are labour-associated effector genes in the myometrium, regardless of the mode of labour (Weiner et al. 2010). However, careful criteria of participant inclusion would be required with numerous potential confounders to consider for a 'normal' PTL cohort such as non-infectious PTL, singleton pregnancy, gestational age, smoking, ethnicity, BMI, medical administration e.g. betamethasone therapy which has anti-inflammatory effects.

Can we inhibit labour processes in an in vivo model and delay labour?

To test whether an inhibitor had the potential to stop PTL, we would need to use an animal model. We would require careful titration of inhibitors and studies of pharmacokinetics in tissues and animal models. Using an established non-infectious PTL model with a progesterone receptor antagonist mifepristone (RU486) (Dudley et al. 1996b; Gomez-Lopez et al. 2014b) we would investigate the effect on timing of labour and inhibition in labouring processes. In combination with the *in vivo* investigations, we would require dose and toxicity testing of the inhibitors for optimal dosing. Any promising potential chemical candidates would then be investigated in different animal model species for toxicological affects for future potential in utilising master regulator candidates in human clinical trials. Several animals would include, rabbits, guinea pigs and sheep.

Long term future directions

Can we improve delivery of therapeutics to the target tissues?

Encapsulation

The development of new therapies in pregnancy is difficult due to the potential for maternal and fetal morbidities. In order to improve not only therapeutic efficacy, but also delivery, drug targeting techniques can be applied. Encapsulating barriers with tissue-specific targeting labels are one such approach. Lipids are easily metabolised and liposomes are biocompatible (Mallick & Choi 2014). Liposomes been successfully used as therapeutic transporters in chemotherapy (Gabizon et al. 2006), with liposomal encapsulated doxorubicin demonstrating increased tumour reduction efficacy in mice, compared to the unbound drug. This technology also has efficacy at reduced doses, identifying the potential in decreasing chemotherapeutic side-effects (Gabizon 2002). The large surface area of a liposome also allows multivalent attachment of targeting peptides. In place of systemic delivery to all vascular beds, the use of organ-specific peptide-mediated delivery of target drugs can improve therapeutic effects, and reduce systemic side effects (Ruoslahti et al., 2010).

Tissue-specific targeting

There are risks to the fetus to consider when applying a master regulator therapeutic systemically, especially one selected to target an upstream molecule with multiple downstream effects. Though our *in silico* prediction are based on genes selectively expressed at labour in the human and mouse uterine tissues, many of the functions, and especially those of the inflammatory nature, are ubiquitously involved in haematological development, cell growth and cell survival. Therapeutic inhibition of such targets may have unforeseen effects on the rapidly developing fetus if therapeutics are able to cross the placental-fetal barrier.

Tissue-specific targeting peptides have previously been identified by screening phage libraries in whole animal models. Using this technique, we can synthesis liposomes that display myometrial/decidual targeting peptides on their surface. This will allow delivery of potential PTL therapies to their site of action, maximising the therapeutic effect. In addition, the encapsulated drug has less general exposure to maternal tissue, which is particularly important where timing and dosage of therapies is crucial. As an example, extended treatment with COX-2 inhibitors often requires drug withdrawal due to reported maternal hypertension, cardiac complications and, in severe cases, kidney failure (Komers et al. 2001). Directed liposomal targeted delivery, would reduce maternal systemic exposure, and could potentially increase its effectiveness as a PTL suppressive drug.

The potential of liposomal encapsulation in tocolysis had been shown in a recent study targeting oxytocin receptors in the myometrium. The targeted encapsulated tocolytic drugs (nifedipine, salbutamol, rolipram and dofetilide) extended pregnancies or increased duration of labour, and, the liposomes were not detected in the fetus localising primarily in the uterine tissues (Paul et al.

2015). The development of targeted liposomal drug delivery and our *in silico* identification of master regulators provide promising therapeutic potential for PTL.

What is preterm labour and is it a condition that should be treated?

A problem in PTL and preterm birth, is that it is currently defined only by gestational age rather than aetiology. This is a major issue which also contributes to the lack of efficacy in available treatments for idiopathic PTL. Future treatments with increased effectiveness would require strategic treatment plans to benefit infant and/or maternal health. This includes the need for stratification of patient samples to investigate causal conditions of PTL, as this may identify alternative genetic regulators based on an aetiology rather than the gestational age; or, the stratification of patients to identify those in which an intervention would benefit the infant and/or maternal outcome, in clinical trials. Maternal side effects on cardiovascular health are of concern over long term tocolysis (Katz & Farmer 1999; de Heus et al. 2009), and fetal distress and long term effects on fetal health have also been reported especially using magnesium sulphate and selective PTGS2 inhibition (van Veen et al. 2005; Hubinont & Debieve 2011; Klauser et al. 2014). This has also been an issue with antibiotic treatment in infection-related PTL being associated with an increased risk of cerebral palsy (Kenyon et al. 2001; Kenyon et al. 2008).

8.0 References

Abbas, Abul K., Kenneth M. Murphy, and Alan Sher. 1996. "Functional Diversity of Helper T Lymphocytes." Nature 383 (6603): 787–93. doi:10.1038/383787a0.

Abdelazim, Ibrahim A, Khaled M Abdelrazak, Mohamed Al-Kadi, Amr H Yehia, and Amr F Abdulkareem. 2014. "Fetal Fibronectin (Quick Check fFN Test) versus Placental Alpha Microglobulin-1 (AmniSure Test) for Detection of Premature Rupture of Fetal Membranes." Archives of Gynecology and Obstetrics 290 (3): 457–64. doi:10.1007/s00404-014-3225-5.

Abramovici, A, J Cantu, and S M Jenkins. 2012. "Tocolytic Therapy for Acute Preterm Labor." Obstet Gynecol Clin North Am 39 (1): 77–87. doi:S0889-8545(11)00126-4 [pii]10.1016/j.ogc.2011.12.003.

Abramovici, Adi, and Sheri Jenkins. 2013. "Network Meta-Analysis Shows That Prostaglandin Inhibitors and Nifedipine Are Best Short-Term Tocolytics for Preterm Delivery." Evidence-Based Medicine 18 (5): 182–83. doi:10.1136/eb-2012-101128.

Ackerman, William E, Xiaolan L Zhang, Brad H Rovin, and Douglas A Kniss. 2005. "Modulation of Cytokine-Induced Cyclooxygenase 2 Expression by PPARG Ligands through NFkappaB Signal Disruption in Human WISH and Amnion Cells." Biology of Reproduction 73 (3): 527–35. doi:10.1095/biolreprod.104.039032.

Aguan, K, J A Carvajal, L P Thompson, and C P Weiner. 2000. "Application of a Functional Genomics Approach to Identify Differentially Expressed Genes in Human Myometrium during Pregnancy and Labour." Mol Hum Reprod 6 (12): 1141–45.

Ahern, J, K E Pickett, S Selvin, and B Abrams. 2003. "Preterm Birth among African American and White Women: A Multilevel Analysis of Socioeconomic Characteristics and Cigarette Smoking." J Epidemiol Community Health 57 (8): 606–11.

Airoldi, James, Vincenzo Berghella, Harish Sehdev, and Jack Ludmir. 2005. "Transvaginal Ultrasonography of the Cervix to Predict Preterm Birth in Women with Uterine Anomalies." Obstetrics and Gynecology 106 (3): 553–56. doi:10.1097/01.AOG.0000173987.59595.e2.

Alexander, Helen A, Suren R Sooranna, Leslie Myatt, and Mark R Johnson. 2012. "Myometrial Tumor Necrosis Factor-α Receptors Increase with Gestation and Labor and Modulate Gene Expression through Mitogen-Activated Kinase and Nuclear Factor-κB." Reproductive Sciences (Thousand Oaks, Calif.) 19 (1): 43–54. doi:10.1177/1933719111413297.

Alijotas-Reig, J, E Llurba, and J Ma Gris. 2014. "Potentiating Maternal Immune Tolerance in Pregnancy: A New Challenging Role for Regulatory T Cells." Placenta 35 (4): 241–48. doi:10.1016/j.placenta.2014.02.004.

Anbe, Hiroshi, Toshiaki Okawa, Noboru Sugawara, Hidenori Takahashi, Akira Sato, Yuri P Vedernikov, George R Saade, and Robert E Garfield. 2007. "Influence of Progesterone on Myometrial Contractility in Pregnant Mice Treated with Lipopolysaccharide." The Journal of Obstetrics and Gynaecology Research 33 (6): 765–71. doi:10.1111/j.1447-0756.2007.00653.x.

Antolovich, G C, I J Clarke, I C McMillen, R A Perry, P M Robinson, M Silver, and R Young. 1990. "Hypothalamo-Pituitary Disconnection in the Fetal Sheep." Neuroendocrinology 51 (1): 1–9.

Areia, Ana Luísa, Sofia Vale-Pereira, Ana Vaz-Ambrósio, Vera Alves, Paulo Rodrigues-Santos, Manuel Santos Rosa, Paulo Moura, and Anabela Mota-Pinto. 2015. "Does Progesterone Administration in Preterm Labor Influence Treg Cells?" Journal of Perinatal Medicine, September. doi:10.1515/jpm-2015-0134.

Arrowsmith, Sarah, Siobhan Quenby, Andrew Weeks, Theodor Burdyga, and Susan Wray. 2012. "Poor Spontaneous and Oxytocin-Stimulated Contractility in Human Myometrium from Postdates Pregnancies." PloS One 7 (5): e36787. doi:10.1371/journal.pone.0036787.

Arthur, P, M J Taggart, and B F Mitchell. 2007. "Oxytocin and Parturition: A Role for Increased Myometrial Calcium and Calcium Sensitization?" Front Biosci 12: 619–33. doi:2087 [pii].

Arthur, Patrice, Michael J Taggart, Barbara Zielnik, Susan Wong, and Bryan F Mitchell. 2008. "Relationship between Gene Expression and Function of Uterotonic Systems in the Rat during Gestation, Uterine Activation and Both Term and Preterm Labour." J Physiol 586 (Pt 24): 6063–76. doi:10.1113/jphysiol.2008.164004.

Astolfi, P, and L A Zonta. 1999. "Risks of Preterm Delivery and Association with Maternal Age, Birth Order, and Fetal Gender." Hum Reprod 14 (11): 2891–94.

Athayde, N, R Romero, E Maymon, R Gomez, P Pacora, H Araneda, and B H Yoon. 1999. "A Role for the Novel Cytokine RANTES in Pregnancy and Parturition." Am J Obstet Gynecol 181 (4): 989–94. doi:S0002937899005773 [pii].

Bagnoli, F, A Mori, C Fommei, G Coriolani, S Badii, and B Tomasini. 2013. "ACTH and Cortisol Cord Plasma Concentrations in Preterm and Term Infants." Journal of Perinatology : Official Journal of the California Perinatal Association 33 (7): 520–24. doi:10.1038/jp.2012.165.

Bansal, Yogita, and Om Silakari. 2012. "The Therapeutic Journey of Benzimidazoles: A Review." Bioorganic & Medicinal Chemistry 20 (21): 6208–36. doi:10.1016/j.bmc.2012.09.013.

Bartlett, S R, R Sawdy, and G E Mann. 1999. "Induction of Cyclooxygenase-2 Expression in Human Myometrial Smooth Muscle Cells by Interleukin-1beta: Involvement of p38 Mitogen-Activated Protein Kinase." J Physiol 520 Pt 2 (October): 399–406. doi:PHY_9752 [pii].

Beck, S, D Wojdyla, L Say, A P Betran, M Merialdi, J H Requejo, C Rubens, R Menon, and P F Van Look. 2010. "The Worldwide Incidence of Preterm Birth: A Systematic Review of Maternal Mortality and Morbidity." Bull World Health Organ 88 (1): 31–38. doi:10.2471/BLT.08.062554.

Belfort, Michael A. 2011. "Indicated Preterm Birth for Placenta Accreta." Seminars in Perinatology 35 (5): 252–56. doi:10.1053/j.semperi.2011.05.002.

Belt, A R, J J Baldassare, M Molnar, R Romero, and F Hertelendy. 1999. "The Nuclear Transcription Factor NF-kappaB Mediates Interleukin-1beta-Induced Expression of Cyclooxygenase-2 in Human Myometrial Cells." Am J Obstet Gynecol 181 (2): 359–66. doi:S0002937899004755 [pii].

Bennett, S L, J B Cullen, D M Sherer, and J R Woods. 1993. "The Ferning and Nitrazine Tests of Amniotic Fluid between 12 and 41 Weeks Gestation." American Journal of Perinatology 10 (2): 101–4. doi:10.1055/s-2007-994637.

Bergenfelz, C, H Janols, M Wullt, K Jirström, A Bredberg, and K Leandersson. 2013. "Wnt5a Inhibits Human Monocyte-Derived Myeloid Dendritic Cell Generation." Scandinavian Journal of Immunology 78 (2): 194–204. doi:10.1111/sji.12075.

Bethin, K E, Y Nagai, R Sladek, M Asada, Y Sadovsky, T J Hudson, and L J Muglia. 2003. "Microarray Analysis of Uterine Gene Expression in Mouse and Human Pregnancy." Mol Endocrinol 17 (8): 1454–69. doi:10.1210/me.2003-0007.

Bevis, K S, and J R Biggio. 2011. "Cervical Conization and the Risk of Preterm Delivery." Am J Obstet Gynecol 205 (1): 19–27. doi:S0002-9378(11)00004-4 [pii]10.1016/j.ajog.2011.01.003.

Bizargity, P, R Del Rio, M Phillippe, C Teuscher, and E A Bonney. 2009. "Resistance to Lipopolysaccharide-Induced Preterm Delivery Mediated by Regulatory T Cell Function in Mice." Biol Reprod 80 (5): 874–81. doi:biolreprod.108.074294 [pii]10.1095/biolreprod.108.074294.

Blencowe, H, S Cousens, M Z Oestergaard, D Chou, A B Moller, R Narwal, A Adler, et al. 2012. "National, Regional, and Worldwide Estimates of Preterm Birth Rates in the Year 2010 with Time Trends since 1990 for Selected Countries: A Systematic Analysis and Implications." Lancet 379 (9832): 2162–72. doi:S0140-6736(12)60820-4 [pii]10.1016/S0140-6736(12)60820-4.

Bless, N M, M Huber-Lang, R F Guo, R L Warner, H Schmal, B J Czermak, T P Shanley, et al. 2000. "Role of CC Chemokines (macrophage Inflammatory Protein-1 Beta, Monocyte Chemoattractant Protein-1, RANTES) in Acute Lung Injury in Rats." J Immunol 164 (5): 2650–59. doi:ji_v164n5p2650 [pii].

Blesson, Chellakkan S, Edgar Büttner, Britt Masironi, and Lena Sahlin. 2012. "Prostaglandin Receptors EP and FP Are Regulated by Estradiol and Progesterone in the Uterus of Ovariectomized Rats." Reproductive Biology and Endocrinology : RB&E 10 (January): 3. doi:10.1186/1477-7827-10-3.

Bokstrom, H, M Brannstrom, M Alexandersson, and A Norstrom. 1997. "Leukocyte Subpopulations in the Human Uterine Cervical Stroma at Early and Term Pregnancy." Hum Reprod 12 (3): 586–90.

Bollapragada, Shrikant, Refaat Youssef, Fiona Jordan, Ian Greer, Jane Norman, and Scott Nelson. 2009. "Term Labor Is Associated with a Core Inflammatory Response in Human Fetal Membranes, Myometrium, and Cervix." American Journal of Obstetrics and Gynecology 200 (1). Mosby, Inc.: 104.e1–11. doi:10.1016/j.ajog.2008.08.032.

Boos, A, J Kohtes, V Janssen, C Mulling, A Stelljes, H Zerbe, M Hassig, and H H Thole. 2006. "Pregnancy Effects on Distribution of Progesterone Receptors, Oestrogen Receptor Alpha, Glucocorticoid Receptors, Ki-67 Antigen and Apoptosis in the Bovine Interplacentomal Uterine Wall and Foetal Membranes." Anim Reprod Sci 91 (1-2): 55–76. doi:S0378-4320(05)00087-4 [pii]10.1016/j.anireprosci.2005.03.012.

Bowen, J M, L Chamley, J A Keelan, and M D Mitchell. 2002. "Cytokines of the Placenta and Extra-Placental Membranes: Roles and Regulation during Human Pregnancy and Parturition." Placenta 23 (4): 257–73. doi:10.1053/plac.2001.0782S0143400401907829 [pii].

Brizot, Maria L, Wagner Hernandez, Adolfo W Liao, Roberto E Bittar, Rossana P V Francisco, Vera L J Krebs, and Marcelo Zugaib. 2015. "Vaginal Progesterone for the Prevention of Preterm Birth in Twin Gestations: A Randomized Placebo-Controlled Double-Blind Study." American Journal of Obstetrics and Gynecology 213 (1): 82.e1–9. doi:10.1016/j.ajog.2015.02.021.

Brown, N L, S A Alvi, M G Elder, P R Bennett, and M H Sullivan. 2000. "The Regulation of Prostaglandin Output from Term Intact Fetal Membranes by Anti-Inflammatory Cytokines." Immunology 99 (1): 124–33.

Buhimschi, Catalin S, Frederik Schatz, Graciela Krikun, Irina A Buhimschi, and Charles J Lockwood. 2010. "Novel Insights into Molecular Mechanisms of Abruption-Induced Preterm Birth." Expert Reviews in Molecular Medicine 12 (January): e35. doi:10.1017/S1462399410001675.

Bukowski, R, G D Hankins, G R Saade, G D Anderson, and S Thornton. 2006. "Labor-Associated Gene Expression in the Human Uterine Fundus, Lower Segment, and Cervix." PLoS Med 3 (6): e169. doi:05-PLME-RA-0327R2 [pii]10.1371/journal.pmed.0030169.

Bulmer, J N, D Pace, and A Ritson. 1988. "Immunoregulatory Cells in Human Decidua: Morphology, Immunohistochemistry and Function." Reprod Nutr Dev 28 (6B): 1599–1613.

Cano-Gauci, D F, and B Sarkar. 1996. "Reversible Zinc Exchange between Metallothionein and the Estrogen Receptor Zinc Finger." FEBS Letters 386 (1): 1–4.

Carl, Joseph W, and Xue-Feng Bai. 2008. "IL27: Its Roles in the Induction and Inhibition of Inflammation." International Journal of Clinical and Experimental Pathology 1: 117–23.

Castillo-Castrejon, Marisol, Noemí Meraz-Cruz, Nardhy Gomez-Lopez, Arturo Flores-Pliego, Jorge Beltrán-Montoya, Martín Viveros-Alcaráz, and Felipe Vadillo-Ortega. 2014. "Choriodecidual Cells from Term Human Pregnancies Show Distinctive Functional Properties Related to the Induction of Labor." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 71 (1): 86–93. doi:10.1111/aji.12179.

Castro-Leyva, Violeta, Aurora Espejel-Nuñez, Gerardo Barroso, Veronica Zaga-Clavellina, Arturo Flores-Pliego, Iyari Morales-Mendez, Silvia Giono-Cerezo, Scott W Walsh, and Guadalupe Estrada-Gutierrez. 2012. "Preserved Ex Vivo Inflammatory Status in Decidual Cells from Women with Preterm Labor and Subclinical Intrauterine Infection." PloS One 7 (8): e43605. doi:10.1371/journal.pone.0043605.

Chaemsaithong, Piya, Ichchha Madan, Roberto Romero, Nandor Gabor Than, Adi L Tarca, Sorin Draghici, Gaurav Bhatti, et al. 2013. "Characterization of the Myometrial Transcriptome in Women with an Arrest of Dilatation during Labor." Journal of Perinatal Medicine 41 (6): 665–81. doi:10.1515/jpm-2013-0086.

Chai, S Y, R Smith, J T Fitter, C Mitchell, X Pan, M Ilicic, K Maiti, T Zakar, and G Madsen. 2014. "Increased Progesterone Receptor A Expression in Labouring Human Myometrium Is Associated with Decreased Promoter Occupancy by the Histone Demethylase JARID1A." Molecular Human Reproduction 20 (5): 442–53. doi:10.1093/molehr/gau005.

Chakrabarty, Anindita, Susanne Tranguch, Takiko Daikoku, Kevin Jensen, Henry Furneaux, and Sudhansu K Dey. 2007. "MicroRNA Regulation of Cyclooxygenase-2 during Embryo Implantation." Proceedings of the National Academy of Sciences of the United States of America 104 (38): 15144–49. doi:10.1073/pnas.0705917104.

Challis, John R, Charles J Lockwood, Leslie Myatt, Jane E Norman, J F Strauss 3rd, Felice Petraglia, and Jerome F Strauss. 2009. "Inflammation and Pregnancy." Reprod Sci 16 (2): 206–15. doi:16/2/206 [pii]10.1177/1933719108329095.

Challis, J, J Newnham, F Petraglia, M Yeganegi, and A Bocking. 2013. "Fetal Sex and Preterm Birth." Placenta 34 (2): 95–99. doi:10.1016/j.placenta.2012.11.007.

Chames, Patrick, Marc Van Regenmortel, Etienne Weiss, and Daniel Baty. 2009. "Therapeutic Antibodies: Successes, Limitations and Hopes for the Future." British Journal of Pharmacology 157 (2): 220–33. doi:10.1111/j.1476-5381.2009.00190.x.

Chan, E Cheng, S Fraser, S Yin, G Yeo, K Kwek, R J Fairclough, and R Smith. 2002. "Human Myometrial Genes Are Differentially Expressed in Labor: A Suppression Subtractive Hybridization Study." The Journal of Clinical Endocrinology and Metabolism 87 (6): 2435–41. doi:10.1210/jcem.87.6.8439.

Chan, Yi-Wah, Hugo A van den Berg, Jonathan D Moore, Siobhan Quenby, and Andrew M Blanks. 2014. "Assessment of Myometrial Transcriptome Changes Associated with Spontaneous Human Labour by High-Throughput RNA-Seq." Experimental Physiology 99 (3): 510–24. doi:10.1113/expphysiol.2013.072868.

Chandran, Sreenath, Michael T. Cairns, Margaret O'Brien, and Terry J. Smith. 2014. "Transcriptomic Effects of Estradiol Treatment on Cultured Human Uterine Smooth Muscle Cells." Molecular and Cellular Endocrinology, June. doi:10.1016/j.mce.2014.05.020.

Chao, K H, M Y Wu, C D Chen, J H Yang, Y S Yang, and H N Ho. 1999. "The Expression of Killer Cell Inhibitory Receptors on Natural Killer Cells and Activation Status of CD4+ and CD8+ T Cells in the Decidua of Normal and Abnormal Early Pregnancies." Human Immunology 60 (9): 791–97.

Charpigny, G, J Leroy, M Breuiller-Fouché, Z Tanfin, S Mhaouty-Kodja, Ph Robin, D Leiber, et al. 2003. "A Functional Genomic Study to Identify Differential Gene Expression in the Preterm and Term Human Myometrium." Biol Reprod 68 (6): 2289–96. doi:10.1095/biolreprod.102.013763 biolreprod.102.013763 [pii].

Chatr-Aryamontri, Andrew, Bobby Joe Breitkreutz, Sven Heinicke, Lorrie Boucher, Andrew Winter, Chris Stark, Julie Nixon, et al. 2013. "The BioGRID Interaction Database: 2013 Update." Nucleic Acids Research 41 (D1): 470–78. doi:10.1093/nar/gks1158.

Chatr-Aryamontri, Andrew, Bobby-Joe Breitkreutz, Rose Oughtred, Lorrie Boucher, Sven Heinicke, Daici Chen, Chris Stark, et al. 2015. "The BioGRID Interaction Database: 2015 Update." Nucleic Acids Research 43 (Database issue): D470–78. doi:10.1093/nar/gku1204.

Chen, Caoyi, Juan C Opazo, Offer Erez, Monica Uddin, Joaquin Santolaya-Forgas, Morris Goodman, Lawrence I Grossman, Roberto Romero, and Derek E Wildman. 2008. "The Human Progesterone Receptor Shows Evidence of Adaptive Evolution Associated with Its Ability to Act as a Transcription Factor." Molecular Phylogenetics and Evolution 47 (2): 637–49. doi:10.1016/j.ympev.2007.12.026.

Chen, Luqiu, Kristy A Grabowski, Jun-Ping Xin, John Coleman, Zan Huang, Baltazar Espiritu, Serhan Alkan, et al. 2004. "IL-4 Induces Differentiation and Expansion of Th2 Cytokine-Producing Eosinophils." Journal of Immunology (Baltimore, Md. : 1950) 172 (4): 2059–66.

Chow, L, and S J Lye. 1994. "Expression of the Gap Junction Protein Connexin-43 Is Increased in the Human Myometrium toward Term and with the Onset of Labor." Am J Obstet Gynecol 170 (3): 788–95. doi:S0002937894005417 [pii].

Christiaens, Inge, Dean B Zaragoza, Larry Guilbert, Sarah A Robertson, Bryan F Mitchell, and David M Olson. 2008. "Inflammatory Processes in Preterm and Term Parturition." J Reprod Immunol 79 (1): 50–57. doi:S0165-0378(08)00049-1 [pii]10.1016/j.jri.2008.04.002.

Cline, Melissa S, Michael Smoot, Ethan Cerami, Allan Kuchinsky, Nerius Landys, Chris Workman, Rowan Christmas, et al. 2007. "Integration of Biological Networks and Gene Expression Data Using Cytoscape." Nature Protocols 2 (10): 2366–82. doi:10.1038/nprot.2007.324.

Clodi, Martin, Greisa Vila, René Geyeregger, Michaela Riedl, Thomas M Stulnig, Joachim Struck, Thomas A Luger, and Anton Luger. 2008. "Oxytocin Alleviates the Neuroendocrine and Cytokine Response to Bacterial Endotoxin in Healthy Men." American Journal of Physiology. Endocrinology and Metabolism 295 (3): E686–91. doi:10.1152/ajpendo.90263.2008.

Cnattingius, Sven. 2004. "The Epidemiology of Smoking during Pregnancy: Smoking Prevalence, Maternal Characteristics, and Pregnancy Outcomes." Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco 6 Suppl 2 (Suppl_2): S125–40. doi:10.1080/14622200410001669187.

Cohen, Philip. 2009. "Targeting Protein Kinases for the Development of Anti-Inflammatory Drugs." Current Opinion in Cell Biology 21 (2): 317–24. doi:10.1016/j.ceb.2009.01.015.

Coleman, S J, L Gerza, C J P Jones, C P Sibley, J D Aplin, and A E P Heazell. 2013. "Syncytial Nuclear Aggregates in Normal Placenta Show Increased Nuclear Condensation, but Apoptosis and Cytoskeletal Redistribution Are Uncommon." Placenta 34 (5): 449–55. doi:10.1016/j.placenta.2013.02.007.

Conde-Agudelo, Agustin, and Roberto Romero. 2015a. "Cervical Phosphorylated Insulin-like Growth Factor Binding Protein-1 Test for the Prediction of Preterm Birth: A Systematic Review and Metaanalysis." American Journal of Obstetrics and Gynecology, July. doi:10.1016/j.ajog.2015.06.060.

Conde-Agudelo, Agustin, and Roberto Romero. 2015b. "Vaginal Progesterone to Prevent Preterm Birth in Pregnant Women with a Sonographic Short Cervix: Clinical and Public Health Implications." American Journal of Obstetrics and Gynecology, October. doi:10.1016/j.ajog.2015.09.102.

Conde-Agudelo, Agustín, Roberto Romero, and Juan Pedro Kusanovic. 2011. "Nifedipine in the Management of Preterm Labor: A Systematic Review and Metaanalysis." American Journal of Obstetrics and Gynecology 204 (2): 134.e1–20. doi:10.1016/j.ajog.2010.11.038.

Cook, J L, D B Zaragoza, D H Sung, and D M Olson. 2000. "Expression of Myometrial Activation and Stimulation Genes in a Mouse Model of Preterm Labor: Myometrial Activation, Stimulation, and Preterm Labor." Endocrinology 141 (5): 1718–28. doi:10.1210/endo.141.5.7474.

Cookson, Victoria J, and Neil R Chapman. 2010. "NF-kappaB Function in the Human Myometrium during Pregnancy and Parturition." Histology and Histopathology 25 (7): 945–56.

Costeloe, K L, E M Hennessy, S Haider, F Stacey, N Marlow, and E S Draper. 2012. "Short Term Outcomes after Extreme Preterm Birth in England: Comparison of Two Birth Cohorts in 1995 and 2006 (the EPICure Studies)." BMJ 345: e7976.

Crowther, C A, J E Hiller, and L W Doyle. 2002. "Magnesium Sulphate for Preventing Preterm Birth in Threatened Preterm Labour." Cochrane Database Syst Rev, no. 4: CD001060. doi:10.1002/14651858.CD001060.

Curry, Allison E, Ida Vogel, Carolyn Drews, Diana Schendel, Kristin Skogstrand, W Dana Flanders, David Hougaard, Jørn Olsen, and Poul Thorsen. 2007. "Mid-Pregnancy Maternal Plasma Levels of Interleukin 2, 6, and 12, Tumor Necrosis Factor-Alpha, Interferon-Gamma, and Granulocyte-Macrophage Colony-Stimulating Factor and Spontaneous Preterm Delivery." Acta Obstet Gynecol Scand 86 (9): 1103–10. doi:781482745 [pii]10.1080/00016340701515423.

da Silva, A A, V M Simoes, M A Barbieri, H Bettiol, F Lamy-Filho, L C Coimbra, and M T Alves. 2003. "Young Maternal Age and Preterm Birth." Paediatr Perinat Epidemiol 17 (4): 332–39. doi:515 [pii].

Danti, Luana, Marina Zonca, Lorena Barbetti, Andrea Lojacono, Silvia Marini, Nazario Cappello, Umberto Bianchi, and Chiara Benedetto. 2014. "Prophylactic Oral Nifedipine to Reduce Preterm Delivery: A

Randomized Controlled Trial in Women at High Risk." Acta Obstetricia et Gynecologica Scandinavica 93 (8): 802–8. doi:10.1111/aogs.12405.
Darne, J, H H McGarrigle, and G C Lachelin. 1987a. "Increased Saliva Oestriol to Progesterone Ratio before Preterm Delivery: A Possible Predictor for Preterm Labor?" Br Med J (Clin Res Ed) 294 (6567): 270–72.
Darne, J, H H McGarrigle, and G C Lachelin. 1987b. "Saliva Oestriol, Oestradiol, Oestrone and Progesterone Levels in Pregnancy: Spontaneous Labour at Term Is Preceded by a Rise in the Saliva Oestriol:progesterone Ratio." Br J Obstet Gynaecol 94 (3): 227–35.
Davidson, Dennis, Alla Zaytseva, Veronika Miskolci, Susana Castro-Alcaraz, Ivana Vancurova, and Hardik Patel. 2013. "Gene Expression Profile of Endotoxin-Stimulated Leukocytes of the Term New Born: Control of Cytokine Gene Expression by Interleukin-10." PloS One 8 (1): e53641. doi:10.1371/journal.pone.0053641.
de Heus, Roel, Ben Willem Mol, Jan-Jaap H M Erwich, Herman P van Geijn, Wilfried J Gyselaers, Myriam Hanssens, Linda Härmark, et al. 2009. "Adverse Drug Reactions to Tocolytic Treatment for Preterm Labour: Prospective Cohort Study." BMJ (Clinical Research Ed.) 338 (January): b744.
De, M, T Sanford, and G W Wood. 1993. "Relationship between Macrophage Colony-Stimulating Factor Production by Uterine Epithelial Cells and Accumulation and Distribution of Macrophages in the Uterus of Pregnant Mice." J Leukoc Biol 53 (3): 240–48.
De Silva, D, M D Mitchell, and J A Keelan. 2010. "Inhibition of Choriodecidual Cytokine Production and Inflammatory Gene Expression by Selective I-kappaB Kinase (IKK) Inhibitors." Br J Pharmacol 160 (7): 1808–22. doi:BPH839 [pii]10.1111/j.1476-5381.2010.00839.x.
Denison, F C, R W Kelly, A A Calder, and S C Riley. 1998. "Cytokine Secretion by Human Fetal Membranes, Decidua and Placenta at Term." Hum Reprod 13 (12): 3560–65.
Di Renzo, Gian Carlo, Lluis Cabero Roura, Fabio Facchinetti, Aris Antsaklis, Gregor Breborowicz, Eduard Gratacos, Peter Husslein, et al. 2011. "Guidelines for the Management of Spontaneous Preterm Labor: Identification of Spontaneous Preterm Labor, Diagnosis of Preterm Premature Rupture of Membranes, and Preventive Tools for Preterm Birth." The Journal of Maternal-Fetal & Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians 24 (5): 659–67. doi:10.3109/14767058.2011.553694.
Di Stefano, Valeria, Bingbing Wang, Nataliya Parobchak, Natalie Roche, and Todd Rosen. 2015. "RelB/p52-Mediated NF-κB Signaling Alters Histone Acetylation to Increase the Abundance of Corticotropin-Releasing Hormone in Human Placenta." Science Signaling 8 (391): ra85. doi:10.1126/scisignal.aaa9806.
Di, W L, G C Lachelin, H H McGarrigle, N S Thomas, and D L Becker. 2001. "Oestriol and Oestradiol Increase Cell to Cell Communication and connexin43 Protein Expression in Human Myometrium." Mol Hum Reprod 7 (7): 671–79.
Diamond, Allaire K, Leigh M Sweet, Karen H Oppenheimer, Diana F Bradley, and Mark Phillippe. 2007. "Modulation of Monocyte Chemotactic Protein-1 Expression during Lipopolysaccharide-Induced Preterm Delivery in the Pregnant Mouse." Reproductive Sciences (Thousand Oaks, Calif.) 14 (6): 548–59. doi:14/6/548 [pii]10.1177/1933719107307792.
Doring, B, Oksana Shynlova, Prudence Tsui, Dominik Eckardt, Ulrike Janssen-Bienhold, Franz Hofmann, Susanne Feil, et al. 2006. "Ablation of connexin43 in Uterine Smooth Muscle Cells of the Mouse Causes Delayed Parturition." J Cell Sci 119 (Pt 9): 1715–22. doi:jcs.02892 [pii]10.1242/jcs.02892.
Dubicke, A, P Andersson, E Fransson, E Andersson, A Sioutas, A Malmstrom, E Sverremark-Ekstrom, and G Ekman-Ordeberg. 2010b. "High-Mobility Group Box Protein 1 and Its Signalling Receptors in Human Preterm and Term Cervix." J Reprod Immunol 84 (1): 86–94. doi:S0165-0378(09)00523-3 [pii]10.1016/j.jri.2009.09.010.
Dubicke, Aurelija, Emma Fransson, Gabriele Centini, Eva Andersson, Birgitta Byström, Anders Malmström, Felice Petraglia, et al. 2010a. "Pro-Inflammatory and Anti-Inflammatory Cytokines in Human Preterm and Term Cervical Ripening." J Reprod Immunol 84 (2): 176–85. doi:10.1016/j.jri.2009.12.004.
Duckitt, K, and S Thornton. 2002. "Nitric Oxide Donors for the Treatment of Preterm Labour." Cochrane Database Syst Rev, no. 3: CD002860. doi:CD002860 [pii]10.1002/14651858.CD002860.
Dudley, D J, D W Branch, S S Edwin, and M D Mitchell. 1996b. "Induction of Preterm Birth in Mice by RU486." Biol Reprod 55 (5): 992–95.
Dudley, D J, C L Chen, D W Branch, E Hammond, and M D Mitchell. 1993. "A Murine Model of Preterm Labor: Inflammatory Mediators Regulate the Production of Prostaglandin E2 and Interleukin-6 by Murine Decidua." Biol Reprod 48 (1): 33–39.
Dudley, D J, D Collmer, M D Mitchell, and M S Trautman. 1996a. "Inflammatory Cytokine mRNA in Human Gestational Tissues: Implications for Term and Preterm Labor." J Soc Gynecol Investig 3 (6): 328–35. doi:S1071557696000421 [pii].

Duhen, Thomas, Rebekka Duhen, Antonio Lanzavecchia, Federica Sallusto, and Daniel J Campbell. 2012. "Functionally Distinct Subsets of Human FOXP3+ Treg Cells That Phenotypically Mirror Effector Th Cells." Blood 119 (19): 4430–40. doi:10.1182/blood-2011-11-392324.

Ehsanipoor, Robert M, Morgan L Swank, Seung Chik Jwa, Deborah A Wing, Gofran Tarabulsi, and Karin J Blakemore. 2015. "Placental α-Microglobulin-1 in Vaginal Secretions of Women with Evidence of Preterm Labor." American Journal of Perinatology, September. doi:10.1055/s-0035-1563710.

Eke, Ahizechukwu C, Tina Chalaan, Ghadear Shukr, George U Eleje, and Charles I Okafor. 2015. "A Systematic Review and Meta-Analysis of Progestogen Use for Maintenance Tocolysis after Preterm Labor in Women with Intact Membranes." International Journal of Gynaecology and Obstetrics: The Official Organ of the International Federation of Gynaecology and Obstetrics, October. doi:10.1016/j.ijgo.2015.06.058.

Ekman, G, P H Perssen, U Ulmsten, and L Wingerup. 1983. "The Impact on Labor Induction of Intracervically Applied PGE2-Gel, Related to Gestational Age in Patients with an Unripe Cervix." Acta Obstet Gynecol Scand Suppl 113: 173–75.

El Fekih, C, N Ouerdiane, C Mrezguia, M Mourali, M Douagi, M Abdennebi, and N Ben Zineb. 2009. "[Premature rupture membrane at term with unfavourable cervix]." Tunis Med 87 (9): 603–6.

Elliott, C L, J A Loudon, N Brown, D M Slater, P R Bennett, and M H Sullivan. 2001. "IL-1beta and IL-8 in Human Fetal Membranes: Changes with Gestational Age, Labor, and Culture Conditions." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 46 (4): 260–67.

Elliott, C L, D M Slater, W Dennes, L Poston, and P R Bennett. 2000. "Interleukin 8 Expression in Human Myometrium: Changes in Relation to Labor Onset and with Gestational Age." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 43 (5): 272–77.

Enquobahrie, D A, M A Williams, C Qiu, S Y Muhie, K Slentz-Kesler, Z Ge, and T Sorenson. 2009. "Early Pregnancy Peripheral Blood Gene Expression and Risk of Preterm Delivery: A Nested Case Control Study." BMC Pregnancy Childbirth 9: 56. doi:1471-2393-9-56 [pii]10.1186/1471-2393-9-56.

Esplin, M Sean, M Bardett Fausett, Morgan R Peltier, Steven Hamblin, Robert M Silver, D Ware Branch, Eli Y Adashi, and David Whiting. 2005. "The Use of cDNA Microarray to Identify Differentially Expressed Labor-Associated Genes within the Human Myometrium during Labor." American Journal of Obstetrics and Gynecology 193 (2): 404–13. doi:10.1016/j.ajog.2004.12.021.

Esplin, M Sean, Tracy A Manuck, Michael W Varner, Bryce Christensen, Joseph Biggio, Radek Bukowski, Samuel Parry, et al. 2015. "Cluster Analysis of Spontaneous Preterm Birth Phenotypes Identifies Potential Associations among Preterm Birth Mechanisms." American Journal of Obstetrics and Gynecology 213 (3): 429.e1–9. doi:10.1016/j.ajog.2015.06.011.

Evans, J J, I L Sin, and F J White. 1982. "Progesterone-Binding Globulin and Progesterone in Guinea-Pigs after Ovariectomy, Abortion and Parturition." J Steroid Biochem 16 (2): 171–73.

Farinelli, C K, D A Wing, J M Szychowski, J Owen, G Hankins, J D Iams, J S Sheffield, A Perez-Delboy, V Berghella, and E R Guzman. 2012. "Association between Body Mass Index and Pregnancy Outcome in a Randomized Trial of Cerclage for Short Cervix." Ultrasound Obstet Gynecol 40 (6): 669–73. doi:10.1002/UOG.11170.

Fehniger, Todd A, Manisha H Shah, Matthew J Turner, Jeffrey B VanDeusen, Susan P Whitman, Megan A Cooper, Kazuhiro Suzuki, Mark Wechser, Frederico Goodsaid, and Michael A Caligiuri. 1999. "Differential Cytokine and Chemokine Gene Expression by Human NK Cells Following Activation with IL-18 or IL-15 in Combination with IL-12: Implications for the Innate Immune Response." The Journal of Immunology 162 (8): 4511–20.

Fenstad, Mona H., Matthew P. Johnson, Linda T. Roten, Per A. Aas, Siri Forsmo, Kjetil Klepper, Christine E. East, et al. 2010. "Genetic and Molecular Functional Characterization of Variants within TNFSF13B, a Positional Candidate Preeclampsia Susceptibility Gene on 13q." PLoS ONE 5. doi:10.1371/journal.pone.0012993.

Fidel Jr., P L, R Romero, J Cutright, N Wolf, R Gomez, H Araneda, M Ramirez, and B H Yoon. 1997. "Treatment with the Interleukin-I Receptor Antagonist and Soluble Tumor Necrosis Factor Receptor Fc Fusion Protein Does Not Prevent Endotoxin-Induced Preterm Parturition in Mice." J Soc Gynecol Investig 4 (1): 22–26. doi:S1071-5576(96)00060-3 [pii]10.1016/S1071-5576(96)00060-3.

Fishman, Shira G, and Stephen T Chasen. 2011. "Risk Factors for Emergent Preterm Delivery in Women with Placenta Previa and Ultrasound Findings Suspicious for Placenta Accreta." Journal of Perinatal Medicine 39 (6): 693–96. doi:10.1515/JPM.2011.086.

Fleming, H, C Namit, and E Gurpide. 1980. "Estrogen Receptors in Epithelial and Stromal Cells of Human Endometrium in Culture." Journal of Steroid Biochemistry 12 (January): 169–74.

Flenady, Vicki, Hanna E Reinebrant, Helen G Liley, Eashan G Tambimuttu, and Dimitri N M Papatsonis. 2014. Cochrane Database of Systematic Reviews. The Cochrane Database of Systematic Reviews. Vol. 6. Chichester, UK: John Wiley & Sons, Ltd. doi:10.1002/14651858.CD004452.pub3.

Fletcher, Jamie I, Michelle Haber, Michelle J Henderson, and Murray D Norris. 2010. "Figure 1 : ABC Transporters in Cancer: More than Just Drug Efflux Pumps : Nature Reviews Cancer." Nature Reviews. Cancer. February. doi:10.1038/nrc2789.

Florio, P, A Franchini, F M Reis, I Pezzani, E Ottaviani, and F Petraglia. 2000. "Human Placenta, Chorion, Amnion and Decidua Express Different Variants of Corticotropin-Releasing Factor Receptor Messenger RNA." Placenta 21 (1): 32–37. doi:10.1053/plac.1999.0461.

Fontenot, Jason D, Marc A Gavin, and Alexander Y Rudensky. 2003. "Foxp3 Programs the Development and Function of CD4+CD25+ Regulatory T Cells." Nature Immunology 4 (4): 330–36. doi:10.1038/ni904.

Fu, X, C Moberg, T Backstrom, and U Ulmsten. 1996. "Effects of Estradiol and Interaction with Progesterone or Oxytocin on Term Human Myometrial Contractile Activity." Acta Obstet Gynecol Scand 75 (1): 13–18.

Fuchs, A R, L G Graddy, A A Kowalski, and M J Fields. 2002. "Oxytocin Induces PGE2 Release from Bovine Cervical Mucosa *in vivo*." Prostaglandins & Other Lipid Mediators 70 (1-2): 119–29.

Fuentes, A, E P Spaziani, and W F O'Brien. 1996. "The Expression of Cyclooxygenase-2 (COX-2) in Amnion and Decidua Following Spontaneous Labor." Prostaglandins 52 (4): 261–67. doi:S0090698096000883 [pii].

Gabizon, A A. 2002. "Liposomal Drug Carrier Systems in Cancer Chemotherapy: Current Status and Future Prospects." J Drug Target 10 (7): 535–38. doi:10.1080/1061186021000043061.

Gabizon, Alberto A, Hilary Shmeeda, and Samuel Zalipsky. 2006. "Pros and Cons of the Liposome Platform in Cancer Drug Targeting*." Journal of Liposome Research 16 (3): 175–83. doi:doi:10.1080/08982100600848769.

Garfield, R E, and S Beier. 1989. "Increased Myometrial Responsiveness to Oxytocin during Term and Preterm Labor." Am J Obstet Gynecol 161 (2): 454–61. doi:0002-9378(89)90541-3 [pii].

Garfield, R E, A M Irani, L B Schwartz, E Bytautiene, and R Romero. 2006. "Structural and Functional Comparison of Mast Cells in the Pregnant versus Nonpregnant Human Uterus." Am J Obstet Gynecol 194 (1): 261–67. doi:S0002-9378(05)00655-1 [pii]10.1016/j.ajog.2005.05.011 [doi].

Garite, T J, and S E Gocke. 1990. "Diagnosis of Preterm Rupture of Membranes: Is Testing for Alpha-Fetoprotein Better than Ferning or Nitrazine?" American Journal of Perinatology 7 (3): 276–78. doi:10.1055/s-2007-999500.

Gaulton, Anna, Louisa J Bellis, A Patricia Bento, Jon Chambers, Mark Davies, Anne Hersey, Yvonne Light, et al. 2012. "ChEMBL: A Large-Scale Bioactivity Database for Drug Discovery." Nucleic Acids Research 40 (Database issue): D1100–1107. doi:10.1093/nar/gkr777.

Genc, M R, and C E Ford. 2010. "The Clinical Use of Inflammatory Markers during Pregnancy." Curr Opin Obstet Gynecol 22 (2): 116–21. doi:10.1097/GCO.0b013e3283374ac8.

Gervasi, M T, T Chaiworapongsa, N Naccasha, S Blackwell, B H Yoon, E Maymon, and R Romero. 2001. "Phenotypic and Metabolic Characteristics of Maternal Monocytes and Granulocytes in Preterm Labor with Intact Membranes." Am J Obstet Gynecol 185 (5): 1124–29. doi:S0002-9378(01)84673-1 [pii]10.1067/mob.2001.117681.

Gilbert, R E, K Pike, S L Kenyon, W Tarnow-Mordi, and D J Taylor. 2005. "The Effect of Prepartum Antibiotics on the Type of Neonatal Bacteraemia: Insights from the MRC ORACLE Trials." BJOG 112 (6): 830–32. doi:BJO00558 [pii]10.1111/j.1471-0528.2005.00558.x.

Gilmour, Jane, and Paul Lavender. 2008. "Control of IL-4 Expression in T Helper 1 and 2 Cells." Immunology 124 (4): 437–44. doi:10.1111/j.1365-2567.2008.02845.x.

Glinianaia, Svetlana V, Rakesh Ghosh, Judith Rankin, Mark S Pearce, Louise Parker, and Tanja Pless-Mulloli. 2013. "No Improvement in Socioeconomic Inequalities in Birthweight and Preterm Birth over Four Decades: A Population-Based Cohort Study." BMC Public Health 13 (January): 345. doi:10.1186/1471-2458-13-345.

Goldenberg, R L, J D Iams, B M Mercer, P J Meis, A Moawad, A Das, M Miodovnik, et al. 2001. "The Preterm Prediction Study: Toward a Multiple-Marker Test for Spontaneous Preterm Birth." American Journal of Obstetrics and Gynecology 185 (3): 643–51. doi:10.1067/mob.2001.116752.

Goldenberg, R L, and J F Culhane. 2005. "Prepregnancy Health Status and the Risk of Preterm Delivery." Arch Pediatr Adolesc Med 159 (1): 89–90. doi:159/1/89 [pii]10.1001/archpedi.159.1.89.

Goldenberg, R L, J F Culhane, J D Iams, and R Romero. 2008. "Epidemiology and Causes of Preterm Birth." Lancet 371 (9606): 75–84. doi:S0140-6736(08)60074-4 [pii]10.1016/S0140-6736(08)60074-4.

Goldman, S, A Weiss, I Almalah, and E Shalev. 2005. "Progesterone Receptor Expression in Human Decidua and Fetal Membranes before and after Contractions: Possible Mechanism for Functional Progesterone Withdrawal." Mol Hum Reprod 11 (4): 269–77. doi:gah161 [pii]10.1093/molehr/gah161.

Gomez, R, R Romero, J K Nien, T Chaiworapongsa, L Medina, Y M Kim, B H Yoon, et al. 2005. "A Short Cervix in Women with Preterm Labor and Intact Membranes: A Risk Factor for Microbial Invasion of the Amniotic Cavity." Am J Obstet Gynecol 192 (3): 678–89. doi:S0002937804018575 [pii]10.1016/j.ajog.2004.10.624.

Gomez-Lopez, Nardhy, Derek StLouis, Marcus A Lehr, Elly N Sanchez-Rodriguez, and Marcia Arenas-Hernandez. 2014a. "Immune Cells in Term and Preterm Labor." Cellular and Molecular Immunology 11 (6): 571–81. doi:10.1038/cmi.2014.46.

Gomez-Lopez, Nardhy, Wing-Chiu Tong, Marcia Arenas-Hernandez, Satomi Tanaka, Omar Hajar, David M Olson, Michael J Taggart, and Bryan F Mitchell. 2014b. "Chemotactic Activity of Gestational Tissues Through Late Pregnancy, Term Labor, and RU486-Induced Preterm Labor in Guinea Pigs." American Journal of Reproductive Immunology (New York, N.Y. : 1989), October. doi:10.1111/aji.12333.

Gomez-Lopez, Nardhy, Rodrigo Vega-Sanchez, Marisol Castillo-Castrejon, Roberto Romero, Karen Cubeiro-Arreola, and Felipe Vadillo-Ortega. 2013. "Evidence for a Role for the Adaptive Immune Response in Human Term Parturition." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 69 (3): 212–30. doi:10.1111/aji.12074.

Gonzalez, Juan M, Hua Xu, Jinghua Chai, Ella Ofori, and Michal A Elovitz. 2009. "Preterm and Term Cervical Ripening in CD1 Mice (Mus Musculus): Similar or Divergent Molecular Mechanisms?" Biol Reprod 81 (6): 1226–32. doi:biolreprod.108.075309 [pii]10.1095/biolreprod.108.075309.

Goodwin, J S, J L Ceuppens, and M A Rodriguez. 1983. "Administration of Nonsteroidal Anti-Inflammatory Agents in Patients with Rheumatoid Arthritis. Effects on Indexes of Cellular Immune Status and Serum Rheumatoid Factor Levels." JAMA 250 (18): 2485–88.

Gorowiec, Marta R, Rob D Catalano, Jane E Norman, Fiona C Denison, and Henry N Jabbour. 2011. "Prokineticin 1 Induces Inflammatory Response in Human Myometrium: A Potential Role in Initiating Term and Preterm Parturition." Am J Pathol 179 (6): 2709–19. doi:S0002-9440(11)00846-7 [pii]10.1016/j.ajpath.2011.08.029.

Groom, K M. 2007. "Preterm Labour. Pharmacological Prevention of Prematurity." Best Pract Res Clin Obstet Gynaecol 21 (5): 843–56. doi:S1521-6934(07)00067-3 [pii]10.1016/j.bpobgyn.2007.03.010.

Gross, G A, T Imamura, C Luedke, S K Vogt, L M Olson, D M Nelson, Y Sadovsky, and L J Muglia. 1998. "Opposing Actions of Prostaglandins and Oxytocin Determine the Onset of Murine Labor." Proc Natl Acad Sci U S A 95 (20): 11875–79.

Guo, Wen-jing, Xun Qu, Mei-xiang Yang, Wei-dong Zhang, Lu Liang, Qian-qian Shao, and Bei-hua Kong. 2008. "Expression of BAFF in the Trophoblast and Decidua of Normal Early Pregnant Women and Patients with Recurrent Spontaneous Miscarriage." Chinese Medical Journal 121 (4): 309–15.

Gupta, D K, T A Sato, J A Keelan, K W Marvin, and M D Mitchell. 2001. "Expression of Prostaglandin H Synthase-1 and -2 in Murine Intrauterine and Gestational Tissues from Mid Pregnancy until Term." Prostaglandins Other Lipid Mediat 66 (1): 17–25.

Gustafsson, C, P Hummerdal, L Matthiesen, G Berg, C Ekerfelt, and J Ernerudh. 2006. "Cytokine Secretion in Decidual Mononuclear Cells from Term Human Pregnancy with or without Labour: ELISPOT Detection of IFN-Gamma, IL-4, IL-10, TGF-Beta and TNF-Alpha." J Reprod Immunol 71 (1): 41–56. doi:S0165-0378(06)00004-0 [pii]10.1016/j.jri.2005.12.009.

Haas, DM, DM Caldwell, P Kirkpatrick, J J McIntosh, and N J Welton. 2012a. "Tocolytic Therapy for Preterm Delivery: Systematic Review and Network Meta-Analysis." BMJ 345: e6226.

Haas, D M, P Kirkpatrick, J J McIntosh, and D M Caldwell. 2012b. "Assessing the Quality of Evidence for Preterm Labor Tocolytic Trials." J Matern Fetal Neonatal Med 25 (9): 1646–52. doi:10.3109/14767058.2011.650249.

Haddad, Ramsi, Gerard Tromp, Helena Kuivaniemi, Tinnakorn Chaiworapongsa, Yeon Mee Kim, Moshe Mazor, and Roberto Romero. 2006. "Human Spontaneous Labor without Histologic Chorioamnionitis Is Characterized by an Acute Inflammation Gene Expression Signature." American Journal of Obstetrics and Gynecology 195 (2): 394.e1–24. doi:10.1016/j.ajog.2005.08.057.

Haluska, G J, T R Wells, J J Hirst, R M Brenner, D W Sadowsky, and M J Novy. 2002. "Progesterone Receptor Localization and Isoforms in Myometrium, Decidua, and Fetal Membranes from Rhesus Macaques: Evidence for Functional Progesterone Withdrawal at Parturition." J Soc Gynecol Investig 9 (3): 125–36. doi:S1071557602001442 [pii].

Hamilton, Sarah A, Y Oomomian, G Stephen, O Shynlova, C L Tower, A Garrod, S J Lye, and R L Jones. 2012. "Macrophages Infiltrate the Human and Rat Decidua during Term and Preterm Labor: Evidence That Decidual Inflammation Precedes Labor." Biol Reprod 86 (2): 39. doi:biolreprod.111.095505 [pii] 10.1095/biolreprod.111.095505.

Hamilton, Sarah A, Clare L Tower, and Rebecca L Jones. 2013. "Identification of Chemokines Associated with the Recruitment of Decidual Leukocytes in Human Labour: Potential Novel Targets for Preterm Labour." PLoS One 8 (2): e56946. doi:10.1371/journal.pone.0056946.

Han, Yu Mi, Roberto Romero, Jung-Sun Kim, Adi L Tarca, Sun Kwon Kim, Sorin Draghici, Juan Pedro Kusanovic, et al. 2008. "Region-Specific Gene Expression Profiling: Novel Evidence for Biological

Heterogeneity of the Human Amnion." Biology of Reproduction 79 (5): 954–61. doi:10.1095/biolreprod.108.069260.
Han, C S, F Schatz, and C J Lockwood. 2011. "Abruption-Associated Prematurity." Clin Perinatol 38 (3): 407–21. doi:S0095-5108(11)00052-2 [pii]10.1016/j.clp.2011.06.001.
Haque, N S, J T Fallon, J J Pan, M B Taubman, and P C Harpel. 2004. "Chemokine Receptor-8 (CCR8) Mediates Human Vascular Smooth Muscle Cell Chemotaxis and Metalloproteinase-2 Secretion." Blood 103 (4): 1296–1304. doi:10.1182/blood-2002-05-1480 [doi]2002-05-1480 [pii].
Haram, Kjell, Jan Helge Mortensen, and John C Morrison. 2014. "Cerclage, Progesterone and α-Hydroxyprogeterone Caproate Treatment in Women at Risk for Preterm Delivery." The Journal of Maternal-Fetal & Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians 27 (16): 1710–15. doi:10.3109/14767058.2013.876003.
Harris, H, R Henderson, R Bhat, and B Komm. 2001. "Regulation of Metallothionein II Messenger Ribonucleic Acid Measures Exogenous Estrogen Receptor-Beta Activity in SAOS-2 and LNCaPLN3 Cells." Endocrinology 142 (2): 645–52. doi:10.1210/endo.142.2.7952.
Hassan, S S, R Romero, E Maymon, S M Berry, S C Blackwell, M C Treadwell, and M Tomlinson. 2001. "Does Cervical Cerclage Prevent Preterm Delivery in Patients with a Short Cervix?" Am J Obstet Gynecol 184 (7): 1325–31. doi:S0002937801173060 [pii].
Hassan, Sonia S, Roberto Romero, Ramsi Haddad, Israel Hendler, Nahla Khalek, Gerard Tromp, Michael P Diamond, Yoram Sorokin, and John Malone. 2006. "The Transcriptome of the Uterine Cervix before and after Spontaneous Term Parturition." American Journal of Obstetrics and Gynecology 195 (3): 778–86. doi:10.1016/j.ajog.2006.06.021.
Havelock, Jon C, Patrick Keller, Ndaya Muleba, Bobbie A Mayhew, Brian M Casey, William E Rainey, and R Ann Word. 2005. "Human Myometrial Gene Expression before and during Parturition." Biology of Reproduction 72 (3): 707–19. doi:10.1095/biolreprod.104.032979.
Hecker, Nikolai, Jessica Ahmed, Joachim von Eichborn, Mathias Dunkel, Karel Macha, Andreas Eckert, Michael K Gilson, Philip E Bourne, and Robert Preissner. 2012. "SuperTarget Goes Quantitative: Update on Drug-Target Interactions." Nucleic Acids Research 40 (Database issue): D1113–17. doi:10.1093/nar/gkr912.
Hediger, M L, T O Scholl, J I Schall, and P M Krueger. 1997. "Young Maternal Age and Preterm Labor." Ann Epidemiol 7 (6): 400–406. doi:S1047-2797(97)00046-X [pii].
Helguera, G, M Eghbali, D Sforza, T Y Minosyan, L Toro, and E Stefani. 2009. "Changes in Global Gene Expression in Rat Myometrium in Transition from Late Pregnancy to Parturition." Physiol Genomics 36 (2): 89–97. doi:10.1152/physiolgenomics.00290.2007.
Hendler, I, R L Goldenberg, B M Mercer, J D Iams, P J Meis, A H Moawad, C A MacPherson, et al. 2005. "The Preterm Prediction Study: Association between Maternal Body Mass Index and Spontaneous and Indicated Preterm Birth." Am J Obstet Gynecol 192 (3): 882–86. doi:S0002937804010397 [pii]10.1016/j.ajog.2004.09.021 [doi].
Heng, Yujing Jan, Craig Edward Pennell, Hon Nian Chua, Jonathan Edward Perkins, and Stephen James Lye. 2014. "Whole Blood Gene Expression Profile Associated with Spontaneous Preterm Birth in Women with Threatened Preterm Labor." PloS One 9 (5): e96901. doi:10.1371/journal.pone.0096901.
Hever, Aniko, Richard B Roth, Peter Hevezi, Maria E Marin, Jose A Acosta, Hector Acosta, Jose Rojas, et al. 2007. "Human Endometriosis Is Associated with Plasma Cells and Overexpression of B Lymphocyte Stimulator." Proceedings of the National Academy of Sciences of the United States of America 104 (30): 12451–56. doi:10.1073/pnas.0703451104.
Hickey, C A, S P Cliver, S F McNeal, and R L Goldenberg. 1997. "Low Pregravid Body Mass Index as a Risk Factor for Preterm Birth: Variation by Ethnic Group." Obstet Gynecol 89 (2): 206–12. doi:S0029-7844(96)00486-3 [pii]10.1016/S0029-7844(96)00486-3 [doi].
Hillier, S L, S S Witkin, M A Krohn, D H Watts, N B Kiviat, and D A Eschenbach. 1993. "The Relationship of Amniotic Fluid Cytokines and Preterm Delivery, Amniotic Fluid Infection, Histologic Chorioamnionitis, and Chorioamnion Infection." Obstet Gynecol 81 (6): 941–48.
Hinton, A C, P L Grigsby, B A Pitzer, D E Brockman, R F Ittenbach, R B Hinton, and L Myatt. 2010. "Hormonal Regulation of Prostaglandin E2 Receptors: Localization and Expression in Rat Cervical Tissue." Reprod Sci 17 (2): 136–46. doi:1933719109348068 [pii]10.1177/1933719109348068 [doi].
Hirota, Y, J Cha, M Yoshie, T Daikoku, and S K Dey. 2011. "Heightened Uterine Mammalian Target of Rapamycin Complex 1 (mTORC1) Signaling Provokes Preterm Birth in Mice." Proc Natl Acad Sci U S A 108 (44): 18073–78. doi:1108180108 [pii]10.1073/pnas.1108180108.
Hirota, Yasushi, Takiko Daikoku, Susanne Tranguch, Huirong Xie, Heather B Bradshaw, and Sudhansu K Dey. 2010. "Uterine-Specific p53 Deficiency Confers Premature Uterine Senescence and Promotes Preterm Birth in Mice." The Journal of Clinical Investigation 120 (3): 803–15. doi:10.1172/JCI40051.

Hirsch, E, Y Filipovich, and M Mahendroo. 2006. "Signaling via the Type I IL-1 and TNF Receptors Is Necessary for Bacterially Induced Preterm Labor in a Murine Model." Am J Obstet Gynecol 194 (5): 1334–40. doi:S0002-9378(05)02449-X [pii]10.1016/j.ajog.2005.11.004.

Ho, Amy Wing Yin, Chun Kwok Wong, and Christopher Wai Kei Lam. 2008. "Tumor Necrosis Factor-Alpha up-Regulates the Expression of CCL2 and Adhesion Molecules of Human Proximal Tubular Epithelial Cells through MAPK Signaling Pathways." Immunobiology 213 (7): 533–44. doi:10.1016/j.imbio.2008.01.003.

Ho, M, O M Faye-Petersen, R L Goldenberg, W A Carlo, S P Cliver, and W W Andrews. 2012. "Elevated Midtrimester Alpha-Fetoprotein and Delivery Markers of Inflammation in a Preterm Population." J Matern Fetal Neonatal Med 25 (11): 2424–27. doi:10.3109/14767058.2012.700342.

Horng, T, G M Barton, and R Medzhitov. 2001. "TIRAP: An Adapter Molecule in the Toll Signaling Pathway." Nature Immunology 2 (9): 835–41. doi:10.1038/ni0901-835.

Hsieh, Vivian Chia-Rong, Shwn-Huey Shieh, Chiu-Ying Chen, Saou-Hsing Liou, Yu-Chen Hsiao, and Trong-Neng Wu. 2015. "Does Social Health Insurance Close the Gap: The Case of Socioeconomic Status and Preterm Low-Birth-Weight Survival." Asia-Pacific Journal of Public Health / Asia-Pacific Academic Consortium for Public Health 27 (5): 497–508. doi:10.1177/1010539515583503.

Hu, Shi-Jun, Gang Ren, Ji-Long Liu, Zhen-Ao Zhao, Yong-Sheng Yu, Ren-Wei Su, Xing-Hong Ma, Hua Ni, Wei Lei, and Zeng-Ming Yang. 2008. "MicroRNA Expression and Regulation in Mouse Uterus during Embryo Implantation." The Journal of Biological Chemistry 283 (34): 23473–84. doi:10.1074/jbc.M800406200.

Hua, Renyi, James E Pease, Weiwei Cheng, Suren R Sooranna, Jonathan M Viney, Scott M Nelson, Les Myatt, Philip R Bennett, and Mark R Johnson. 2013. "Human Labour Is Associated with a Decline in Myometrial Chemokine Receptor Expression: The Role of Prostaglandins, Oxytocin and Cytokines." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 69 (1): 21–32. doi:10.1111/aji.12025.

Hua, Renyi, James E Pease, Suren R Sooranna, Jonathan M Viney, Scott M Nelson, Les Myatt, Philip R Bennett, and Mark R Johnson. 2012. "Stretch and Inflammatory Cytokines Drive Myometrial Chemokine Expression via NF-kappaB Activation." Endocrinology 153 (1): 481–91. doi:en.2011-1506 [pii]10.1210/en.2011-1506.

Hubinont, C, and F Debieve. 2011. "Prevention of Preterm Labour: 2011 Update on Tocolysis." J Pregnancy 2011: 941057. doi:10.1155/2011/941057.

Igarashi, Toshio M, Kaylon L Bruner-Tran, Grant R Yeaman, Bruce A Lessey, Dean P Edwards, Esther Eisenberg, and Kevin G Osteen. 2005. "Reduced Expression of Progesterone Receptor-B in the Endometrium of Women with Endometriosis and in Cocultures of Endometrial Cells Exposed to 2,3,7,8-Tetrachlorodibenzo-P-Dioxin." Fertility and Sterility 84 (1): 67–74. doi:10.1016/j.fertnstert.2005.01.113.

Ingemarsson, I. 2003. "Gender Aspects of Preterm Birth." BJOG 110 Suppl : 34–38. doi:S1470032803000223 [pii].

Irizarry, Rafael A, Benjamin M Bolstad, Francois Collin, Leslie M Cope, Bridget Hobbs, and Terence P Speed. 2003. "Summaries of Affymetrix GeneChip Probe Level Data." Nucleic Acids Research 31 (4): e15.

Ishihara, O, H Numari, M Saitoh, Y Arai, H Takanashi, H Kitagawa, and K Kinoshita. 1996. "Prostaglandin E2 Production by Endogenous Secretion of Interleukin-1 in Decidual Cells Obtained before and after the Labor." Prostaglandins 52 (3): 199–208.

Isnardi, Isabelle, Yen-Shing Ng, Iva Srdanovic, Roja Motaghedi, Sergei Rudchenko, Horst von Bernuth, Shen-Ying Zhang, et al. 2008. "IRAK-4- and MyD88-Dependent Pathways Are Essential for the Removal of Developing Autoreactive B Cells in Humans." Immunity 29 (5): 746–57. doi:10.1016/j.immuni.2008.09.015.

Ivaska, Johanna, Hanna-Mari Pallari, Jonna Nevo, and John E Eriksson. 2007. "Novel Functions of Vimentin in Cell Adhesion, Migration, and Signaling." Experimental Cell Research 313 (10): 2050–62. doi:10.1016/j.yexcr.2007.03.040.

Jenei, Veronika, Victoria Sherwood, Jillian Howlin, Rickard Linnskog, Annette Säfholm, Lena Axelsson, and Tommy Andersson. 2009. "A T-Butyloxycarbonyl-Modified Wnt5a-Derived Hexapeptide Functions as a Potent Antagonist of Wnt5a-Dependent Melanoma Cell Invasion." Proceedings of the National Academy of Sciences of the United States of America 106 (46): 19473–78. doi:10.1073/pnas.0909409106.

Jiang, C W, P M Sarrel, D C Lindsay, P A Poole-Wilson, and P Collins. 1991. "Endothelium-Independent Relaxation of Rabbit Coronary Artery by 17 Beta-Oestradiol *in vitro*." British Journal of Pharmacology 104 (4): 1033–37.

Jiang, Tony T, Vandana Chaturvedi, James M Ertelt, Jeremy M Kinder, Dayna R Clark, Amy M Valent, Lijun Xin, and Sing Sing Way. 2014. "Regulatory T Cells: New Keys for Further Unlocking the Enigma of Fetal Tolerance and Pregnancy Complications." Journal of Immunology (Baltimore, Md. : 1950) 192 (11): 4949–56. doi:10.4049/jimmunol.1400498.

Jørgensen, Jan Stener, Louise Katrine Kjær Weile, and Ronald Francis Lamont. 2014. "Preterm Labor: Current Tocolytic Options for the Treatment of Preterm Labor." Expert Opinion on Pharmacotherapy 15 (5): 585–88. doi:10.1517/14656566.2014.880110.

Jung, Jeenah, Aaron W Lifland, Eric J Alonas, Chiara Zurla, and Philip J Santangelo. 2013. "Characterization of mRNA-Cytoskeleton Interactions in Situ Using FMTRIP and Proximity Ligation." PloS One 8 (9): e74598. doi:10.1371/journal.pone.0074598.

Kalkhoven, E, S Wissink, P T van der Saag, and B van der Burg. 1996. "Negative Interaction between the RelA(p65) Subunit of NF-kappaB and the Progesterone Receptor." J Biol Chem 271 (11): 6217–24.

Kamat, S, P Veena, and R Rani. 2014. "Comparison of Nifedipine and Progesterone for Maintenance Tocolysis after Arrested Preterm Labour." Journal of Obstetrics and Gynaecology : The Journal of the Institute of Obstetrics and Gynaecology 34 (4): 322–25. doi:10.3109/01443615.2013.874407.

Kamphuis, Esmé I, Bouchra Koullali, Michel Hof, Christianne J M de Groot, Brenda M Kazemier, Sarah Robertson, Ben W J Mol, and Anita C Ravelli. 2015. "Fetal Gender of the First Born and the Recurrent Risk of Spontaneous Preterm Birth." American Journal of Perinatology, September. doi:10.1055/s-0035-1563716.

Kara, M, S Ozden, P Arioglu, and A Cetin. 1998. "The Significance of Amniotic Fluid Interleukin-6 Levels in Preterm Labour." The Australian & New Zealand Journal of Obstetrics & Gynaecology 38 (4): 403–6.

Kasama, T, Y Miwa, T Isozaki, T Odai, M Adachi, and S L Kunkel. 2005. "Neutrophil-Derived Cytokines: Potential Therapeutic Targets in Inflammation." Curr Drug Targets Inflamm Allergy 4 (3): 273–79.

Kastner, P, A Krust, B Turcotte, U Stropp, L Tora, H Gronemeyer, and P Chambon. 1990. "Two Distinct Estrogen-Regulated Promoters Generate Transcripts Encoding the Two Functionally Different Human Progesterone Receptor Forms A and B." The EMBO Journal 9 (5): 1603–14.

Katoh, Masuko, and Masaru Katoh. 2009. "Transcriptional Mechanisms of WNT5A Based on NF-kappaB, Hedgehog, TGFbeta, and Notch Signaling Cascades." International Journal of Molecular Medicine 23 (6): 763–69.

Katz, V L, and R M Farmer. 1999. "Controversies in Tocolytic Therapy." Clinical Obstetrics and Gynecology 42 (4): 802–19.

Kayisli, Umit A, Neal G Mahutte, and Aydin Arici. 2002. "Uterine Chemokines in Reproductive Physiology and Pathology." Am J Reprod Immunol 47 (4): 213–21.

Keelan, J A, M Blumenstein, R J A Helliwell, T A Sato, K W Marvin, and M D Mitchell. 2003. "Cytokines, Prostaglandins and Parturition--a Review." Placenta 24 Suppl A (April): S33–46. doi:S0143400402909483 [pii].

Keelan, J A, K W Marvin, T A Sato, M Coleman, L M McCowan, and M D Mitchell. 1999. "Cytokine Abundance in Placental Tissues: Evidence of Inflammatory Activation in Gestational Membranes with Term and Preterm Parturition." American Journal of Obstetrics and Gynecology 181 (6): 1530–36.

Kendziorski, C, R A Irizarry, K-S Chen, J D Haag, and M N Gould. 2005. "On the Utility of Pooling Biological Samples in Microarray Experiments." Proceedings of the National Academy of Sciences of the United States of America 102 (12): 4252–57. doi:10.1073/pnas.0500607102.

Kenyon, S, P Brocklehurst, D Jones, N Marlow, A Salt, and D Taylor. 2008. "MRC ORACLE Children Study. Long Term Outcomes Following Prescription of Antibiotics to Pregnant Women with Either Spontaneous Preterm Labour or Preterm Rupture of the Membranes." BMC Pregnancy Childbirth 8: 14. doi:1471-2393-8-14 [pii]10.1186/1471-2393-8-14.

Kenyon, S L, D J Taylor, and W Tarnow-Mordi. 2001. "Broad-Spectrum Antibiotics for Spontaneous Preterm Labour: The ORACLE II Randomised Trial. ORACLE Collaborative Group." Lancet 357 (9261): 989–94. doi:S0140673600042343 [pii].

Khan, K A, S Petrou, M Dritsaki, S J Johnson, B Manktelow, E S Draper, L K Smith, et al. 2015. "Economic Costs Associated with Moderate and Late Preterm Birth: A Prospective Population-Based Study." BJOG : An International Journal of Obstetrics and Gynaecology 122 (11): 1495–1505. doi:10.1111/1471-0528.13515.

Khanam, T, and G Burnstock. 2007. "Changes in Expression of P2X1 Receptors and Connexin 43 in the Rat Myometrium during Pregnancy." Fertil Steril 88 (4 Suppl): 1174–79. doi:S0015-0282(07)00239-7 [pii]10.1016/j.fertnstert.2007.01.130 [doi].

Khanjani, Shirin, Mandeep K Kandola, Tamsin M Lindstrom, Suren R Sooranna, Manuela Melchionda, Yun S Lee, Vasso Terzidou, Mark R Johnson, and Phillip R Bennett. 2011. "NF-κB Regulates a Cassette of Immune/inflammatory Genes in Human Pregnant Myometrium at Term." Journal of Cellular and Molecular Medicine 15 (4): 809–24. doi:10.1111/j.1582-4934.2010.01069.x.

Kilarski, W M, X Fu, T Backstrom, G M Roomans, and U Ulmsten. 1996. "Progesterone, Oestradiol and Oxytocin and Their *in vitro* Effect on Maintaining the Number of Gap Junction Plaques in Human Myometrium at Term." Acta Physiol Scand 157 (4): 461–69.

Kilarski, W M, J Hongpaisan, D Semik, and G M Roomans. 2000. "Effect of Progesterone and Oestradiol on Expression of connexin43 in Cultured Human Myometrium Cells." Folia Histochem Cytobiol 38 (1): 3–9.

Kim, Sung Hye, David A MacIntyre, Maria Firmino Da Silva, Andrew M Blanks, Yun S Lee, Steven Thornton, Phillip R Bennett, and Vasso Terzidou. 2015. "Oxytocin Activates NF-κB-Mediated Inflammatory Pathways in Human Gestational Tissues." Molecular and Cellular Endocrinology 403 (March): 64–77. doi:10.1016/j.mce.2014.11.008.

King, J A, H Huddart, and W G Staff. 1998. "Effect of Choline Ester Analogues, Noradrenaline and Nifedipine on Normal and Hypertrophied Human Urinary Bladder Detrusor Muscle." Gen Pharmacol 30 (1): 131–36. doi:S0306362397000323 [pii].

Klauser, Chad K, Christian M Briery, Rick W Martin, LeDon Langston, Everett F Magann, and John C Morrison. 2014. "A Comparison of Three Tocolytics for Preterm Labor: A Randomized Clinical Trial." The Journal of Maternal-Fetal & Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians 27 (8): 801–6. doi:10.3109/14767058.2013.847416.

Klemetti, R, M Gissler, M Niinimaki, and E Hemminki. 2012. "Birth Outcomes after Induced Abortion: A Nationwide Register-Based Study of First Births in Finland." Hum Reprod 27 (11): 3315–20. doi:des294 [pii]10.1093/humrep/des294 [doi].

Knox, Craig, Vivian Law, Timothy Jewison, Philip Liu, Son Ly, Alex Frolkis, Allison Pon, et al. 2011. "DrugBank 3.0: A Comprehensive Resource for 'Omics' Research on Drugs." Nucleic Acids Research 39 (Database issue): D1035–41. doi:10.1093/nar/gkq1126.

Kokubu, K, E Hondo, N Sakaguchi, E Sagara, and Y Kiso. 2005. "Differentiation and Elimination of Uterine Natural Killer Cells in Delayed Implantation and Parturition Mice." J Reprod Dev 51 (6): 773–76. doi:JST.JSTAGE/jrd/17033 [pii].

Komers, R, S Anderson, and M Epstein. 2001. "Renal and Cardiovascular Effects of Selective Cyclooxygenase-2 Inhibitors." Am J Kidney Dis 38 (6): 1145–57. doi:S0272-6386(01)90143-X [pii]10.1053/ajkd.2001.29203.

Krucker, T, G R Siggins, and S Halpain. 2000. "Dynamic Actin Filaments Are Required for Stable Long-Term Potentiation (LTP) in Area CA1 of the Hippocampus." Proceedings of the National Academy of Sciences of the United States of America 97 (12): 6856–61. doi:10.1073/pnas.100139797.

Kurdi, Ahmed M, Rateb A Mesleh, Malak M Al-Hakeem, Tareq Y Khashoggi, and Hani M Khalifa. 2004. "Multiple Pregnancy and Preterm Labor." Saudi Medical Journal 25 (5): 632–37.

Kyrklund-Blomberg, Nina B., and Sven Cnattingius. 1998. "Preterm Birth and Maternal Smoking: Risks Related to Gestational Age and Onset of Delivery." American Journal of Obstetrics and Gynecology 179 (4): 1051–55. doi:10.1016/S0002-9378(98)70214-5.

La Rocca, Claudia, Fortunata Carbone, Salvatore Longobardi, and Giuseppe Matarese. 2014. "The Immunology of Pregnancy: Regulatory T Cells Control Maternal Immune Tolerance toward the Fetus." Immunology Letters 162 (1 Pt A): 41–48. doi:10.1016/j.imlet.2014.06.013.

Langfelder, Peter, Rui Luo, Michael C Oldham, and Steve Horvath. 2011. "Is My Network Module Preserved and Reproducible?" PLoS Computational Biology 7 (1): e1001057. doi:10.1371/journal.pcbi.1001057.

Lappas, M, C Riley, R Lim, G Barker, G E Rice, R Menon, and M Permezel. 2011. "MAPK and AP-1 Proteins Are Increased in Term Pre-Labour Fetal Membranes Overlying the Cervix: Regulation of Enzymes Involved in the Degradation of Fetal Membranes." Placenta 32 (12): 1016–25. doi:S0143-4004(11)00456-5 [pii]10.1016/j.placenta.2011.09.011.

Lawn, J E, M G Gravett, T M Nunes, C E Rubens, and C Stanton. 2010. "Global Report on Preterm Birth and Stillbirth (1 of 7): Definitions, Description of the Burden and Opportunities to Improve Data." BMC Pregnancy Childbirth 10 Suppl 1: S1. doi:1471-2393-10-S1-S1 [pii]10.1186/1471-2393-10-S1-S1.

Leber, A, A Teles, and A C Zenclussen. 2010. "Regulatory T Cells and Their Role in Pregnancy." Am J Reprod Immunol 63 (6): 445–59. doi:10.1111/j.1600-0897.2010.00821.x.

Lee, Seung Mi, Roberto Romero, Jeong Woo Park, Sun Min Kim, Chan-Wook Park, Steven J Korzeniewski, Tinnakorn Chaiworapongsa, and Bo Hyun Yoon. 2012a. "The Clinical Significance of a Positive Amnisure Test in Women with Preterm Labor and Intact Membranes." The Journal of Maternal-Fetal & Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians 25 (9): 1690–98. doi:10.3109/14767058.2012.657279.

Lee, Yun, Suren R Sooranna, Vasso Terzidou, Mark Christian, Jan Brosens, Kaisa Huhtinen, Matti Poutanen, Geraint Barton, Mark R Johnson, and Phillip R Bennett. 2012b. "Interactions between Inflammatory Signals and the Progesterone Receptor in Regulating Gene Expression in Pregnant Human Uterine Myocytes." Journal of Cellular and Molecular Medicine 16 (10): 2487–2503. doi:10.1111/j.1582-4934.2012.01567.x.

Lei, K, L Chen, B J Cryar, R Hua, S R Sooranna, J J Brosens, P R Bennett, and M R Johnson. 2011. "Uterine Stretch and Progesterone Action." J Clin Endocrinol Metab 96 (6): E1013–24. doi:jc.2010-2310 [pii]10.1210/jc.2010-2310 [doi].

Lei, Kaiyu, Li Chen, Ektoras X Georgiou, Suren R Sooranna, Shirin Khanjani, Jan J Brosens, Phillip R Bennett, and Mark R Johnson. 2012. "Progesterone Acts via the Nuclear Glucocorticoid Receptor to Suppress IL-1β-Induced COX-2 Expression in Human Term Myometrial Cells." PloS One 7 (11): e50167. doi:10.1371/journal.pone.0050167.

Leitich, H, C Egarter, A Kaider, M Hohlagschwandtner, P Berghammer, and P Husslein. 1999. "Cervicovaginal Fetal Fibronectin as a Marker for Preterm Delivery: A Meta-Analysis." Am J Obstet Gynecol 180 (5): 1169–76. doi:S0002937899002707 [pii].

Leitner, Kirstin, Mofeedah Al Shammary, Michael McLane, Michael V Johnston, Michal A Elovitz, and Irina Burd. 2014. "IL-1 Receptor Blockade Prevents Fetal Cortical Brain Injury but Not Preterm Birth in a Mouse Model of Inflammation-Induced Preterm Birth and Perinatal Brain Injury." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 71 (5): 418–26. doi:10.1111/aji.12216.

Lembet, A, D Eroglu, T Ergin, E Kuscu, H Zeyneloglu, S Batioglu, and A Haberal. 2002. "New Rapid Bed-Side Test to Predict Preterm Delivery: Phosphorylated Insulin-like Growth Factor Binding Protein-1 in Cervical Secretions." Acta Obstet Gynecol Scand 81 (8): 706–12. doi:aog810804 [pii].

Leveno, K J, B B Little, and F G Cunningham. 1990. "The National Impact of Ritodrine Hydrochloride for Inhibition of Preterm Labor." Obstetrics and Gynecology 76 (1): 12–15.

Li, Bei, Yuqiang Shi, Jianhong Shu, Junling Gao, Ping Wu, and Shao-Jun Tang. 2013. "Wingless-Type Mammary Tumor Virus Integration Site Family, Member 5A (Wnt5a) Regulates Human Immunodeficiency Virus Type 1 (HIV-1) Envelope Glycoprotein 120 (gp120)-Induced Expression of pro-Inflammatory Cytokines via the Ca2+/calmodulin-Dependent Protein." The Journal of Biological Chemistry 288 (19): 13610–19. doi:10.1074/jbc.M112.381046.

Li, C, and W H Wong. 2001. "Model-Based Analysis of Oligonucleotide Arrays: Expression Index Computation and Outlier Detection." Proceedings of the National Academy of Sciences of the United States of America 98 (1): 31–36. doi:10.1073/pnas.011404098.

Li, Li-Ping, Yi-Chuan Fang, Guo-Fa Dong, Yi Lin, and Shigeru Saito. 2012. "Depletion of Invariant NKT Cells Reduces Inflammation-Induced Preterm Delivery in Mice." Journal of Immunology (Baltimore, Md. : 1950) 188 (9): 4681–89. doi:10.4049/jimmunol.1102628.

Li, Ruth, William E Ackerman, Taryn L Summerfield, Lianbo Yu, Parul Gulati, Jie Zhang, Kun Huang, Roberto Romero, and Douglas A Kniss. 2011. "Inflammatory Gene Regulatory Networks in Amnion Cells Following Cytokine Stimulation: Translational Systems Approach to Modeling Human Parturition." PloS One 6 (6): e20560. doi:10.1371/journal.pone.0020560.

Likis, F E, D R Edwards, J C Andrews, A L Woodworth, R N Jerome, C J Fonnesbeck, J N McKoy, and K E Hartmann. 2012. "Progestogens for Preterm Birth Prevention: A Systematic Review and Meta-Analysis." Obstet Gynecol 120 (4): 897–907. doi:10.1097/AOG.0b013e3182699a15.

Lim, H, B C Paria, S K Das, J E Dinchuk, R Langenbach, J M Trzaskos, and S K Dey. 1997. "Multiple Female Reproductive Failures in Cyclooxygenase 2-Deficient Mice." Cell 91 (2): 197–208. doi:S0092-8674(00)80402-X [pii].

Lim, Ratana, Gillian Barker, and Martha Lappas. 2014. "The TLR2 Ligand FSL-1 and the TLR5 Ligand Flagellin Mediate pro-Inflammatory and pro-Labour Response via MyD88/TRAF6/NF-κB-Dependent Signalling." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 71 (5): 401–17. doi:10.1111/aji.12229.

Lim, Sheri, David A MacIntyre, Yun S Lee, Shirin Khanjani, Vasso Terzidou, T G Teoh, and Phillip R Bennett. 2012. "Nuclear Factor Kappa B Activation Occurs in the Amnion prior to Labour Onset and Modulates the Expression of Numerous Labour Associated Genes." PloS One 7 (4): e34707. doi:10.1371/journal.pone.0034707.

Lin, Y, M Xie, Y Chen, J Di, and Y Zeng. 2006. "Preterm Delivery Induced by LPS in Syngeneically Impregnated BALB/c and NOD/SCID Mice." J Reprod Immunol 71 (2): 87–101. doi:S0165-0378(06)00012-X [pii]10.1016/j.jri.2006.01.005.

Lipchina, Inna, Yechiel Elkabetz, Markus Hafner, Robert Sheridan, Aleksandra Mihailovic, Thomas Tuschl, Chris Sander, Lorenz Studer, and Doron Betel. 2011. "Genome-Wide Identification of microRNA Targets in Human ES Cells Reveals a Role for miR-302 in Modulating BMP Response." Genes & Development 25 (20): 2173–86. doi:10.1101/gad.17221311.

Liu, Xuejun, Marta Milo, Neil D Lawrence, and Magnus Rattray. 2005. "A Tractable Probabilistic Model for Affymetrix Probe-Level Analysis across Multiple Chips." Bioinformatics (Oxford, England) 21 (18): 3637–44. doi:10.1093/bioinformatics/bti583.

Liu, Xuejun, Marta Milo, Neil D Lawrence, and Magnus Rattray. 2006. "Probe-Level Measurement Error Improves Accuracy in Detecting Differential Gene Expression." Bioinformatics (Oxford, England) 22 (17): 2107–13. doi:10.1093/bioinformatics/btl361.

Lockwood, C J, U A Kayisli, C Stocco, W Murk, E Vatandaslar, L F Buchwalder, and F Schatz. 2012. "Abruption-Induced Preterm Delivery Is Associated with Thrombin-Mediated Functional Progesterone Withdrawal in Decidual Cells." Am J Pathol. doi:S0002-9440(12)00678-5 [pii]10.1016/j.ajpath.2012.08.036.

Lockwood, C J, P Toti, F Arcuri, M Paidas, L Buchwalder, G Krikun, and F Schatz. 2005. "Mechanisms of Abruption-Induced Premature Rupture of the Fetal Membranes: Thrombin-Enhanced Interleukin-8 Expression in Term Decidua." Am J Pathol 167 (5): 1443–49. doi:S0002-9440(10)61230-8 [pii]10.1016/S0002-9440(10)61230-8 [doi].

Lopes, P, O Besse, P Sagot, F Dantal, P de Morel, N Panel, and M F Lerat. 1991. "[The value of the administration of prostaglandin E2 on the biodegradable support of the maturation of the cervix uteri and the induction of labor]." J Gynecol Obstet Biol Reprod (Paris) 20 (6): 827–32.

Loudon, J A Z, S R Sooranna, P R Bennett, and M R Johnson. 2004. "Mechanical Stretch of Human Uterine Smooth Muscle Cells Increases IL-8 mRNA Expression and Peptide Synthesis." Molecular Human Reproduction 10 (12): 895–99. doi:10.1093/molehr/gah112.

Lu, Michael C, and Belinda Chen. 2004. "Racial and Ethnic Disparities in Preterm Birth: The Role of Stressful Life Events." American Journal of Obstetrics and Gynecology 191 (3): 691–99. doi:10.1016/j.ajog.2004.04.018.

Lye, S J, B J Nicholson, M Mascarenhas, L MacKenzie, and T Petrocelli. 1993. "Increased Expression of Connexin-43 in the Rat Myometrium during Labor Is Associated with an Increase in the Plasma Estrogen:progesterone Ratio." Endocrinology 132 (6): 2380–86.

MacIntyre, David A, Yun S Lee, Roberta Migale, Bronwen R Herbert, Simon N Waddington, Donald Peebles, Henrik Hagberg, Mark R Johnson, and Phillip R Bennett. 2014. "Activator Protein 1 Is a Key Terminal Mediator of Inflammation-Induced Preterm Labor in Mice." FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 28 (5): 2358–68. doi:10.1096/fj.13-247783.

Mackay, Fabienne, and Pascal Schneider. 2009. "Cracking the BAFF Code." Nature Reviews Immunology 9 (7): 491–502. doi:10.1038/nri2572.

Mackenzie, A P, F Schatz, G Krikun, E F Funai, S Kadner, and C J Lockwood. 2004. "Mechanisms of Abruption-Induced Premature Rupture of the Fetal Membranes: Thrombin Enhanced Decidual Matrix Metalloproteinase-3 (stromelysin-1) Expression." Am J Obstet Gynecol 191 (6): 1996–2001. doi:S0002937804008671 [pii]10.1016/j.ajog.2004.08.003.

Mackler, A M, G Iezza, M R Akin, P McMillan, and S M Yellon. 1999. "Macrophage Trafficking in the Uterus and Cervix Precedes Parturition in the Mouse." Biol Reprod 61 (4): 879–83.

Makhseed, M, R Raghupathy, S El-Shazly, F Azizieh, J A Al-Harmi, and M M Al-Azemi. 2003. "Pro-Inflammatory Maternal Cytokine Profile in Preterm Delivery." Am J Reprod Immunol 49 (5): 308–18.

Makino, S, D B Zaragoza, B F Mitchell, S Robertson, and D M Olson. 2007. "Prostaglandin F2alpha and Its Receptor as Activators of Human Decidua." Semin Reprod Med 25 (1): 60–68. doi:10.1055/s-2006-956776.

Makita, Naoyuki, Yoshiyuki Hizukuri, Kyoko Yamashiro, Masao Murakawa, and Yasuhiro Hayashi. 2015. "IL-10 Enhances the Phenotype of M2 Macrophages Induced by IL-4 and Confers the Ability to Increase Eosinophil Migration." International Immunology 27 (3): 131–41. doi:10.1093/intimm/dxu090.

Malinin, Nikolay L., Elzbieta Pluskota, and Tatiana V. Byzova. 2012. "Integrin Signaling in Vascular Function." Current Opinion in Hematology. doi:10.1097/MOH.0b013e3283523df0.

Mallick, Sudipta, and Joon Sig Choi. 2014. "Liposomes: Versatile and Biocompatible Nanovesicles for Efficient Biomolecules Delivery." Journal of Nanoscience and Nanotechnology 14 (1): 755–65.

Manabe, Y, A Manabe, and A Takahashi. 1982. "F Prostaglandin Levels in Amniotic Fluid during Balloon-Induced Cervical Softening and Labor at Term." Prostaglandins 23 (2): 247–56.

Manabe, Y, A Manabe, and A Takahashi. 1983. "Effect of Indomethacin on Stretch-Induced Uterine Activity in the Post-Partum." Prostaglandins 25 (5): 653–59.

Manabe, Y, N Sakaguchi, and A Nakajima. 1981. "Initiation of Uterine Contractions by Purely Mechanical Stretching of the Uterus at Midpregnancy." International Journal of Biological Research in Pregnancy.

Manaster, Irit, Saar Mizrahi, Debra Goldman-Wohl, Hen Y Sela, Noam Stern-Ginossar, Dikla Lankry, Raizy Gruda, et al. 2008. "Endometrial NK Cells Are Special Immature Cells That Await Pregnancy." J Immunol 181 (3): 1869–76. doi:181/3/1869 [pii].

Mangham, Lindsay J, Stavros Petrou, Lex W Doyle, Elizabeth S Draper, and Neil Marlow. 2009. "The Cost of Preterm Birth throughout Childhood in England and Wales." Pediatrics 123 (2): e312–27. doi:10.1542/peds.2008-1827.

Manuck, T A, H D Major, M W Varner, R Chettier, L Nelson, and M S Esplin. 2010. "Progesterone Receptor Genotype, Family History, and Spontaneous Preterm Birth." Obstet Gynecol 115 (4): 765–70. doi:10.1097/AOG.0b013e3181d53b8300006250-201004000-00015 [pii].

Martinez de Tejada, B, A Karolinski, M C Ocampo, C Laterra, I Hösli, D Fernández, D Surbek, et al. 2015. "Prevention of Preterm Delivery with Vaginal Progesterone in Women with Preterm Labour (4P): Randomised Double-Blind Placebo-Controlled Trial." BJOG : An International Journal of Obstetrics and Gynaecology 122 (1): 80–91. doi:10.1111/1471-0528.13061.

Marvin, K W, J A Keelan, R L Eykholt, T A Sato, and M D Mitchell. 2002. "Use of cDNA Arrays to Generate Differential Expression Profiles for Inflammatory Genes in Human Gestational Membranes Delivered at Term and Preterm." Mol Hum Reprod 8 (4): 399–408.

Masat, Elisa, Chiara Gasparini, Chiara Agostinis, Fleur Bossi, Oriano Radillo, Francesco De Seta, Nicola Tamassia, Marco A Cassatella, and Roberta Bulla. 2015. "RelB Activation in Anti-Inflammatory Decidual Endothelial Cells: A Master Plan to Avoid Pregnancy Failure?" Scientific Reports 5 (January): 14847. doi:10.1038/srep14847.

Mazor, M, R Hershkovitz, W Chaim, J Levy, Y Sharony, J R Leiberman, and M Glezerman. 1994. "Human Preterm Birth Is Associated with Systemic and Local Changes in progesterone/17 Beta-Estradiol Ratios." Am J Obstet Gynecol 171 (1): 231–36. doi:0002-9378(94)90474-X [pii].

McCall, M. N., B. M. Bolstad, and R. A. Irizarry. 2010. "Frozen Robust Multiarray Analysis (fRMA)." Biostatistics 11 (2): 242–53. doi:10.1093/biostatistics/kxp059.

McCall, Matthew N, Harris A Jaffee, Susan J Zelisko, Neeraj Sinha, Guido Hooiveld, Rafael A Irizarry, and Michael J Zilliox. 2014. "The Gene Expression Barcode 3.0: Improved Data Processing and Mining Tools." Nucleic Acids Research 42 (Database issue): D938–43. doi:10.1093/nar/gkt1204.

McCall, Matthew N, Karan Uppal, Harris A Jaffee, Michael J Zilliox, and Rafael A Irizarry. 2011. "The Gene Expression Barcode: Leveraging Public Data Repositories to Begin Cataloging the Human and Murine Transcriptomes." Nucleic Acids Research 39 (Database issue): D1011–15. doi:10.1093/nar/gkq1259.

McKillen, K, S Thornton, and C W Taylor. 1999. "Oxytocin Increases the [Ca2+]i Sensitivity of Human Myometrium during the Falling Phase of Phasic Contractions." Am J Physiol 276 (2 Pt 1): E345–51.

McLean, M, A Bisits, J Davies, W Walters, A Hackshaw, K De Voss, and R Smith. 1999. "Predicting Risk of Preterm Delivery by Second-Trimester Measurement of Maternal Plasma Corticotropin-Releasing Hormone and Alpha-Fetoprotein Concentrations." Am J Obstet Gynecol 181 (1): 207–15. doi:S000293789900441X [pii].

McLean, M, A Bisits, J Davies, R Woods, P Lowry, and R Smith. 1995. "A Placental Clock Controlling the Length of Human Pregnancy." Nat Med 1 (5): 460–63.

McNutt, C M, B J Nicholson, and S J Lye. 1994. "ACTH-Induced Preterm Labour in the Ewe Is Associated with Increased mRNA and Protein Levels of Myometrial Gap Junction Protein, Connexin-43." The Journal of Endocrinology 141 (2): 195–202.

Mehra, Suwan, Erol Amon, Sarah Hopkins, Jeffrey A. Gavard, and Jaye Shyken. 2015. "Transvaginal Cervical Length and Amniotic Fluid Index: Can It Predict Delivery Latency Following Preterm Premature Rupture of Membranes?" American Journal of Obstetrics and Gynecology 212 (3): 400.e1–400.e9. doi:10.1016/j.ajog.2015.01.022.

Meis, P J. 2005. "17 Hydroxyprogesterone for the Prevention of Preterm Delivery." Obstet Gynecol 105 (5 Pt 1): 1128–35. doi:105/5/1128 [pii]10.1097/01.AOG.0000160432.95395.8f.

Menon, Ramkumar. 2008. "Spontaneous Preterm Birth, a Clinical Dilemma: Etiologic, Pathophysiologic and Genetic Heterogeneities and Racial Disparity." Acta Obstetricia et Gynecologica Scandinavica 87 (6): 590–600. doi:10.1080/00016340802005126.

Menzies, F M, M C Shepherd, R J Nibbs, and S M Nelson. 2011. "The Role of Mast Cells and Their Mediators in Reproduction, Pregnancy and Labour." Hum Reprod Update 17 (3): 383–96. doi:dmq053 [pii]10.1093/humupd/dmq053.

Menzies, Fiona M, Claire A Higgins, Malcolm C Shepherd, Robert J B Nibbs, and Scott M Nelson. 2012. "Mast Cells Reside in Myometrium and Cervix, but Are Dispensable in Mice for Successful Pregnancy and Labor." Immunol Cell Biol 90 (3): 321–29. doi:10.1038/icb.2011.40.

Merlino, Amy A, Toni N Welsh, Huiqing Tan, Li Juan Yi, Vernon Cannon, Brian M Mercer, and Sam Mesiano. 2007. "Nuclear Progesterone Receptors in the Human Pregnancy Myometrium: Evidence That Parturition Involves Functional Progesterone Withdrawal Mediated by Increased Expression of Progesterone Receptor-A." The Journal of Clinical Endocrinology and Metabolism 92 (5): 1927–33. doi:10.1210/jc.2007-0077.

Mesiano, Sam, Eng-Cheng Chan, John T Fitter, Kenneth Kwek, George Yeo, and Roger Smith. 2002. "Progesterone Withdrawal and Estrogen Activation in Human Parturition Are Coordinated by Progesterone Receptor A Expression in the Myometrium." The Journal of Clinical Endocrinology and Metabolism 87 (6): 2924–30. doi:10.1210/jcem.87.6.8609.

Miller, David A., Janet A. Chollet, and T.Murphy Goodwin. 1997. "Clinical Risk Factors for Placenta Previa–placenta Accreta." American Journal of Obstetrics and Gynecology 177 (1): 210–14. doi:10.1016/S0002-9378(97)70463-0.

Min, K S, S Mukai, M Ohta, S Onosaka, and K Tanaka. 1992. "Glucocorticoid Inhibition of Inflammation-Induced Metallothionein Synthesis in Mouse Liver." Toxicology and Applied Pharmacology 113 (2): 293–98.

Mitchell, J A, and S J Lye. 2001. "Regulation of connexin43 Expression by c-Fos and c-Jun in Myometrial Cells." Cell Communication & Adhesion 8 (4-6): 299–302.

Mitchell, M D, R J Romero, S S Edwin, and M S Trautman. 1995. "Prostaglandins and Parturition." Reprod Fertil Dev 7 (3): 623–32.

Mittal, P, R Romero, A L Tarca, S Draghici, C L Nhan-Chang, T Chaiworapongsa, J Hotra, et al. 2011. "A Molecular Signature of an Arrest of Descent in Human Parturition." Am J Obstet Gynecol 204 (2): 177 e15–33. doi:10.1016/j.ajog.2010.09.025.

Mittal, Pooja, Roberto Romero, Adi L Tarca, Juan Gonzalez, Sorin Draghici, Yi Xu, Zhong Dong, et al. 2010. "Characterization of the Myometrial Transcriptome and Biological Pathways of Spontaneous Human Labor at Term." J Perinat Med 38 (6): 617–43. doi:10.1515/JPM.2010.097 [doi].

Montalbano, Alina P, Samuel Hawgood, and Carole R Mendelson. 2013. "Mice Deficient in Surfactant Protein A (SP-A) and SP-D or in TLR2 Manifest Delayed Parturition and Decreased Expression of Inflammatory and Contractile Genes." Endocrinology 154 (1): 483–98. doi:10.1210/en.2012-1797.

Montenegro, D, R Romero, S S Kim, A L Tarca, S Draghici, J P Kusanovic, J S Kim, et al. 2009. "Expression Patterns of microRNAs in the Chorioamniotic Membranes: A Role for microRNAs in Human Pregnancy and Parturition." J Pathol 217 (1): 113–21. doi:10.1002/path.2463.

Moreau, C, M Kaminski, P Y Ancel, J Bouyer, B Escande, G Thiriez, P Boulot, et al. 2005. "Previous Induced Abortions and the Risk of Very Preterm Delivery: Results of the EPIPAGE Study." BJOG 112 (4): 430–37. doi:BJO00478 [pii]10.1111/j.1471-0528.2004.00478.x.

Morgan, T K, J E Tolosa, L Mele, R J Wapner, C Y Spong, Y Sorokin, D J Dudley, et al. 2012. "Placental Villous Hypermaturation Is Associated with Idiopathic Preterm Birth." J Matern Fetal Neonatal Med. doi:10.3109/14767058.2012.746297.

Mor-Vaknin, Nirit, Antonello Punturieri, Kajal Sitwala, and David M Markovitz. 2003. "Vimentin Is Secreted by Activated Macrophages." Nature Cell Biology 5 (1). Nature Publishing Group: 59–63. doi:10.1038/ncb898.

Mosher, Andrea A, Kelly J Rainey, Mark A Giembycz, Stephen Wood, and Donna M Slater. 2012. "Prostaglandin E2 Represses Interleukin 1 Beta-Induced Inflammatory Mediator Output from Pregnant Human Myometrial Cells through the EP2 and EP4 Receptors." Biol Reprod 87 (1): 1–10,7. doi:biolreprod.112.100099 [pii]10.1095/biolreprod.112.100099 [doi].

Mosimann, Beatrice, Marion Wagner, Hassan Shehata, Leona C Y Poon, Brian Ford, Kypros H Nicolaides, and Amolak S Bansal. 2013. "Natural Killer Cells and Their Activation Status in Normal Pregnancy." International Journal of Reproductive Medicine 2013 (January): 906813. doi:10.1155/2013/906813.

Muhle, R A, P Pavlidis, W N Grundy, and E Hirsch. 2001. "A High-Throughput Study of Gene Expression in Preterm Labor with a Subtractive Microarray Approach." American Journal of Obstetrics and Gynecology 185 (3): 716–24. doi:10.1067/mob.2001.117183.

Mulhair, L, J Carter, L Poston, P Seed, and A Briley. 2009. "Prospective Cohort Study Investigating the Reliability of the AmnioSense Method for Detection of Spontaneous Rupture of Membranes." BJOG : An International Journal of Obstetrics and Gynaecology 116 (2): 313–18. doi:10.1111/j.1471-0528.2008.01828.x.

Murphy, Shaun P, Nazeeh N Hanna, Loren D Fast, Sunil K Shaw, Göran Berg, James F Padbury, Roberto Romero, and Surendra Sharma. 2009. "Evidence for Participation of Uterine Natural Killer Cells in the Mechanisms Responsible for Spontaneous Preterm Labor and Delivery." Am J Obstet Gynecol 200 (3): 308.e1–9. doi:S0002-9378(08)02055-3 [pii]10.1016/j.ajog.2008.10.043.

Nadeau-Vallée, Mathieu, Christiane Quiniou, Julia Palacios, Xin Hou, Atefeh Erfani, Ankush Madaan, Mélanie Sanchez, et al. 2015. "Novel Noncompetitive IL-1 Receptor-Biased Ligand Prevents Infection- and Inflammation-Induced Preterm Birth." Journal of Immunology (Baltimore, Md. : 1950) 195 (7): 3402–15. doi:10.4049/jimmunol.1500758.

Nagamatsu, T, and D J Schust. 2010. "The Immunomodulatory Roles of Macrophages at the Maternal-Fetal Interface." Reprod Sci 17 (3): 209–18. doi:1933719109349962 [pii]10.1177/1933719109349962.

Nam, Ka Hyun, Ja Young Kwon, Young-Han Kim, and Yong-Won Park. 2010. "Pregnancy Outcome after Cervical Conization: Risk Factors for Preterm Delivery and the Efficacy of Prophylactic Cerclage." Journal of Gynecologic Oncology 21 (4): 225–29. doi:10.3802/jgo.2010.21.4.225.

Nepusz, Tamás, Haiyuan Yu, and Alberto Paccanaro. 2012. "Detecting Overlapping Protein Complexes in Protein-Protein Interaction Networks." Nature Methods 9 (5): 471–72. doi:10.1038/nmeth.1938.

Ng, Beng Kwang, Pei Shan Lim, Mohamad Nasir Shafiee, Nur Azurah Abdul Ghani, Nor Azlin Mohamed Ismail, Mohd Hashim Omar, and Muhammad Abdul Jamil Muhammad Yassin. 2013. "Comparison between Amnisure Placental Alpha Microglobulin-1 Rapid Immunoassay and Standard Diagnostic Methods for Detection of Rupture of Membranes." BioMed Research International 2013 (January): 1–7. doi:10.1155/2013/587438.

Nieminen, Mikko, Tiina Henttinen, Marika Merinen, Fumiko Marttila–Ichihara, John E. Eriksson, Sirpa Jalkanen, Fumiko Marttila-Ichihara, John E. Eriksson, and Sirpa Jalkanen. 2006. "Vimentin Function in Lymphocyte Adhesion and Transcellular Migration." Nature Cell Biology 8 (2): 156–62. doi:10.1038/ncb1355.

Norman, J E, and A H Shennan. 2013. "Prevention of Preterm Birth--Why Can't We Do Any Better?" Lancet 381 (9862): 184–85. doi:10.1016/s0140-6736(12)61956-4.

Norman, Jane E, Andrew Shennan, Phillip Bennett, Steven Thornton, Stephen Robson, Neil Marlow, John Norrie, et al. 2012. "Trial Protocol OPPTIMUM- Does Progesterone Prophylaxis for the Prevention of Preterm Labour Improve Outcome?" BMC Pregnancy and Childbirth 12 (1): 79. doi:10.1186/1471-2393-12-79.

Norstrom, A. 1982. "Influence of Prostaglandin D2 on the Biosynthesis of Connective Tissue Constituents in the Pregnant Human Cervix." Prostaglandins 23 (3): 361–67.

Norwitz, E R, and A B Caughey. 2011. "Progesterone Supplementation and the Prevention of Preterm Birth." Rev Obstet Gynecol 4 (2): 60–72.

Norwitz, E R, P M Starkey, A Lopez Bernal, and A C Turnbull. 1991. "Identification by Flow Cytometry of the Prostaglandin-Producing Cell Populations of Term Human Decidua." J Endocrinol 131 (2): 327–34.

Novy, M J. 1977. "Endocrine and Pharmacological Factors Which Influence the Onset of Labour in Rhesus Monkeys." Ciba Found Symp, no. 47: 259–95.

O'Brien, John M, and David F Lewis. 2015. "Prevention of Preterm Birth with Vaginal Progesterone or 17-Alpha-Hydroxyprogesterone Caproate: A Critical Examination of Efficacy and Safety." American Journal of Obstetrics and Gynecology, November. doi:10.1016/j.ajog.2015.10.934.

O'Brien, Margaret, John J Morrison, and Terry J Smith. 2008. "Upregulation of PSCDBP, TLR2, TWIST1, FLJ35382, EDNRB, and RGS12 Gene Expression in Human Myometrium at Labor." Reproductive Sciences (Thousand Oaks, Calif.) 15 (4): 382–93. doi:10.1177/1933719108316179.

O'Brien, W F. 1995. "The Role of Prostaglandins in Labor and Delivery." Clinics in Perinatology 22 (4): 973–84.

Ochedalski, Tomasz, and Agnieszka Lachowicz. 2004. "Maternal and Fetal Hypothalamo-Pituitary-Adrenal Axis: Different Response Depends upon the Mode of Parturition." Neuro Endocrinology Letters 25 (4): 278–82.

Ochedalski, T, K Zylińska, T Laudański, and A Lachowicz. 2001. "Corticotrophin-Releasing Hormone and ACTH Levels in Maternal and Fetal Blood during Spontaneous and Oxytocin-Induced Labour." European Journal of Endocrinology / European Federation of Endocrine Societies 144 (2): 117–21.

Olson, D M, and C Ammann. 2007. "Role of the Prostaglandins in Labour and Prostaglandin Receptor Inhibitors in the Prevention of Preterm Labour." Front Biosci 12: 1329–43. doi:2151 [pii].

Olson, D M, D B Zaragoza, M C Shallow, J L Cook, B F Mitchell, P Grigsby, and J Hirst. 2003. "Myometrial Activation and Preterm Labour: Evidence Supporting a Role for the Prostaglandin F Receptor--a Review." Placenta 24 Suppl A (April): S47–54. doi:S0143400402909380 [pii].

Osman, Inass, Anne Young, Marie Anne Ledingham, Andrew J Thomson, Fiona Jordan, Ian A Greer, and Jane E Norman. 2003. "Leukocyte Density and pro-Inflammatory Cytokine Expression in Human Fetal Membranes, Decidua, Cervix and Myometrium before and during Labour at Term." Mol Hum Reprod 9 (1): 41–45.

Osmers, R G, J Bläser, W Kuhn, and H Tschesche. 1995. "Interleukin-8 Synthesis and the Onset of Labor." Obstetrics and Gynecology 86 (2): 223–29.

Owen, J, G Hankins, J D Iams, V Berghella, J S Sheffield, A Perez-Delboy, R S Egerman, et al. 2009. "Multicenter Randomized Trial of Cerclage for Preterm Birth Prevention in High-Risk Women with Shortened Midtrimester Cervical Length." Am J Obstet Gynecol 201 (4): 375 e1–8. doi:S0002-9378(09)00940-5 [pii]10.1016/j.ajog.2009.08.015 [doi].

Padilla, L, K Reinicke, H Montesino, F Villena, H Asencio, M Cruz, and M I Rudolph. 1990. "Histamine Content and Mast Cells Distribution in Mouse Uterus: The Effect of Sexual Hormones, Gestation and Labor." Cell Mol Biol 36 (1): 93–100.

Palliser, H K, G T Ooi, J J Hirst, G Rice, N L Dellios, R M Escalona, and I R Young. 2004. "Changes in the Expression of Prostaglandin E and F Synthases at Induced and Spontaneous Labour Onset in the Sheep." J Endocrinol 180 (3): 469–77.

Panter, K R, M E Hannah, K S Amankwah, A Ohlsson, A L Jefferies, and D Farine. 1999. "The Effect of Indomethacin Tocolysis in Preterm Labour on Perinatal Outcome: A Randomised Placebo-Controlled Trial." Br J Obstet Gynaecol 106 (5): 467–73.

Park, Jong Sung, Fabia Gamboni-Robertson, Qianbin He, Daiva Svetkauskaite, Jae-Yeol Kim, Derek Strassheim, Jang-Won Sohn, et al. 2006. "High Mobility Group Box 1 Protein Interacts with Multiple Toll-like Receptors." American Journal of Physiology. Cell Physiology 290 (3): C917–24. doi:10.1152/ajpcell.00401.2005.

Paul, Jonathan, Susan Hua, and Roger Smith. 2015. "A Targeted Drug Delivery System for the Uterus." Reproductive Sciences 22 (1).

Peacock, J L, J M Bland, and H R Anderson. 1995. "Preterm Delivery: Effects of Socioeconomic Factors, Psychological Stress, Smoking, Alcohol, and Caffeine." BMJ 311 (7004): 531–35.

Pearson, Richard D, Xuejun Liu, Guido Sanguinetti, Marta Milo, Neil D Lawrence, and Magnus Rattray. 2009. "Puma: A Bioconductor Package for Propagating Uncertainty in Microarray Analysis." BMC Bioinformatics 10 (January): 211. doi:10.1186/1471-2105-10-211.

Peng, Hsiu-Huei, Chuan-Chi Kao, Shuenn-Dyh Chang, An-Shine Chao, Yao-Lung Chang, Chao-Nin Wang, Po-Jen Cheng, Yun-Shien Lee, Tzu-Hao Wang, and Hsin-Shih Wang. 2011. "The Effects of Labor on Differential Gene Expression in Parturient Women, Placentas, and Fetuses at Term Pregnancy." The Kaohsiung Journal of Medical Sciences 27 (11): 494–502. doi:10.1016/j.kjms.2011.06.012.

Peng, Xuejun, Constance L Wood, Eric M Blalock, Kuey Chu Chen, Philip W Landfield, and Arnold J Stromberg. 2003. "Statistical Implications of Pooling RNA Samples for Microarray Experiments." BMC Bioinformatics 4 (June): 26. doi:10.1186/1471-2105-4-26.

Pennell, C E, J J Henderson, M J O'Neill, S McChlery, D A Doherty, and J E Dickinson. 2009. "Induction of Labour in Nulliparous Women with an Unfavourable Cervix: A Randomised Controlled Trial Comparing Double and Single Balloon Catheters and PGE2 Gel." BJOG 116 (11): 1443–52. doi:BJO2279 [pii]10.1111/j.1471-0528.2009.02279.x.

Pennell, Craig E, Bo Jacobsson, Scott M Williams, Rebecca M Buus, Louis J Muglia, Siobhan M Dolan, Nils-Halvdan Morken, Hilmi Ozcelik, Stephen J Lye, and Caroline Relton. 2007. "Genetic Epidemiologic Studies of Preterm Birth: Guidelines for Research." American Journal of Obstetrics and Gynecology 196 (2): 107–18. doi:10.1016/j.ajog.2006.03.109.

Penrod, L V, R E Allen, J L Turner, S W Limesand, and M J Arns. 2012. "Effects of Oxytocin, Lipopolysaccharide (LPS), and Polyunsaturated Fatty Acids on Prostaglandin Secretion and Gene Expression in Equine Endometrial Explant Cultures." Domest Anim Endocrinol. doi:S0739-7240(12)00138-5 [pii]10.1016/j.domaniend.2012.09.002 [doi].

Pepe, G J, and E D Albrecht. 1995. "Actions of Placental and Fetal Adrenal Steroid Hormones in Primate Pregnancy." Endocr Rev 16 (5): 608–48.

Perez, A, M D Gurbindo, S Resino, A Aguaron, and M A Munoz-Fernandez. 2007. "NK Cell Increase in Neonates from the Preterm to the Full-Term Period of Gestation." Neonatology 92 (3): 158–63. doi:000101567 [pii]10.1159/000101567.

Peterman, E. E., P. 2nd Taormina, M. Harvey, and L. H. Young. 2004. "Go 6983 Exerts Cardioprotective Effects in Myocardial Ischemia/Reperfusion." Journal of Cardiovascular Pharmacology 43 (5): 645–56.

Peterson, Cora, Scott D Grosse, Rui Li, Andrea J Sharma, Hilda Razzaghi, William H Herman, and Suzanne M Gilboa. 2015. "Preventable Health and Cost Burden of Adverse Birth Outcomes Associated with Pregestational Diabetes in the United States." American Journal of Obstetrics and Gynecology 212 (1): 74.e1–9. doi:10.1016/j.ajog.2014.09.009.

Petrocelli, T, and S J Lye. 1993. "Regulation of Transcripts Encoding the Myometrial Gap Junction Protein, Connexin-43, by Estrogen and Progesterone." Endocrinology 133 (1): 284–90.

Phillips, R J, H Al-Zamil, L P Hunt, M A Fortier, and A López Bernal. 2011. "Genes for Prostaglandin Synthesis, Transport and Inactivation Are Differentially Expressed in Human Uterine Tissues, and the Prostaglandin F Synthase AKR1B1 Is Induced in Myometrial Cells by Inflammatory Cytokines." Molecular Human Reproduction 17 (1): 1–13. doi:10.1093/molehr/gaq057.

Phillips, Robert J, Michel A Fortier, and Andrés López Bernal. 2014. "Prostaglandin Pathway Gene Expression in Human Placenta, Amnion and Choriodecidua Is Differentially Affected by Preterm and Term Labour and by Uterine Inflammation." BMC Pregnancy and Childbirth 14 (1): 241. doi:10.1186/1471-2393-14-241.

Piccinni, M P. 2005. "T Cells in Pregnancy." Chem Immunol Allergy 89: 3–9. doi:87904 [pii]10.1159/000087904 [doi].

Poligone, B, and A S Baldwin. 2001. "Positive and Negative Regulation of NF-kappaB by COX-2: Roles of Different Prostaglandins." The Journal of Biological Chemistry 276 (42): 38658–64. doi:10.1074/jbc.M106599200.

Powers JP, S Li, Jaen JC, Liu J, Walker NP, Z Wang, and Wesche H. 2006. "Discovery and Initial SAR of Inhibitors of Interleukin-1 Receptor-Associated Kinase-4." Bioorganic & Medicinal Chemistry Letters 16 (11): 2842–45. doi:10.1016/j.bmcl.2006.03.020.

Rawdanowicz, T J, A L Hampton, H Nagase, D E Woolley, and L A Salamonsen. 1994. "Matrix Metalloproteinase Production by Cultured Human Endometrial Stromal Cells: Identification of Interstitial Collagenase, Gelatinase-A, Gelatinase-B, and Stromelysin-1 and Their Differential Regulation by Interleukin-1 Alpha and Tumor Necrosis Factor-A." The Journal of Clinical Endocrinology and Metabolism 79 (2): 530–36. doi:10.1210/jcem.79.2.8045973.

Re, F, and J L Strominger. 2001. "Toll-like Receptor 2 (TLR2) and TLR4 Differentially Activate Human Dendritic Cells." The Journal of Biological Chemistry 276 (40): 37692–99. doi:10.1074/jbc.M105927200.

Reece, M S, J A McGregor, K G Allen, M M Mathias, and M A Harris. 1996. "Prostaglandins in Selected Reproductive Tissues in Preterm and Full-Term Gestations." Prostaglandins Leukot Essent Fatty Acids 55 (5): 303–7. doi:S0952-3278(96)90035-6 [pii].

Reese, J, B C Paria, N Brown, X Zhao, J D Morrow, and S K Dey. 2000. "Coordinated Regulation of Fetal and Maternal Prostaglandins Directs Successful Birth and Postnatal Adaptation in the Mouse." Proc Natl Acad Sci U S A 97 (17): 9759–64. doi:97/17/9759 [pii].

Rehman, K S, S Yin, B A Mayhew, R A Word, and W E Rainey. 2003. "Human Myometrial Adaptation to Pregnancy: cDNA Microarray Gene Expression Profiling of Myometrium from Non-Pregnant and Pregnant Women." Mol Hum Reprod 9 (11): 681–700.

Renthal, N E, C C Chen, K C Williams, R D Gerard, J Prange-Kiel, and C R Mendelson. 2010. "miR-200 Family and Targets, ZEB1 and ZEB2, Modulate Uterine Quiescence and Contractility during Pregnancy and Labor." Proceedings of the National Academy of Sciences 107 (48): 20828–33. doi:10.1073/pnas.1008301107.

Rezapour, M, J Hongpaisan, X Fu, T Backstrom, G M Roomans, and U Ulmsten. 1996. "Effects of Progesterone and Oxytocin on Intracellular Elemental Composition of Term Human Myometrium *in vitro*." Eur J Obstet Gynecol Reprod Biol 68 (1-2): 191–97.

Rhyasen, Garrett W, Lyndsey Bolanos, Jing Fang, Andres Jerez, Mark Wunderlich, Carmela Rigolino, Lesley Mathews, et al. 2013. "Targeting IRAK1 as a Therapeutic Approach for Myelodysplastic Syndrome." Cancer Cell 24 (1): 90–104. doi:10.1016/j.ccr.2013.05.006.

Riley, S C, R Leask, F C Denison, K Wisely, A A Calder, and D C Howe. 1999. "Secretion of Tissue Inhibitors of Matrix Metalloproteinases by Human Fetal Membranes, Decidua and Placenta at Parturition." J Endocrinol 162 (3): 351–59. doi:JOE3366 [pii].

Robertson, Sarah A, Inge Christiaens, Camilla L Dorian, Dean B Zaragoza, Alison S Care, Anke M Banks, and David M Olson. 2010. "Interleukin-6 Is an Essential Determinant of on-Time Parturition in the Mouse." Endocrinology 151 (8): 3996–4006. doi:en.2010-0063 [pii]10.1210/en.2010-0063.

Robertson, S A, R J Skinner, and A S Care. 2006. "Essential Role for IL-10 in Resistance to Lipopolysaccharide-Induced Preterm Labor in Mice." J Immunol 177 (7): 4888–96. doi:177/7/4888 [pii].

Rodewald, Hans-Reimer, and Thorsten B Feyerabend. 2012. "Widespread Immunological Functions of Mast Cells: Fact or Fiction?" Immunity 37 (1): 13–24. doi:10.1016/j.immuni.2012.07.007.

Roh, C R, W J Oh, B K Yoon, and J H Lee. 2000. "Up-Regulation of Matrix Metalloproteinase-9 in Human Myometrium during Labour: A Cytokine-Mediated Process in Uterine Smooth Muscle Cells." Mol Hum Reprod 6 (1): 96–102.

Romero, R, J Espinoza, J P Kusanovic, F Gotsch, S Hassan, O Erez, T Chaiworapongsa, and M Mazor. 2006. "The Preterm Parturition Syndrome." BJOG 113 Suppl : 17–42. doi:BJO1120 [pii]10.1111/j.1471-0528.2006.01120.x.

Romero, R, M Mazor, and B Tartakovsky. 1991. "Systemic Administration of Interleukin-1 Induces Preterm Parturition in Mice." Am J Obstet Gynecol 165 (4 Pt 1): 969–71.

Romero, R, K Nicolaides, A Conde-Agudelo, A Tabor, J M O'Brien, E Cetingoz, E Da Fonseca, et al. 2012. "Vaginal Progesterone in Women with an Asymptomatic Sonographic Short Cervix in the Midtrimester Decreases Preterm Delivery and Neonatal Morbidity: A Systematic Review and Metaanalysis of Individual Patient Data." Am J Obstet Gynecol 206 (2): 124 e1–19. doi:S0002-9378(11)02358-1 [pii]10.1016/j.ajog.2011.12.003.

Romero, R, W Sepulveda, M Mazor, F Brandt, D B Cotton, C A Dinarello, and M D Mitchell. 1992. "The Natural Interleukin-1 Receptor Antagonist in Term and Preterm Parturition." American Journal of Obstetrics and Gynecology 167 (4 Pt 1): 863–72.

Romero, Roberto, Lami Yeo, Piya Chaemsaithong, Tinnakorn Chaiworapongsa, and Sonia S Hassan. 2014. "Progesterone to Prevent Spontaneous Preterm Birth." Seminars in Fetal & Neonatal Medicine 19 (1): 15–26. doi:10.1016/j.siny.2013.10.004.

Roos, C, E Borowiack, M Kowalska, A Zapalska, Bw Mol, L Mignini, C Meads, J Walczak, and Ks Khan. 2013. "What Do We Know about Tocolytic Effectiveness and How Do We Use This Information in Guidelines? A Comparison of Evidence Grading." BJOG : An International Journal of Obstetrics and Gynaecology 120 (13): 1588–96; discussion 1597–98. doi:10.1111/1471-0528.12388.

Ruanphoo, P, and V Phupong. 2015. "Evaluation of the Performance of the Insulin-like Growth Factor-Binding Protein-1/alpha-Fetoprotein Test in Diagnosing Ruptured Fetal Membranes in Pregnant Women." Journal of Perinatology : Official Journal of the California Perinatal Association 35 (8). Nature America, Inc.: 558–60. doi:10.1038/jp.2015.6.

Rush, R W. 1979. "Incidence of Preterm Delivery in Patients with Previous Preterm Delivery And/or Abortion." S Afr Med J 56 (25): 1085–87.

Sadler, L C, T Davison, and L M McCowan. 2000. "A Randomised Controlled Trial and Meta-Analysis of Active Management of Labour." BJOG : An International Journal of Obstetrics and Gynaecology 107 (7): 909–15.

Sadowsky, Drew W, Kristina M Adams, Michael G Gravett, Steven S Witkin, and Miles J Novy. 2006. "Preterm Labor Is Induced by Intraamniotic Infusions of Interleukin-1beta and Tumor Necrosis Factor-Alpha but Not by Interleukin-6 or Interleukin-8 in a Nonhuman Primate Model." Am J Obstet Gynecol 195 (6): 1578–89. doi:S0002-9378(06)00831-3 [pii]10.1016/j.ajog.2006.06.072 [doi].

Saif, Z, N A Hodyl, M J Stark, P J Fuller, T Cole, N Lu, and V L Clifton. 2015. "Expression of Eight Glucocorticoid Receptor Isoforms in the Human Preterm Placenta Vary with Fetal Sex and Birthweight." Placenta 36 (7): 723–30. doi:10.1016/j.placenta.2015.05.001.

Saini, V, A Marchese, and M Majetschak. 2010. "CXC Chemokine Receptor 4 Is a Cell Surface Receptor for Extracellular Ubiquitin." J Biol Chem 285 (20): 15566–76. doi:M110.103408 [pii]10.1074/jbc.M110.103408.

Saito, S, T Kasahara, Y Kato, Y Ishihara, and M Ichijo. 1993. "Elevation of Amniotic Fluid Interleukin 6 (IL-6), IL-8 and Granulocyte Colony Stimulating Factor (G-CSF) in Term and Preterm Parturition." Cytokine 5 (1): 81–88. doi:1043-4666(93)90027-3 [pii].

Saito, Rintaro, Michael E Smoot, Keiichiro Ono, Johannes Ruscheinski, Peng-Liang Wang, Samad Lotia, Alexander R Pico, Gary D Bader, and Trey Ideker. 2012. "A Travel Guide to Cytoscape Plugins." Nature Methods 9 (11): 1069–76. doi:10.1038/nmeth.2212.

Sakai, N, T Tabb, and R E Garfield. 1992. "Studies of Connexin 43 and Cell-to-Cell Coupling in Cultured Human Uterine Smooth Muscle." Am J Obstet Gynecol 167 (5): 1267–77.

Sakurai, Takayuki, Ryo Takai, Heinrich Bürgin, Kana Ishihara, Yuichiro Sakamoto, Jun Amano, Yoshinobu Higuchi, et al. 2012. "The Effects of Interleukin-6 Signal Blockade on Fertility, Embryo-Fetal Development, and Immunization *In vivo*." Birth Defects Research Part B: Developmental and Reproductive Toxicology 95 (4): 304–17. doi:10.1002/bdrb.21019.

Salamonsen, L A, J Zhang, A Hampton, and L Lathbury. 2000. "Regulation of Matrix Metalloproteinases in Human Endometrium." Human Reproduction (Oxford, England) 15 Suppl 3 (August): 112–19.

Salomonis, Nathan, Nathalie Cotte, Alexander C Zambon, Katherine S Pollard, Karen Vranizan, Scott W Doniger, Gregory Dolganov, and Bruce R Conklin. 2005. "Identifying Genetic Networks Underlying Myometrial Transition to Labor." Genome Biology 6 (2): R12. doi:10.1186/gb-2005-6-2-r12.

Samatar, Ahmed A., and Poulikos I. Poulikakos. 2014. "Targeting RAS–ERK Signalling in Cancer: Promises and Challenges." Nature Reviews Drug Discovery 13 (12): 928–42. doi:10.1038/nrd4281.

Sauvé, Karine, Julie Lepage, Mélanie Sanchez, Nikolaus Heveker, and André Tremblay. 2009. "Positive Feedback Activation of Estrogen Receptors by the CXCL12-CXCR4 Pathway." Cancer Research 69 (14): 5793–5800. doi:10.1158/0008-5472.CAN-08-4924.

Schindler, U, and V R Baichwal. 1994. "Three NF-Kappa B Binding Sites in the Human E-Selectin Gene Required for Maximal Tumor Necrosis Factor Alpha-Induced Expression." Molecular and Cellular Biology 14 (9): 5820–31.

Schmidt-Weber, C B, S I Alexander, L E Henault, L James, and A H Lichtman. 1999. "IL-4 Enhances IL-10 Gene Expression in Murine Th2 Cells in the Absence of TCR Engagement." Journal of Immunology (Baltimore, Md. : 1950) 162 (1): 238–44.

Schmitz, T, E Dallot, M J Leroy, M Breuiller-Fouche, F Ferre, and D Cabrol. 2001. "EP(4) Receptors Mediate Prostaglandin E(2)-Stimulated Glycosaminoglycan Synthesis in Human Cervical Fibroblasts in Culture." Mol Hum Reprod 7 (4): 397–402.

Schroeder, Andreas, Odilo Mueller, Susanne Stocker, Ruediger Salowsky, Michael Leiber, Marcus Gassmann, Samar Lightfoot, Wolfram Menzel, Martin Granzow, and Thomas Ragg. 2006. "The RIN: An RNA Integrity Number for Assigning Integrity Values to RNA Measurements." BMC Molecular Biology 7 (January): 3. doi:10.1186/1471-2199-7-3.

Searle, R F, and C J Matthews. 1988. "Differential Expression of Class II Major Histocompatibility Complex and Thy 1.2 Antigens on Mouse Decidua." Placenta 9 (1): 57–64.

Seibert, K, and J L Masferrer. 1994. "Role of Inducible Cyclooxygenase (COX-2) in Inflammation." Receptor 4 (1): 17–23.

Senftleben, U, Y Cao, G Xiao, F R Greten, G Krähn, G Bonizzi, Y Chen, et al. 2001. "Activation by IKKalpha of a Second, Evolutionary Conserved, NF-Kappa B Signaling Pathway." Science (New York, N.Y.) 293 (5534): 1495–99. doi:10.1126/science.1062677.

Sennstrom, M B, G Ekman, G Westergren-Thorsson, A Malmstrom, B Bystrom, U Endresen, N Mlambo, et al. 2000. "Human Cervical Ripening, an Inflammatory Process Mediated by Cytokines." Mol Hum Reprod 6 (4): 375–81.

Shankar, R, M P Johnson, N A Williamson, F Cullinane, A W Purcell, E K Moses, and S P Brennecke. 2010. "Molecular Markers of Preterm Labor in the Choriodecidua." Reprod Sci 17 (3): 297–310. doi:1933719109353454 [pii]10.1177/1933719109353454.

Shannon, Paul, Andrew Markiel, Owen Ozier, Nitin S Baliga, Jonathan T Wang, Daniel Ramage, Nada Amin, Benno Schwikowski, and Trey Ideker. 2003. "Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks." Genome Research 13 (11): 2498–2504. doi:10.1101/gr.1239303.

Sharp, Gemma C, Hongwu Ma, Philippa T K Saunders, and Jane E Norman. 2013. "A Computational Model of Lipopolysaccharide-Induced Nuclear Factor Kappa B Activation: A Key Signalling Pathway in Infection-Induced Preterm Labour." PloS One 8 (7): e70180. doi:10.1371/journal.pone.0070180.

Shynlova, O, M Chow, and S J Lye. 2009. "Expression and Organization of Basement Membranes and Focal Adhesion Proteins in Pregnant Myometrium Is Regulated by Uterine Stretch." Reprod Sci 16 (10): 960–69. doi:1933719109338220 [pii]10.1177/1933719109338220.

Shynlova, Oksana, Anna Dorogin, Yunqing Li, and Stephen Lye. 2014. "Inhibition of Infection-Mediated Preterm Birth by Administration of Broad Spectrum Chemokine Inhibitor in Mice." Journal of Cellular and Molecular Medicine 18 (9): 1816–29. doi:10.1111/jcmm.12307.

Shynlova, Oksana, Yu-Hui H Lee, Khetsopon Srikhajon, and Stephen J Lye. 2013a. "Physiologic Uterine Inflammation and Labor Onset: Integration of Endocrine and Mechanical Signals." Reproductive Sciences (Thousand Oaks, Calif.) 20 (2): 154–67. doi:10.1177/1933719112446084.

Shynlova, Oksana, Tamara Nedd-Roderique, Yunqing Li, Anna Dorogin, and Stephen J Lye. 2013b. "Myometrial Immune Cells Contribute to Term Parturition, Preterm Labour and Post-Partum Involution in Mice." Journal of Cellular and Molecular Medicine 17 (1): 90–102. doi:10.1111/j.1582-4934.2012.01650.x.

Shynlova, Oksana, Tamara Nedd-Roderique, Yunqing Li, Anna Dorogin, Tina Nguyen, and Stephen J. Lye. 2013c. "Infiltration of Myeloid Cells into Decidua Is a Critical Early Event in the Labour Cascade and Post-Partum Uterine Remodelling." Journal of Cellular and Molecular Medicine 17 (2): 311–24. doi:10.1111/jcmm.12012.

Shynlova, Oksana, Prudence Tsui, Anna Dorogin, and Stephen J Lye. 2008. "Monocyte Chemoattractant Protein-1 (CCL-2) Integrates Mechanical and Endocrine Signals That Mediate Term and Preterm Labor." J Immunol 181 (2): 1470–79. doi:181/2/1470 [pii].

Shynlova, O, P Tsui, A Dorogin, B L Langille, and S J Lye. 2007a. "The Expression of Transforming Growth Factor Beta in Pregnant Rat Myometrium Is Hormone and Stretch Dependent." Reproduction 134 (3): 503–11. doi:134/3/503 [pii]10.1530/REP-07-0004.

Shynlova, O, S J Williams, H Draper, B G White, D J MacPhee, and S J Lye. 2007b. "Uterine Stretch Regulates Temporal and Spatial Expression of Fibronectin Protein and Its Alpha 5 Integrin Receptor in Myometrium of Unilaterally Pregnant Rats." Biol Reprod 77 (5): 880–88. doi:biolreprod.107.062356 [pii]10.1095/biolreprod.107.062356.

Simian, Marina, Mina J Bissell, Mary Helen Barcellos-Hoff, and Gopalan Shyamala. 2009. "Estrogen and Progesterone Receptors Have Distinct Roles in the Establishment of the Hyperplastic Phenotype in PR-A Transgenic Mice." Breast Cancer Research : BCR 11 (5): R72. doi:10.1186/bcr2408.

Sitras, V, R H Paulssen, H Grønaas, A Vårtun, and G Acharya. 2008. "Gene Expression Profile in Labouring and Non-Labouring Human Placenta near Term." Molecular Human Reproduction 14 (1): 61–65. doi:10.1093/molehr/gam083.

Skinner, K A, and J R Challis. 1985. "Changes in the Synthesis and Metabolism of Prostaglandins by Human Fetal Membranes and Decidua at Labor." Am J Obstet Gynecol 151 (4): 519–23. doi:0002-9378(85)90281-9 [pii].

Slater, E P, A C Cato, M Karin, J D Baxter, and M Beato. 1988. "Progesterone Induction of Metallothionein-IIA Gene Expression." Molecular Endocrinology (Baltimore, Md.) 2 (6): 485–91. doi:10.1210/mend-2-6-485.

Smith, G C, E Celik, M To, O Khouri, and K H Nicolaides. 2008. "Cervical Length at Mid-Pregnancy and the Risk of Primary Cesarean Delivery." N Engl J Med 358 (13): 1346–53. doi:358/13/1346 [pii]10.1056/NEJMoa0706834.

Smith, Roger, Julia I Smith, Xiaobin Shen, Patricia J Engel, Maria E Bowman, Shaun A McGrath, Andrew M Bisits, Patrick McElduff, Warwick B Giles, and David W Smith. 2009. "Patterns of Plasma Corticotropin-Releasing Hormone, Progesterone, Estradiol, and Estriol Change and the Onset of Human Labor." The Journal of Clinical Endocrinology and Metabolism 94 (6): 2066–74. doi:10.1210/jc.2008-2257.

Smoot, Michael E, Keiichiro Ono, Johannes Ruscheinski, Peng-Liang Wang, and Trey Ideker. 2011. "Cytoscape 2.8: New Features for Data Integration and Network Visualization." Bioinformatics (Oxford, England) 27 (3): 431–32. doi:10.1093/bioinformatics/btq675.

Sooranna, S R, Y Lee, L U Kim, A R Mohan, P R Bennett, and M R Johnson. 2004. "Mechanical Stretch Activates Type 2 Cyclooxygenase via Activator Protein-1 Transcription Factor in Human Myometrial Cells." Molecular Human Reproduction 10 (2): 109–13.

Stanek, Jerzy. 2013. "Hypoxic Patterns of Placental Injury: A Review." Archives of Pathology & Laboratory Medicine 137 (5): 706–20. doi:10.5858/arpa.2011-0645-RA.

Starkey, P M, I L Sargent, and C W Redman. 1988. "Cell Populations in Human Early Pregnancy Decidua: Characterization and Isolation of Large Granular Lymphocytes by Flow Cytometry." Immunology 65 (1): 129–34.

St-Denis, A, F Chano, P Tremblay, Y St-Pierre, and A Descoteaux. 1998. "Protein Kinase C-Alpha Modulates Lipopolysaccharide-Induced Functions in a Murine Macrophage Cell Line." The Journal of Biological Chemistry 273 (49): 32787–92.

Steinborn, A, H Gunes, S Roddiger, and E Halberstadt. 1996a. "Elevated Placental Cytokine Release, a Process Associated with Preterm Labor in the Absence of Intrauterine Infection." Obstet Gynecol 88 (4 Pt 1): 534–39.

Steinborn, A, M Kuhnert, and E Halberstadt. 1996b. "Immunmodulating Cytokines Induce Term and Preterm Parturition." J Perinat Med 24 (4): 381–90.

Steinborn, A, A Niederhut, C Solbach, R Hildenbrand, C Sohn, and M Kaufmann. 1999. "Cytokine Release from Placental Endothelial Cells, a Process Associated with Preterm Labour in the Absence of Intrauterine Infection." Cytokine 11 (1): 66–73. doi:S1043-4666(98)90399-4 [pii]10.1006/cyto.1998.0399.

Steinborn, A, C von Gall, R Hildenbrand, H J Stutte, and M Kaufmann. 1998. "Identification of Placental Cytokine-Producing Cells in Term and Preterm Labor." Obstet Gynecol 91 (3): 329–35. doi:S0029784497006807 [pii].

Stephen, Gillian L, Sylvia Lui, Sarah A Hamilton, Clare L Tower, Lynda K Harris, Adam Stevens, and Rebecca L Jones. 2014. "Transcriptomic Profiling of Human Choriodecidua During Term Labor: Inflammation as a Key Driver of Labor." American Journal of Reproductive Immunology (New York, N.Y. : 1989), October. doi:10.1111/aji.12328.

Stirrat, Laura I, James R O'Reilly, Sarah M Barr, Ruth Andrew, Simon C Riley, Alexander F Howie, Maria Bowman, et al. 2015. "Decreased Maternal Hypothalamic-Pituitary-Adrenal Axis Activity in Very Severely Obese Pregnancy: Associations with Birthweight and Gestation at Delivery." Psychoneuroendocrinology 63 (September): 135–43. doi:10.1016/j.psyneuen.2015.09.019.

Sugimoto, Y, A Yamasaki, E Segi, K Tsuboi, Y Aze, T Nishimura, H Oida, et al. 1997. "Failure of Parturition in Mice Lacking the Prostaglandin F Receptor." Science 277 (5326): 681–83.

Suzuki, Nobutaka, Shinobu Suzuki, Gordon S Duncan, Douglas G Millar, Teiji Wada, Christine Mirtsos, Hidetoshi Takada, et al. 2002. "Severe Impairment of Interleukin-1 and Toll-like Receptor Signalling in Mice Lacking IRAK-4." Nature 416 (6882): 750–56. doi:10.1038/nature736.

Svare, J A, L F Andersen, H Madsen, and J Langhoff-Roos. 1991. "[Idiopathic preterm delivery and subclinical genital infection]." Ugeskr Laeger 153 (18): 1260–62.

Sykes, L, D A MacIntyre, X J Yap, T G Teoh, and P R Bennett. 2012a. "The Th1:th2 Dichotomy of Pregnancy and Preterm Labour." Mediators Inflamm 2012: 967629. doi:10.1155/2012/967629.

Sykes, L., D.A. MacIntyre, X. J. Yap, S. Ponnampalam, T. G. Teoh, and P. R. Bennett. 2012b. "Changes in the Th1:Th2 Cytokine Bias in Pregnancy and the Effects of the Anti-Inflammatory Cyclopentenone Prostaglandin 15-Deoxy-Δ(12,14)-Prostaglandin J2." Mediators Inflamm 2012 (January): 416739. doi:10.1155/2012/416739.

Szalay-Beko, Máté, Robin Palotai, Balázs Szappanos, István a Kovács, Balázs Papp, and Péter Csermely. 2012. "ModuLand Plug-in for Cytoscape: Determination of Hierarchical Layers of Overlapping Network Modules and Community Centrality." Bioinformatics (Oxford, England) 28 (16): 2202–4. doi:10.1093/bioinformatics/bts352.

Tabanelli, S, B Tang, and E Gurpide. 1992. "*In vitro* Decidualization of Human Endometrial Stromal Cells." The Journal of Steroid Biochemistry and Molecular Biology 42 (3-4): 337–44.

Tabb, T, G Thilander, A Grover, E Hertzberg, and R Garfield. 1992. "An Immunochemical and Immunocytologic Study of the Increase in Myometrial Gap Junctions (and Connexin 43) in Rats and Humans during Pregnancy." Am J Obstet Gynecol 167 (2): 559–67.

Taché, Véronique, D Yvette LaCoursiere, Aasia Saleemuddin, and Mana M Parast. 2011. "Placental Expression of Vascular Endothelial Growth Factor Receptor-1/soluble Vascular Endothelial Growth Factor Receptor-1 Correlates with Severity of Clinical Preeclampsia and Villous Hypermaturity." Human Pathology 42 (9): 1283–88. doi:10.1016/j.humpath.2010.11.018.

Taggart, Michael J, Patrice Arthur, Barbara Zielnik, and Bryan F Mitchell. 2012. "Molecular Pathways Regulating Contractility in Rat Uterus through Late Gestation and Parturition." American Journal of Obstetrics and Gynecology 207 (1): 76.e15–24. doi:10.1016/j.ajog.2012.04.036.

Tagore, Shephali, and Kenneth Kwek. 2010. "Comparative Analysis of Insulin-like Growth Factor Binding Protein-1 (IGFBP-1), Placental Alpha-Microglobulin-1 (PAMG-1) and Nitrazine Test to Diagnose Premature Rupture of Membranes in Pregnancy." Journal of Perinatal Medicine 38 (6): 609–12. doi:10.1515/JPM.2010.099.

Talge, Nicole M, Claudia Holzman, Jianling Wang, Victoria Lucia, Joseph Gardiner, and Naomi Breslau. 2010. "Late-Preterm Birth and Its Association with Cognitive and Socioemotional Outcomes at 6 Years of Age." Pediatrics 126 (6): 1124–31. doi:10.1542/peds.2010-1536.

Tan, Huiqing, Lijuan Yi, Neal S Rote, William W Hurd, and Sam Mesiano. 2012. "Progesterone Receptor-A and -B Have Opposite Effects on Proinflammatory Gene Expression in Human Myometrial Cells: Implications for Progesterone Actions in Human Pregnancy and Parturition." J Clin Endocrinol Metab 97 (5): E719–30. doi:jc.2011-3251 [pii]10.1210/jc.2011-3251 [doi].

Tanaka, Y, H Narahara, N Takai, J Yoshimatsu, T Anai, and I Miyakawa. 1998. "Interleukin-1beta and Interleukin-8 in Cervicovaginal Fluid during Pregnancy." American Journal of Obstetrics and Gynecology 179 (3 Pt 1): 644–49.

Taylor, A, J Verhagen, K Blaser, M Akdis, and C A Akdis. 2006. "Mechanisms of Immune Suppression by Interleukin-10 and Transforming Growth Factor-Beta: The Role of T Regulatory Cells." Immunology 117 (4): 433–42. doi:IMM2321 [pii]10.1111/j.1365-2567.2006.02321.x [doi].

Terzidou, V, A M Blanks, S H Kim, S Thornton, and P R Bennett. 2011. "Labor and Inflammation Increase the Expression of Oxytocin Receptor in Human Amnion." Biol Reprod 84 (3): 546–52. doi:biolreprod.110.086785 [pii]10.1095/biolreprod.110.086785 [doi].

"The Role of Antibacterials in Women at Risk of Preterm Birth." 2011. Drug Ther Bull 49 (9): 105–8. doi:dtb.2011.02.0056 [pii]10.1136/dtb.2011.02.0056.

Thomson, A J, J F Telfer, A Young, S Campbell, C J Stewart, I T Cameron, I A Greer, and J E Norman. 1999. "Leukocytes Infiltrate the Myometrium during Human Parturition: Further Evidence That Labour Is an Inflammatory Process." Hum Reprod 14 (1): 229–36.

Tibbetts, T A, O M Conneely, and B W O'Malley. 1999. "Progesterone via Its Receptor Antagonizes the pro-Inflammatory Activity of Estrogen in the Mouse Uterus." Biology of Reproduction 60 (5): 1158–65.

Tong, Dan, Xuerong Lu, Hong-Xing Wang, Isabelle Plante, Ed Lui, Dale W Laird, Donglin Bai, and Gerald M Kidder. 2009. "A Dominant Loss-of-Function GJA1 (Cx43) Mutant Impairs Parturition in the Mouse." Biology of Reproduction 80 (6): 1099–1106. doi:10.1095/biolreprod.108.071969.

Toplis, P J, and C D Sims. 1979. "Prospective Study of Different Methods and Routes of Administration of Prostaglandin E2 to Improve the Unripe Cervix." Prostaglandins 18 (1): 127–36.

Törnblom, Susanne Abelin, Aurelija Klimaviciute, Birgitta Byström, Milan Chromek, Annelie Brauner, Gunvor Ekman-Ordeberg, S A Tornblom, and B Bystrom. 2005. "Non-Infected Preterm Parturition Is Related to Increased Concentrations of IL-6, IL-8 and MCP-1 in Human Cervix." Reproductive Biology and Endocrinology : RB&E 3 (January): 39. doi:10.1186/1477-7827-3-39.

Tough, S C, C Newburn-Cook, D W Johnston, L W Svenson, S Rose, and J Belik. 2002. "Delayed Childbearing and Its Impact on Population Rate Changes in Lower Birth Weight, Multiple Birth, and Preterm Delivery." Pediatrics 109 (3): 399–403.

Tsatsaris, V, B Carbonne, and D Cabrol. 2004. "Atosiban for Preterm Labour." Drugs 64 (4): 375–82. doi:6443 [pii].

Tsiartas, P, R M Holst, U B Wennerholm, H Hagberg, D M Hougaard, K Skogstrand, B D Pearce, P Thorsen, M Kacerovsky, and B Jacobsson. 2012. "Prediction of Spontaneous Preterm Delivery in Women with Threatened Preterm Labour: A Prospective Cohort Study of Multiple Proteins in Maternal Serum." BJOG 119 (7): 866–73. doi:10.1111/j.1471-0528.2012.03328.x.

Tsuboi, K, Y Sugimoto, A Iwane, K Yamamoto, S Yamamoto, and A Ichikawa. 2000. "Uterine Expression of Prostaglandin H2 Synthase in Late Pregnancy and during Parturition in Prostaglandin F Receptor-Deficient Mice." Endocrinology 141 (1): 315–24.

Tsuruta, Daisuke, and Jonathan C R Jones. 2003. "The Vimentin Cytoskeleton Regulates Focal Contact Size and Adhesion of Endothelial Cells Subjected to Shear Stress." Journal of Cell Science 116: 4977–84. doi:10.1242/jcs.00823.

Ustun, C, I Kocak, S Baris, A Uzel, and F Saltik. 2001. "Subclinical Chorioamnionitis as an Etiologic Factor in Preterm Deliveries." Int J Gynaecol Obstet 72 (2): 109–15. doi:S0020-7292(00)00280-0 [pii].

Vadillo-Ortega, F, and G Estrada-Gutierrez. 2005. "Role of Matrix Metalloproteinases in Preterm Labour." BJOG 112 Suppl: 19–22. doi:BJO00579 [pii] 10.1111/j.1471-0528.2005.00579.x.

van Veen, A J, M J Pelinck, M G van Pampus, and J J H M Erwich. 2005. "Severe Hypotension and Fetal Death due to Tocolysis with Nifedipine." BJOG : An International Journal of Obstetrics and Gynaecology 112 (4): 509–10. doi:10.1111/j.1471-0528.2004.00480.x.

Vašák, Milan, and Gabriele Meloni. 2011. "Chemistry and Biology of Mammalian Metallothioneins." Journal of Biological Inorganic Chemistry. doi:10.1007/s00775-011-0799-2.
Vince, G.S. S, P.M. M Starkey, M.C. C Jackson, I.L. L Sargent, and C.W.G. W Redman. 1990. "Flow Cytometric Characterisation of Cell Populations in Human Pregnancy Decidua and Isolation of Decidual Macrophages." Journal of Immunological Methods 132 (2): 181–89. doi:10.1016/0022-1759(90)90028-T.
Vogel, Joshua P, Juan Manuel Nardin, Therese Dowswell, Helen M West, and Olufemi T Oladapo. 2014. "Combination of Tocolytic Agents for Inhibiting Preterm Labour." The Cochrane Database of Systematic Reviews 7 (January): CD006169. doi:10.1002/14651858.CD006169.pub2.
Vrachnis, Nikolaos, Fotodotis M Malamas, Stavros Sifakis, Efthymios Deligeoroglou, and Zoe Iliodromiti. 2011. "The Oxytocin-Oxytocin Receptor System and Its Antagonists as Tocolytic Agents." International Journal of Endocrinology 2011 (January): 350546. doi:10.1155/2011/350546.
Waller, D K, L S Lustig, G C Cunningham, L B Feuchtbaum, and E B Hook. 1996. "The Association between Maternal Serum Alpha-Fetoprotein and Preterm Birth, Small for Gestational Age Infants, Preeclampsia, and Placental Complications." Obstetrics and Gynecology 88 (5): 816–22.
Wang, M L, D J Dorer, M P Fleming, and E A Catlin. 2004. "Clinical Outcomes of near-Term Infants." Pediatrics 114 (2): 372–76. doi:114/2/372 [pii].
Wang, Xiaobo, and Gail E Sonenshein. 2005. "Induction of the RelB NF-kappaB Subunit by the Cytomegalovirus IE1 Protein Is Mediated via Jun Kinase and c-Jun/Fra-2 AP-1 Complexes." Journal of Virology 79 (1): 95–105. doi:10.1128/JVI.79.1.95-105.2005.
Wang, Xiaowei, Athanasia Spandidos, Huajun Wang, and Brian Seed. 2012. "PrimerBank: A PCR Primer Database for Quantitative Gene Expression Analysis, 2012 Update." Nucleic Acids Research 40 (Database issue): D1144–49. doi:10.1093/nar/gkr1013.
Wang, Yanli, Tugba Suzek, Jian Zhang, Jiyao Wang, Siqian He, Tiejun Cheng, Benjamin A Shoemaker, Asta Gindulyte, and Stephen H Bryant. 2014. "PubChem BioAssay: 2014 Update." Nucleic Acids Research 42 (Database issue): D1075–82. doi:10.1093/nar/gkt978.
Wang, Yijie, Xiaokui Mo, Melissa G Piper, Hongmei Wang, Narasimham L Parinandi, Denis Guttridge, and Clay B Marsh. 2011. "M-CSF Induces Monocyte Survival by Activating NF-κB p65 Phosphorylation at Ser276 via Protein Kinase C." PloS One 6 (12): e28081. doi:10.1371/journal.pone.0028081.
Wang, Jinfeng, Suming Zhang, Li Li, and Licai Zhang. 2015. "Involvement of Wnt5a within the Cerebrospinal Fluid-Contacting Nucleus in Nerve Injury-Induced Neuropathic Pain." The International Journal of Neuroscience 125 (2): 147–53. doi:10.3109/00207454.2014.915399.
Watari, M, H Watari, M E DiSanto, S Chacko, G P Shi, and J F Strauss 3rd. 1999. "Pro-Inflammatory Cytokines Induce Expression of Matrix-Metabolizing Enzymes in Human Cervical Smooth Muscle Cells." Am J Pathol 154 (6): 1755–62. doi:S0002-9440(10)65431-4 [pii]10.1016/S0002-9440(10)65431-4.
Wathes, D C, S C Borwick, P M Timmons, S T Leung, and S Thornton. 1999. "Oxytocin Receptor Expression in Human Term and Preterm Gestational Tissues prior to and Following the Onset of Labour." J Endocrinol 161 (1): 143–51. doi:JOE03116 [pii].
Watson, D L, S J Kim, and M D Humphrey. 1998. "Study of Cervicovaginal Fetal Fibronectin Status to Guide Treatment of Threatened Preterm Labour." Aust N Z J Obstet Gynaecol 38 (2): 185–87.
Wegienka, G, S Havstad, K R Bobbitt, K J Woodcroft, E M Zoratti, D R Ownby, and C Cole Johnson. 2011. "Within-Woman Change in Regulatory T Cells from Pregnancy to the Postpartum Period." J Reprod Immunol 88 (1): 58–65. doi:S0165-0378(10)00355-4 [pii]10.1016/j.jri.2010.06.157 [doi].
Weiner, C P, C W Mason, Y Dong, I A Buhimschi, P W Swaan, and C S Buhimschi. 2010. "Human Effector/initiator Gene Sets That Regulate Myometrial Contractility during Term and Preterm Labor." Am J Obstet Gynecol 202 (5): 474 e1–20. doi:S0002-9378(10)00247-4 [pii]10.1016/j.ajog.2010.02.034.
Willecke, Klaus, Jürgen Eiberger, Joachim Degen, Dominik Eckardt, Alessandro Romualdi, Martin Güldenagel, Urban Deutsch, and Goran Söhl. 2002. "Structural and Functional Diversity of Connexin Genes in the Mouse and Human Genome." Biological Chemistry 383 (5): 725–37. doi:10.1515/BC.2002.076.
Williams, K C, N E Renthal, J C Condon, R D Gerard, and C R Mendelson. 2012. "MicroRNA-200a Serves a Key Role in the Decline of Progesterone Receptor Function Leading to Term and Preterm Labor." Proc Natl Acad Sci U S A 109 (19): 7529–34. doi:1200650109 [pii]10.1073/pnas.1200650109.
Winkler, M, B Kemp, D C Fischer, H Maul, M Hlubek, and W Rath. 2001. "Tissue Concentrations of Cytokines in the Lower Uterine Segment during Preterm Parturition." J Perinat Med 29 (6): 519–27. doi:10.1515/JPM.2001.072.
Winkler, M, B Kemp, S Hauptmann, and W Rath. 1997. "Parturition: Steroids, Prostaglandin E2, and Expression of Adhesion Molecules by Endothelial Cells." Obstetrics and Gynecology 89 (3): 398–402. doi:10.1016/S0029-7844(96)00500-5.
Winterhager, E, R Stutenkemper, O Traub, E Beyer, and K Willecke. 1991. "Expression of Different Connexin Genes in Rat Uterus during Decidualization and at Term." Eur J Cell Biol 55 (1): 133–42.

Witczak, Magdalena, A Torbe, Ryszard Czajka, and Andrzej Torbé. 2003. "[Maternal serum and amniotic fluid IL-1 alpha, IL-1 beta, IL-6 and IL-8 levels in preterm and term labor complicated by PROM]." Ginekol Pol 74 (10): 1343–47.

Witter, F R. 2000. "Prostaglandin E2 Preparations for Preinduction Cervical Ripening." Clin Obstet Gynecol 43 (3): 469–74.

Wu, A L, J Wang, A Zheleznyak, and E J Brown. 1999. "Ubiquitin-Related Proteins Regulate Interaction of Vimentin Intermediate Filaments with the Plasma Membrane." Mol Cell 4 (4): 619–25. doi:S1097-2765(00)80212-9 [pii].

Wu, H C, C M Shen, Y Y Wu, Y S Yuh, and K E Kua. 2009. "Subclinical Histologic Chorioamnionitis and Related Clinical and Laboratory Parameters in Preterm Deliveries." Pediatr Neonatol 50 (5): 217–21. doi:S1875-9572(09)60066-8 [pii]10.1016/S1875-9572(09)60066-8.

Wu, X, D J Li, M M Yuan, Y Zhu, and M Y Wang. 2004. "[The expression of chemokine receptors in CD56(bright) CD16- natural killer cells and the mechanism of their recruitment in decidua]." Zhonghua Yi Xue Za Zhi 84 (12): 1018–23.

Wu, Xiang, Hong Shen, Ling Yu, Mei Peng, Wei-Si Lai, and Yi-Ling Ding. 2007. "Corticotropin-Releasing Hormone Activates Connexin 43 via Activator Protein-1 Transcription Factor in Human Myometrial Smooth Muscle Cells." American Journal of Physiology. Endocrinology and Metabolism 293 (6): E1789–94. doi:10.1152/ajpendo.00249.2007.

Xie, N, L Liu, Y Li, C Yu, S Lam, O Shynlova, M Gleave, J R Challis, S Lye, and X Dong. 2012. "Expression and Function of Myometrial PSF Suggest a Role in Progesterone Withdrawal and the Initiation of Labor." Mol Endocrinol 26 (8): 1370–79. doi:me.2012-1088 [pii]10.1210/me.2012-1088.

Xu, Chen, Xingji You, Weina Liu, Qianqian Sun, Xiaoying Ding, Ying Huang, and Xin Ni. 2015. "Prostaglandin F2α Regulates the Expression of Uterine Activation Proteins via Multiple Signalling Pathways." Reproduction (Cambridge, England) 149 (1): 139–46. doi:10.1530/REP-14-0479.

Xu, Wei, Anja Roos, Nicole Schlagwein, Andrea M Woltman, Mohamed R Daha, and Cees van Kooten. 2006. "IL-10-Producing Macrophages Preferentially Clear Early Apoptotic Cells." Blood 107 (12): 4930–37. doi:10.1182/blood-2005-10-4144.

Yagel, S. 2009. "The Developmental Role of Natural Killer Cells at the Fetal-Maternal Interface." Am J Obstet Gynecol 201 (4): 344–50. doi:S0002-9378(09)00221-X [pii]10.1016/j.ajog.2009.02.030.

Yang, Yee Hwa, Sandrine Dudoit, Percy Luu, David M Lin, Vivian Peng, John Ngai, and Terence P Speed. 2002. "Normalization for cDNA Microarray Data: A Robust Composite Method Addressing Single and Multiple Slide Systematic Variation." Nucleic Acids Research 30 (4): e15.

Yellon, S M, C A Ebner, and M A Elovitz. 2009. "Medroxyprogesterone Acetate Modulates Remodeling, Immune Cell Census, and Nerve Fibers in the Cervix of a Mouse Model for Inflammation-Induced Preterm Birth." Reprod Sci 16 (3): 257–64. doi:1933719108325757 [pii]10.1177/1933719108325757.

Yellon, S M, C A Ebner, and Y Sugimoto. 2008. "Parturition and Recruitment of Macrophages in Cervix of Mice Lacking the Prostaglandin F Receptor." Biol Reprod 78 (3): 438–44. doi:biolreprod.107.063404 [pii]10.1095/biolreprod.107.063404.

Yin, Zongzhi, Alaa A Sada, Ossama M Reslan, Neha Narula, and Raouf A Khalil. 2012. "Increased MMPs Expression and Decreased Contraction in the Rat Myometrium during Pregnancy and in Response to Prolonged Stretch and Sex Hormones." American Journal of Physiology. Endocrinology and Metabolism 303 (1): E55–70. doi:10.1152/ajpendo.00553.2011.

Yoshimura, Kazuaki, and Emmet Hirsch. 2005. "Effect of Stimulation and Antagonism of Interleukin-1 Signaling on Preterm Delivery in Mice." Journal of the Society for Gynecologic Investigation 12 (7): 533–38. doi:10.1016/j.jsgi.2005.06.006.

Young, Anne, Andrew J Thomson, MarieAnne Ledingham, Fiona Jordan, Ian A Greer, and Jane E Norman. 2002. "Immunolocalization of Proinflammatory Cytokines in Myometrium, Cervix, and Fetal Membranes during Human Parturition at Term." Biol Reprod 66 (2): 445–49.

Yu, Chih Wen, Je Hsin Chen, and Lih Yuan Lin. 1997. "Metal-Induced Metallothionein Gene Expression Can Be Inactivated by Protein Kinase C Inhibitor." FEBS Letters 420 (1): 69–73. doi:10.1016/S0014-5793(97)01489-0.

Yuan, M, F Jordan, I B McInnes, M M Harnett, and J E Norman. 2009. "Leukocytes Are Primed in Peripheral Blood for Activation during Term and Preterm Labour." Mol Hum Reprod 15 (11): 713–24. doi:gap054 [pii]10.1093/molehr/gap054.

Zachariades, E, D Mparmpakas, Y Pang, M Rand-Weaver, P Thomas, and E Karteris. 2012. "Changes in Placental Progesterone Receptors in Term and Preterm Labour." Placenta 33 (5): 367–72. doi:10.1016/j.placenta.2012.01.002.

Zaragoza, Dean B., Robyn R. Wilson, Bryan F. Mitchell, and David M. Olson. 2006. "The Interleukin 1beta-Induced Expression of Human Prostaglandin F2alpha Receptor Messenger RNA in Human Myometrial-

Derived ULTR Cells Requires the Transcription Factor, NFkappaB." Biology of Reproduction 75 (5): 697–704. doi:10.1095/biolreprod.106.053439.

Zeitlin, J, K Szamotulska, N Drewniak, AD Mohangoo, J Chalmers, L Sakkeus, L Irgens, M Gatt, M Gissler, and B Blondel. 2013. "Preterm Birth Time Trends in Europe: A Study of 19 Countries." BJOG: An International Journal of Obstetrics & Gynaecology 120 (11): 1356–65. doi:10.1111/1471-0528.12281.

Zenclussen, A C, K Gerlof, M L Zenclussen, A Sollwedel, A Z Bertoja, T Ritter, K Kotsch, J Leber, and H D Volk. 2005. "Abnormal T-Cell Reactivity against Paternal Antigens in Spontaneous Abortion: Adoptive Transfer of Pregnancy-Induced CD4+CD25+ T Regulatory Cells Prevents Fetal Rejection in a Murine Abortion Model." Am J Pathol 166 (3): 811–22. doi:10.1016/s0002-9440(10)62302-4.

Zhang, Cuicui, Miaohua Mo, Wenwen Ding, Wenjuan Liu, Dewen Yan, Jianxin Deng, Xinping Luo, and Jie Liu. 2014. "High-Mobility Group Box 1 (HMGB1) Impaired Cardiac Excitation-Contraction Coupling by Enhancing the Sarcoplasmic Reticulum (SR) Ca(2+) Leak through TLR4-ROS Signaling in Cardiomyocytes." Journal of Molecular and Cellular Cardiology 74 (September): 260–73. doi:10.1016/j.yjmcc.2014.06.003.

Zhang, Jianhong, Zhilin Chen, Graeme N Smith, and B Anne Croy. 2011a. "Natural Killer Cell-Triggered Vascular Transformation: Maternal Care before Birth?" Cellular & Molecular Immunology 8 (1): 1–11. doi:10.1038/cmi.2010.38.

Zhang, Wei-She, Qing-Sheng Xie, Xin-Hua Wu, and Qing-Hua Liang. 2011b. "Neuromedin B and Its Receptor Induce Labor Onset and Are Associated with the RELA (NFKB P65)/IL6 Pathway in Pregnant Mice." Biology of Reproduction 84 (1): 113–17. doi:10.1095/biolreprod.110.085746.

Zhao, Baohui, Deanna Koon, Allyson L Curtis, Jessica Soper, and Kathleen E Bethin. 2007. "Identification of 9 Uterine Genes That Are Regulated during Mouse Pregnancy and Exhibit Abnormal Levels in the Cyclooxygenase-1 Knockout Mouse." Reprod Biol Endocrinol 5 (January): 28. doi:10.1186/1477-7827-5-28.

Zhao, Yin, Kaori Koga, Yutaka Osuga, Gentaro Izumi, Masashi Takamura, Miyuki Harada, Tetsuya Hirata, et al. 2013. "Cyclic Stretch Augments Production of Neutrophil Chemokines and Matrix Metalloproteinases-1 (MMP-1) from Human Decidual Cells, and the Production Was Reduced by Progesterone." American Journal of Reproductive Immunology (New York, N.Y. : 1989) 69 (5): 454–62. doi:10.1111/aji.12092.

Zilliox, Michael J, and Rafael A Irizarry. 2007. "A Gene Expression Bar Code for Microarray Data." Nature Methods 4 (11): 911–13. doi:10.1038/nmeth1102.

Zlotnik, Albert, Osamu Yoshie, and Hisayuki Nomiyama. 2006. "The Chemokine and Chemokine Receptor Superfamilies and Their Molecular Evolution." Genome Biology 7 (12): 243. doi:10.1186/gb-2006-7-12-243.

APPENDIX

Appendix table 1 Summary of large scale gene studies in human uterine tissues including key findings and use of pathway analysis.

**** indicates studies that included in silico network analysis.**

Author	Tissue	Analysis	Array type	Key findings of gene expression	Key analysis and pathways
Aguan et al., 2000	Myometrium	TNL and TL (N=3). cDNA gel blot. Gene expression quantified with phosphoimager	Atlas Human Blot (Clontech). 588 unique genes	Increased in TL: IGFBP, Ca(2+)/CaM binding PKC substrate, and angiotensin converting enzyme. Decreased: IGF-II, calgranulin A and B (S100A8 and 9), G-protein coupled receptor, IL12, T cell regulation IL27B.	-
Bethin et al., 2003	Myometrium	TL and PTL (26-33wk) vs matched PTNL (N=3 each). Microarray on pooled samples. In situ hybridisation in tissues for genes of interest. Hierarchical clustering analysis using MATLAB 6.12. Functional cluster analysis with GenMAPP and literature/database search.	Affymetrix Human Genome U95A Array (12626 unique genes)	There were 478 genes were significantly expressed in the labour groups but differing in magnitude of expression. These include the upregulation of transcription factors: c-Fos, MafF, enigma, ER factor-1, LIM protein, and Jun B. There was reduced prostaglandin dehydrogenase (PGDH) expression in PTL and TL.	Compared to PTNL, the major pathway clusters in TL and PTL were increased for immune/inflammatory signalling, protease inhibitor, cell adhesion functions.
Chaemsaithong et al., 2013	Myometrium	TL (n=29) and TL with arrest of dilatation (AODIL; n=14). Microarray on individual samples. Gene ontology with GO stats software. Pathway analysis with (KEGG) pathway database and MetaCore analysis. Validation using qRT-PCR.	Illumina Human HT-12 v3 expression microarray (25 000 unique genes)	42 differentially expressed genes were identified in AODIL. Increased myometrial expression with TL and AODIL validated by qRT-PCR: NOS3, HIF1A, CCL2, ANGPTL4, ADAMTS9, GPR4, MT1A, MT2A, selectin E.	Gene ontology enrichment in angiogenesis regulation, response to hypoxia, inflammatory response, and chemokine-mediated signalling. Top pathways overrepresented in: transcription repressor activity, Hsp90, NOS activity, CCL2 signalling, muscle contraction and thyroid signalling. However the RNA quality used for microarray and PCR had an A260/280 nm absorbance ratio of 1.66, a 28S/18S ratio of 0.2, and a RIN of 3.8

Chan et al., 2002.	Myometrium upper vs lower	TNL and TL (n= 3). cDNA gel blot. Validated genes with qRT-PCR Southern blots with RT-PCR products. Northern blots.	Dot blot hybridization. Able to identify 400 Clones	There were 30 differentially expressed clones: 14 upregulated by labour including OXTR, MMP9 and fibronectin, 6 were immune regulation and inflammatory IL-8, MnSOD, and MMP-9, interferon-inducible 1-8d gene, elongation factor 1A, and nudeophosmin. Highlighted were 'two constitutively expressed cyclophilin and alpha-actin were altered by labour'.	-
Chan et al., 2014	Myometrium	TNL and TL (n= 5).RNA sequencing with analysis with transcriptsper million (TPM). PCA performed on data.	RNA sequencing	Transcripts per million to account for overrepresentation, gene variants and alternative splicing. TL has 764 differentially expressed genes compared to TNL. Genes increased with labour associated with chemotaxis, leucocyte infiltration, neutrophils and macrophages. The analysis identified splice variants and gene isoforms in smooth muscle contractility between TL and TNL.	PCA identified labour as distinct between TNL and TL. Upregulated are PGE2 pathway genes, and decreased included PTGER3 and OXTR.
Charpigny et al., 2003	Myometrium	TNL, TL and PTL (28- 31.5wk) (n=4) . Microarray on individual samples. Only genes found in all three groups were used for cluster analysis. Hierarchical clustering using CLUSTER and TreeView (Stanford University, Eisen et al 1998). Pathway analysis by OMIM database (Online Mendelian Inheritance in MAN, OMIM). Validation by qRT-PCR.	Atlas Human 1.2 and 1.2II (Clontech). 1176 known genes each.	There were more changes in differential expression between TL and TNL than between PTL and TL. Genes differentially expressed during TL include upregulated genes: COPB, SRD5A, PRKAA1, ATOH1, TP53, RCN2 and CALM1 and down-regulated: GLRB, DLG2, MEL, PDE1B, AACT.	Of the differentially expressed TL and PTL genes, the majority were clustered in cell proliferation and differentiation, immune system and contraction. Functional groups were decreased in the contractile smooth muscle, uterine autonomic nervous system and proliferation-related. There were 27 genes significantly decreased in TL than PTL. The major clusters were in cell proliferation and differentiation, oncogenesis and immunomodulation.

Cook et al., 2015	Myometrium	TNL and TL (n= 8). miRNA microarray on individual sample. TNL vs TL with oxytocin treatment. Cluster pattern analysis with Principle Components Analysis (PCA). Cell target analysis using miRTarBase platform.	Exiqon miRCURY LNA miRNA microarray Verison 8 (1309 unique miRNAs)	Labour with and without oxytocin treatment. *In vivo* and *in vitro*. There were 12 target miRNAs, the most differential expression was between TNL and TL. hsa-miR-146b-3p was increased all labour conditions compared to TNL. During labour, oxytocin mediates: hsa-miR-146b-3p, hsa-miR-196b-3p, hsa-miR-223-3p, hsa-miR-873-5p, and hsa-miR-876-5p.	.
Esplin, et al., 2005.	Myometrium	TNL and TL (n= 5) . Microarray on individual samples (n=5 and a pooled control), RT-PCR, northern blot, ELISA and in situ hybridisation.	cDNA blot array (6912 clones)	During labour, 56 myometrial genes were differentially expressed. These include: TGFB2, C18orf1, LMCD1 BDNF VMP1 PCDH1 ARHE DUSP5 SERPINE1 THBS2 PAPPA CCL13 PCP4 RUNX1 TIMP1 ZCWCC3 CRIM1 FBN2 CDKN1A CCL18 TNC SERPINE2. Northern blot analysis and qRT-PCR validated: THBS1, PBEF1, SOD2 and NNMT in the TL myometrium.	-
Mittal et al., 2010.	Myometrium	TNL and TL. For the microarray n=19-20; (For all other experiments, n=9-11) . Microarray on individual samples. Pathway analysis with Kyoto Encydopedia of Genes and Genomes (KEGG) pathway database and signalling Pathway Impact Analysis (SPIA). Data mining with MetaCore database (Gene Go, St. Joseph, MI). Validation with RTqPCR. ELISA: IL-6, CCL2, and IL-8. Immunoblotting: CXCL6 & SOCS3.	Illumina Human HT-12 v3 expression microarray (25 000 unique genes)	qRT-PCR validated 29 of 31 genes from microarray. 11 established in labor including IL-8, IL-6, PTGS2, CCL2, HBEGF, IL-1RA, CXCL6, NFKBIZ, and SOCS3. down-regulated genes included FKBP5 and ALDH2. ELISA protein assay supported transcript data. CCL2 and IL-6 concentrations were higher in TL vs TNL. Immunoblotting analysis supported increased CXCL6 and SOCS3 during TL.	Using gene ontology, cluster analysis, biological function and enrichment included the major categories: cytokine-cytokine receptor interaction; Jak-STAT signalling pathway; complement and coagulation cascade; and ascorbate and aldarate metabolism. The overrepresentation analysis, included interleukin-17 signalling. In the impact analysis a major **network module** was associated with I-kB, NFkB, and several NFkB targets supporting a highly inflammmatory associated role in the myometrial transcriptome of spontaneous term labour.

Mittal et al., 2011	Myometrium	TNL, TL, and TL with arrest of descent. Microarray (n=19-21); For all other experiments, n=5-10). Microarray on individual samples. Gene ontology and pathway analysis with (KEGG) pathway database and the signalling Pathway Impact Analysis (SPIA). Data mining with MetaCore database (Gene Go, St. Joseph, MI). Validation with qRT-PCR. ELISA: IL-6.	Illumina Human HT-12 v3 expression microarray (25 000 unique genes)	Arrest of descent is unique from spontaneous term labor with differential expression of 400 genes. The transcriptome involved in in?ammation and muscle contraction. qRT-PCR validated increased IL-6, PTGS2, and HIF1A. Protein concentrations of IL-6 con?rmed the microarray and PCR results, increasing with an arrest of descent.	Enriched biological processes in chemotaxis, and molecular functions such as chemokine activity. Arrest of descent impact analysis identified the biology was associated with cytokine-cytokine receptor interaction, complement and coagulation cascade, regulation of actin cytoskeleton, and focal adhesion.
Rehman et al., 2003	Myometrium	TNL (n=4); and non-pregnant (n=14). Microarray pooled. Array analysed by Genomic expression microarray (GEM) and literature. Validation with northern blot analysis. Non pregnant sample from hysterectomy with benign complications.	UniGEM V human microarray (7075 cDNA elements)	602 transcripts were up-regulated in TNL, 8 were down-regulated >/=2.0-fold. Major changes in the microarray include: IGFBP-1, IGF-1 and 2, Wnt5a, tropomyosin 2, caldesmon, and IREB2. Northern blot validated increase of PAI-1 and MFGE8, and decrease of sFRP4 and ER alpha.	At term, the myometrial gene expression ontology included growth and differentiation, signal transduction, cell to cell contact, inflammation, cellular structure and contractility. Pathway signalling were involved in IGF/ prolactin signalling, Wnt signalling pathways, plasminogen activator inhibition, integrin/EGF binding, ER/AP-1 expression, adhesion molecules, myosin/caldesmon expression.
Weiner et al., 2010	Myometrium	TNL, TL, PTNL, PTL (25-32 weeks) with or without infection (n=6 all). Microarray on individual samples. Gene ontology with GO stats software and pathway-network analysis using MetaCore analytical suite.	Affymetrix Human Genome U133 Plus 2.0 Array (38 500 unique genes)	Selectively expressed at TL (vs TNL/PTL) upregulation of PROK2, EREG, BDKRB1, IL13RA2, and MS. Down-regulation of IL9R. Selectively expressed at PTL (vs PTNL/TL) upregulation of CatSper2, PTPRZ1, GSTT2, NDRG4, and CD3E. qRT-PCR validated TL and PTL upregulation of contractility maintainance HNT, KCNAB2, and RRAD.	Identification of a labour activating effector gene set (TL and PTL) of 67 genes. Expression dominated by inflammatory ontology, in the effector and TL (including chemotaxis, cell adhesion, leukocyte chemotaxis, signal transduction, muscle contraction, proliferation, regulation of angiogensis). Preterm also inflammatory but distinct including: TCR, IL10, TREM1, response to hypoxia and oxidative stress, histamine signalling.

Myometrium and cervix

Author	Tissue	Analysis	Array type	Key findings of gene expression	Key analysis and pathways
Havelock et al., 2005.	Myometrium fundus, lower; Cervical cells	TNL and TL (n=5). Microarray. Validation by qRT-PCR. Immunohistochemistry (IHC) of PTGS2 and S100A9	UniGEM-V Human microarray (9182 cDNA probes)	Few labour-related changes. The predominant changes were in inflammatory response including: S100A9, MX1, G1P3, IFITM1, FCGR3A, and SERPINA3.	-
Bollapragada et al., 2009	Myometrium and cervix	TNL and TL (n=9 each). Microarray with validation by qRT-PCR. Pathway analysis using Ingenuity Pathway Analysis (IPA).	Affymetrix Human Genome U133 Plus 2.0 Array (38 500 unique genes)	Common at TL between myometrium and cervix were 110 genes upregulated and 29 down-regulated. Upregulated at TL include 248 myometrial genes and 274 in cervix. down-regulated at TL include 170 myometrial genes. Chemokine expression common in both tissues. qRT-PCR validated increased expression of CXCL3, CXCL5, CXCL 8, CCL2, and CCL20 at TL.	The most significantly pathway upregulated was in?ammatory pathway signalling at TL. Gene ontology indicated this involved cellular movement and immune response. Other pathways include signalling in fibrosis/hepatic stellate cell activation, glucocorticoid receptor, IL-6, IL-10, eicosanoid, LXR/RXR activation, leucocyte extravasation and hepatic cholestasis
Bukowski et al., 2006.	Myometrium and cervix	TNL and TL (n= 6 - 7). Microarray on individual samples. Comparison of upper and lower myometrium and cervix. Cluster analysis: K-means non-hierarchical, and hierarchical clustering. Pathway analysis using GenBank, UniGene, Gene Ontology databases and IPA. Validation with RT-PCR.	Affymetrix Human Genome U95A Array (12626 unique genes)	500 significantly expressed TL genes and only 6% were shared between myometrium and cervix. Most changes in gene expression were down-regulated and were not shared between the fundus and lower myometrium.	Differential expression between myometrial segments and TNL vs TL. In the fundus, the top clusters included CCR5, IKBKE, IL-1β, CCR7, IL-8. The myometrial lower included cytokine inducible kinase (PrK), FGF7, LIF, TCL1A, and TNFRF1. In the cervix, top clusters included TNF13, TRAF, TNF4, STAT5B, and PTPN1.
Hassan et al., 2006	Cervix	TNL and TL (n=7-9). Microarray on individual samples. Validation by qRT-PCR on a different cohort (n=9-10).	Affymetrix Human Genome U133 Plus 2.0 Array (38 500 unique genes)	1192 genes were differentially expressed in the TL cervix. The top significantly upregulated genes in the microarray at TL were: IL-8 x20; IL-6 x22; VEGF x2.8; TLR2; x3.2; TLR4 x1.7; and down-regulated were TLR3 x-2.4,TLR5 x-1.9. qRT-PCR validated upregulation of IL-6, IL-8 VEGF,TLR3 and TLR5.	After TL (vs TNL), genes were upregulated in functions related to neutrophil chemotaxis. Gene ontology enriched gene expression changes into categories involving: response to biotic stimulus, apoptosis, epidermis development and steroid metabolism.

Choriodecidua

Author	Tissue	Analysis	Array type	Key findings of gene expression	Key analysis and pathways
Shankar et al., 2010.	Chorio-decidua	iPTL (+infection; n=6), sPTL (-infection, n=4) (<35 weeks; n=12), aPTL (-infection, +abruption, n=2) vs PTNL (matched; n=7). Microarray on individual samples. Hierarchical clustering and pathway analysis using IPA. Validation using 2D gel electrophoresis and mass spectrometry on TL and PTL.	Illumina Sentrix Human Whole Genome (WG-6) Series 2 BeadChips (47 000 transcripts)	Compared to PTNL, many differentially transcripts were inflammatory mediators: TNFα, IL-6, SOCS3, LIF, CCL3, CCL3L3, CCL4, CCL4, CXCL1, CXCL2. iPTL had high expression of IL-6, SOCS3, TNF-α, SOCS1, PBEF1, IL-8, NF-κB. sPTL immune activity was characterized by SOCS3, PBEF1, TNFa, IL-6, TNFAIP3, IL6ST, IL2RB, CXCR4, CCL3, CXCL14, CCL4L1, IFNAR2, IFNGR2, ACVRL1 and 1B, TLR2, NFkBIZ, NFkBIA, PTGS2, PLA2G10, PLA2G2A. Two proteins were upregulated with TL: osteoglycin and PGRMC2. PTL proteins upregulated galectin 1, annexin 3, annexin 5 and PDI.	Functional genomics identified preterm subgroup profiles. iPTL deliveries were associated with extensive NFκB activation. sPTL group upregulated cytokine/chemokine receptors. aPTL delivery characterized by the complement cascade and thrombosis. All conditions were mediated by TLR signalling: iPTL through TLR1 and 2. sPTL only through TLR2. Prostaglandin biosynthesis from phospholipids and arachidonic acids were distinct between all groups: iPTL delivery through aiPLA2γ and PLCXD1; sPTL through PLA2G2A and PLA2G10; aPTL through PLA1A and PLCE1, TXA, PPARγ and PI3K.
Stephen et al., 2014	Chorio-decidua	TNL and TL (n=6 each). Microarray on pooled samples. Validation by qRT-PCR (n=10-11). Pathway and network analysis with IPA.	Affymetrix Human Genome U133 Plus 2.0 Array (38 500 unique genes)	Large exploratory list of inflammatory, cell proliferation, structure and growth genes. qRT-PCR validated ICAM1, CXCR4, CD44, TLR4, SOCS3, BCL2A, and IDO.	Two major pathways of the differentially expressed genes: the inflammatory response (with STAT1 and 3, HMGB1, NfkB and NR3C1 as **central network hubs**) and proliferation of cells (which included PGR, TP53 and MYCB as central hubs). The genes also enriched in: leukocyte trafficking, cytokine signalling, cell fate, tissue remodelling, and translation. Up-stream networks of these genes suggested mediation by several non-coding microRNAs: miRNA-21, miRNA-46, miRNA-141, and miRNA-200. Transcription factors upstream of genes included: NFkB, STAT1 &3, NR3C1 and HMGB1. Unmasking genes with lower fold change in expression identified MYCN, MYC, PGR, TP53 and FOS.

Amnion

Author	Tissue	Analysis	Array type	Key findings of gene expression	Key analysis and pathways
Han et al., 2008	Amnion	TNL and TL (n=10). Reflected and placental amnion and response to LPS. Microarray on pooled samples (in threes) for each condition. RT2 Profiler PCR Array validation. PCA analysis. Immunoblotting analysis. Pathway analysis using KEGG pathways. Direction of gene-gene signalling interaction with impact analysis with Pathway Express software.	Affymetrix Human Genome U133 Plus 2.0 Array (38 500 unique genes)	839 genes were differentially expressed between placental amnion and reflected amnion. At TL vs TNL there were 17 differentially expressed genes including: IL-1A, IL-1B, IL-6, TNF, CXCL1, CXCL2, and CXCL3. PCR array analysis focused on 84 genes involved in NFkB and validated upregulation of IL-1B, IL-6, IL-8, IRAK2, TLR4, and TGFBR1 at TL. IL-1B is differentially expressed at TNL compared to TNL with LPS. Protein expression of TGFB1, 2, 3, BR1, BR2 and phosphorylated SMAD1, 5, and 9 were higher in reflected amnion than in placental amnion.	Pathway analysis identified amnion regional differences in signalling pathways in cell adhesion, JAK/STAT signalling, MAPK signalling, and TGFβ pathways. Gene ontology analysis enriched 106 biological processes during labour, the top processes in immune response and inflammatory response. Pathway impact analysis identified 5 significant pathways, including cytokine-cytokine receptor interaction and apoptosis as major cellular processes within the immune response.

Amnion and choriodecidua

Author	Tissue	Analysis	Array type	Key findings of gene expression	Key analysis and pathways
Marvin et al., 2002	Amnion and chorio-decidua	TNL, TL, PTL without chorioamnionitis and PTL with chorioamnionitis (n=4, all). Microarray on individual samples. Gene ontology described from literature.	R&D Systems Human Cytokine Expression Arrays (847 unique genes)	-	The pathway profiles were created by the authors based on the differentially expressed genes of TL compared to TNL and known functions. Gene functions in non-infection related labour identified include pro-inflammatory cytokines and anti-inflammatory cytokines, cytokine receptors, chemotactic signals, matrix metalloproteinases, and cell adhesion molecules.

Chorioamnion, decidua and blood

Author	Tissue	Analysis	Array type	Key findings of gene expression	Key analysis and pathways
Haddad et al., 2006	Chorio-aminion and maternal blood	TNL and TL (n=12). Microarray on individual samples. Hierarchical clustering with Cluster 3.0 and Java TreeView. Gene ontology analysis using GO Tree Machine software. Validation with qRT-PCR.	Affymetrix HG-U133A and HG-U133B array (34 000 unique genes)	197 unique transcripts in TL versus TNL. Many genes involved in acute inflammation during labour. Analysis of interval after rupture of membranes and duration of labour, to determine if prolonged labour induced the abundant inflammatory activity and found no association. Top ranking genes include: CXCL2, IL-8, BMP2, CXCL3, BMP3, CRYBB2, KIAA0826, CXCL1, CCL20, IER3, TLR2, MT2 and MT1. Validation with qRT-PCR included: IL-8, SOD2, PBEF1, and PHLDA1.	From 30 significantly upregulated genes there were 6 enriched gene ontology catergories: response to wounding, taxis, response to parasite, response to biotic stimulus, response to abiotic stimulus, and viral genome replication. Cluster analysis indicated categories of chemokine activity, cytokine activity, or cytokine binding. In the labour blood samples, there was no enrichment in gene ontology. Among the down-regulated genes, there was no enrichment for GO categories. Subclasses of gene expression level between individuals (i.e. low, average and high expressors describe magnitude of expression between distinct groups within TL).
Montenegro et al., 2009	Chorio-aminion, decidual cells, whole fetal membranes, placenta and cord	TNL, TL and PTL (n=10). Microarray on individual chorioamnion samples. Computational analyses using miRBase Targets. Luciferase assay with reporter constructs. Validation using qRT-PCR and TaqMan assay for 5S ribosomal RNA. IHC performed on chorioamnion, placenta, and umbilical cord. Immunoblot analysis on phospholipase A2, Dicer.	Exiqon miRCURY locked nudeic acid (LNA) microarrays. 455 unique miRNA's	TL and PTL had differential expression of ten miRNAs, all down-regulated 25, 338, 101, 449, 154, 199a, 135a, 142-3p, 202, 136. qRT-PCR validated miR338, miR-449, miR-136, and miR-199a at term. PCA shows mima's in labour are very distinctly expressed between PTL and TL. miR-338 was decreased at term with target analyses identifying PLA2G4B. Inhibition with anti-miR-338 increased decidual PLA2G4B mRNA and protein expression. Luciferase assay identified miR-338 binding to the 3UTR of PLA2G4B supported the decrease of PLA2G4B. Dicer decreased at TL in the chorioamniotic membranes.	-

Placenta and blood

Author	Tissue	Analysis	Array type	Key findings of gene expression	Key analysis and pathways
Peng et al., 2011	Maternal blood, cord blood, and placenta	TNL and TL term (n=10). Microarray analysis on individual samples. Samples also compared to commercial Human Universal Reference Total RNA (BD Clontech). Pathway analysis with MetaCore Analytical Suite (GeneGo). Immunoblot anaylis for GDF15 and ADM	Genomic Medicine Research Core Laboratory Human 7K version 2 microarrays (7334 clones, with 3452 unique genes)	Genes involved in inflammation, hypoxia, uterine stress and contractions. Few of the genes had shared expression between maternal blood, cord blood or placenta, indicating functions and pathways are differentially activated during human labor.	In the maternal blood, the functions enriched were: cytokine and NFkB signalling pathways, regulation of TLR4, and SOCS3. Laboured cord blood upregulated genes were involved in: responding to stress and stimuli, regulation of T-cell receptor beta locus, and the FK506 binding protein 8. In the laboured placenta: nitric oxide transport, gas transport, response to hydrostatic pressure, oxygen transport, acute phase responses, and the TNF-mediated signalling pathway.
** Enquobahrie et al., 2009.	Maternal Blood	Second trimester (16wk) blood from threatened PTL (n=14) and TL (n=16). Microarray on individual samples. Hierarchical cluster anlaysis: CLUSTER and TreeView. PCA analysis. Inferred protein-protein interactions using Cytoprophet, a Cytoscape plug-in. Common promoter sequence analysis with ConTra MAPPER.	Affymetrix Human Genome U133 Plus 2.0 Array (38 500 unique genes)	99 genes were upregulated and 110 down-regulated in PTB cases. Genes included PTGER4, prolactin, Protein C, cystathionine-beta-synthase, TNFSF13B, FLT1, TEX1 (testis expressed gene 1), Transgelin and methylthioadenosine phosphorylase. qRT-PCR validated: ABP1, CBS, FCER1A, PTGDS, SLC16A7 and QRSL1.	The differentially expressed PTB genes were involved in functions related to immune system and inflammation, organ development, tissue morphology, metabolism (lipid, carbohydrate and amino acid) and cell signalling. **Network analysis** identified genes significantly associated with preterm birth compared to term. Top genes include: NFKB, P38MAPK, Mapk, Akt, VEGF, retinoic acid, HOXA9, and TNF. Common promoter sequences were found to binding sites of TFAP2A, EGR1, Sp1 and 3.

** Heng et al., 2014	Maternal Blood	Threatened PTL with: spontaneous preterm birth in 48 hrs (n=48), one week, and those at term (n=106). Microarray on individual samples. Gene ontology with GO stats software. Pathway analyses using Reactome Functional Interaction and DAVID Bioinformatics Resources 6.7 (BioCarta, KEGG and Panther). Network analysis using Cytoscape plug-in (version 4.0 beta) community networks and clusters. Validated by qRT-PCR	Affymetrix Human Genome U133 Plus 2.0 Array (38 500 unique genes)	The top genes predicted to be highly associated with threatened PTL leading to PTB: ZDHHC19, HPGD, GPR84, OPLAH, METTL18, TDRD9, ATP9A, GALNT14 and GOLGA8A. qRT-PCR validated all 28 genes in 96 samples.	The 469 differentially expressed genes functionally clustered into metabolic process, response to stress, immune system process and signal transduction. Network analysis predicted nine of the genes associated with dinical blood markers for identification of PTL results with high sensitivity and specificity compared to fetal fibronectin alone sensitivity (91.7% vs 83.3%) and specificity (88% vs 66%). Addition of all three markers did not improve sensitivity but increased specificity to 94%.