Placental genomics: regulatory roles of histamine in pre-eclampsia

Placental Genomics: Regulatory Roles of Histamine in Pre-eclampsia

Obed Brew

A commentary submitted in partial fulfilment of the requirements of
University of West London for the degree of
Doctor of Philosophy

2017

Acknowledgements

My sincere thanks to Dr Mark Sullivan and Professor Anthony Woodman for their dedicated and continual support for this work. My sincere thanks also to my family for their patience and endurance.

Acts 20:24

Abstract

Aim: Pre-eclampsia (PE) is a multifactorial pregnancy related disorder and a major cause of perinatal mortality. Mothers who develop PE present with clinical symptoms akin to experimentally induced elevated histamine. Maternal blood histamine is elevated, while Diamine Amine Oxidase (DAO) level is diminished in PE. The abnormalities in placental development and functions linked to aetiology of PE have similarities with effects of elevated histamine in other tissues, yet the effects of the elevated histamine on placental function were not directly investigated. Therefore, a series of studies were undertaken and published to increase our knowledge and elucidate our understanding on: (1) the functionality of elevated histamine in the placenta; (2) the causes of the elevated histamine in PE placentae, and (3) of the effects of the elevated histamine on placental gene expression and the implications for PE.

Methods: A series of published high-throughput methodologies have been critically discussed in this commentary. Molecular biotechnology and bioinformatics methodologies such as, *ex vivo* elevated histamine placental explant model, gene cloning, RT-qPCR, *In situ* hybridization, microarrays, enzymological analysis, ELISA, Immunohistochemistry, RNA expression array assay analysis, integrated meta-gene analyses, Gene Set Enrichment Analysis, Gene Ontology and biological pathway analyses, Leading Edge Metagene analysis, Systematic Reviews with Meta-analysis and Causal effect analysis have been discussed. The rationale for the appropriate use of these methods to investigate the topological expression of placental histamine receptors, regulation of histamine synthesis and production, the effects of elevated histamine on gene expression, and functional roles of the elevated histamine regulated genes in human placenta is also critically discussed.

Results: The findings show that defective Histamine-DAO-Axis (dHDA) underpins PE, and this defect may be precipitated in early pregnancy, thus predating the onset of the clinical manifestation of PE in mothers who later develop the complication; histamine receptors H1 and H2, and DAO messages are expressed in juxtapositions at the foeto-maternal interface, Histidine Decarboxylase (HDC) HDC activity is elevated in PE placentae; and while elevated histamine up-regulates the proteins for Th1-like cytokines, it also down-regulates DAO message expression after prolonged exposure in the placenta. The findings further show that lipopolysaccharides and pro-inflammatory cytokines including IL-10 and INF-γ increase histamine production in the placenta; and the histamine has a positive feedback loop regulatory relation with the pro-inflammatory cytokines.

Furthermore, the validation of Elevated Histamine Model (EHM), an *in vitro* model designed for studying histamine effect in placenta showed that placental micro explants ($\sim$50 mg) in long-term culture (explants that have undergone syncytiotrophoblast regeneration) at the liquid-gas interface in 8% oxygen is an optimum culture condition to study effects of histamine in the placenta. EHM produced RNA with quality akin to time zero pre-culture explants.

The works also led to the identifications of a core set of significant genes that are consistently expressed in both normal (NP) in PE placentae but at varying levels, and a further subset of significant genes expressed consistently only in PE placentae (PE specific genes). Comparison of EHM significant genes with the PE specific genes confirmed the presence of 270 consistently expressed genes that appear to underpin the effects of elevated histamine in the placenta with implications for PE pathogenesis. Further analyses of these EHM genes in PE placentae showed that the natural process by which pre-eclampsia develops is affected by the amount of histamine in the maternal blood and placenta, and the natural process by which pre-eclampsia develops is indirectly affected by histamine via covariate functional groups regulated by specific histamine regulated PE placental genes.

Conclusion: Histamine receptor genes are expressed at the foeto-maternal interface; DAO genes are expressed in the placenta; HDC activity levels are increased in PE placentae; there is cross-talk between histamine, DAO and cytokines in the placenta; elevated histamine regulated the expression of specific genes in the placenta and these genes are abnormally expressed in PE placentae; the functions of the histamine regulated genes also identified in PE placentae are involved in tissue morphology and possibly poor placentation, metabolic defects, endothelial dysfunction, inflammation, immunologic response, angiogenic and anti-angiogenic response in PE placentae. Therefore it is reasonable to conclude that the elevated histamine observed in PE would have pathophysiological roles in PE and early detection leading to effective control of maternal blood histamine levels has survival values and it's thus recommended.

Table of Contents

Contents

Abstract 4
Table of Figures 9
List of Tables 9
List of Abbreviations 10
Chapter 1 12
Introduction 12
1.1 Background 12
1.2 Theories of Pre-eclampsia Pathophysiology and the Role of Histamine 14
1.3 How could Histamine be the missing link in Pre-eclampsia Pathogenesis? 16
1.4 Academic Biography 21
1.5 Portfolio of Publications 23
1.6 Themes for Commentary 25
Chapter 2 Theme 1: Histamine links with Pre-eclampsia: Evidence from Systematic Reviews 26
2.1 Introduction 27
Account of originality at the time of publication 27
2.2 Contributions made by the reviews to the understanding of the impact of Maternal Blood Histamine on pre-eclamptic pregnancy outcome 28
2.2.1 The Roles of Pre-formed and De Novo Histamine in Pregnancy 28
2.2.2 The Sources of Histamine during Pregnancy 30
2.2.3 Histamine Metabolism in Pre-eclampsia 31
2.2.4 Association of diminished maternal DAO activity and increasing blood histamine with Pre-eclampsia 35
2.3 Conclusion 41
Chapter 3 Theme 2: Methodological Considerations- Model for studying *ex vivo* placental gene expression in response to histamine 42
3.1 Introduction: 43
3.2 Sub-section (1): Contributions made to the knowledge and understanding of Patients, the Placenta and Tissue Culture (#2 - #11) 44
3.2.1 The Patient 44
3.2.2 Justification for using Placental Explants 45
3.2.3 Tissue sampling 46
3.2.4 Effects of Culture Duration, Explant Size and Oxygen on Explant Morphology (Publication #3) 47
3.2.5 Modelling Defective Histamine-DAO-Axis (publications #9 - #11) 50
3.3 Sub-section 2: Justification for evolution of methodological techniques and contributions knowledge: - Transition from Single Gene to Multi-gene Analysis 52
3.4 Sub-section 3: Microarrays and Bioinformatics: Considerations, Rationale and Contributions to Knowledge of Placental Gene Expression 53
3.4.1 Microarray Hybridisation (Publications #4, #9 and #11) 53
3.4.2 Rationale for using Loop microarray design (Publications #4, #9 and #11) 54

3.4.3 Dealing with the impacts of variances (Publications #4, #9 and #11) 57
3.4.4 Validation of the Microarray Design: Biological Relevance of Pre-culture explant as baseline control for histamine treated cultured explants (Publication #3 & #4) 59
3.4.5 Rationale for Bioinformatics methodologies (Publications #2, #4 and #11) 65
3.5 Conclusion....70
Chapter 4 Theme 3: Understanding causes of elevated Histamine in Pregnancy and Pre-eclampsia....72
4.1 Introduction: 73
4.2 HDC activity in PE placentae (Publication #5) 74
4.3 Effects of Cytokines and Endotoxins on Histamine Production in the Placenta (Publication #6)....76
4.4 Histamine receptors and DAO mRNA are expressed in human placenta (Publication #7) 79
4.5 Conclusion....80
Chapter 5 Theme 4: Effects of histamine on placental genes expression: Implications for pre-eclampsia 81
5.1 Introduction 82
5.2 Histamine has regulatory feedback loop with Placental DAO (Publication #6 & #8)....83
5.3 Histamine regulates placental Th-1/Th-2 regulation (Publication #6) 85
5.4 Uncovering the complexities of PE genetic pathways (Publication #2) 89
5.5 Histamine regulates specific genes in pre-eclamptic placenta (Publications #9, 10 & #11) 94
5.5.1 Global Changes in EHM Gene Expression 94
5.5.2 Determination of the plausible effects of histamine in PE Placentae 97
5.5.3 Identification of causal effect of histamine specific genes in PE 103
5.6 Discussions and Conclusion 106
Chapter 6 Final Conclusion 110
6.1 Reflection on contributions made by the publications to the knowledge and Science of Placental Genomics and Pre-eclampsia 110
6.1.1 The placenta expresses histamine receptors, and histamine production and elimination in the PE placentae are impaired 110
6.1.2 Elevated histamine in the placenta has functional roles: it regulates production of Th-1/Th-2 like cytokines in the placenta 112
6.1.3 Optimum culture condition is fundamental for appropriate investigation of histamine effect in human placenta and implications in PE 113
6.1.1 Histamine has complex functions in the placenta: it regulates genetic pathways that have implications in PE placentae....115
6.1.2 Is histamine the missing link in PE pathophysiology? 116
6.2 Reflection on my contributions and my professional development as a research practitioner 119
6.3 Suggestions for future developments 123
6.3.1 Early Maternal Blood Pregnancy Histamine and links with Preeclampsia 123
6.3.2 Histamine Intolerance and Pregnancy Outcome 124
6.3.3 Genetic Variations in histamine pathways and Pregnancy Outcomes 126
6.3.4 Validation of HSPE genes and curation of histamine regulated pathways in PE placentae 126
6.3.5 Histamine effects on CD3ξ signal transduction in placental T and NK cells 127
Reference....128

Appendix 144

Table of Figures

Figure 1-1: Theoretical Concepts for Pre-eclampsia Pathogenesis 15
Figure 1-2: Summary of Systemic Effects of Elevated Histamine 18
Figure 1-3: Conceptual Framework for Histamine in Pre-eclampsia Pathogenesis 20
Figure 2-1: Metabolism of histamine by ascorbic acid 32
Figure 2-2: Mean plot of normal maternal blood histamine levels 36
Figure 2-3: Rodent Oestrous and Gestational Histamine 36
Figure 2-4: Normal and PE Maternal Blood Histamine levels superimposed 39
Figure 2-5: Normal Trimesters and Pre-eclamptic Maternal Blood Histamine 39
Figure 2-6: Normal and PE Maternal Blood Histamine levels Compared 40
Figure 3-1: Microarray Experiment Culture flowchart 54
Figure 3-2: Microarray Experiment Design 56
Figure 3-3: Biological Relevance Network Analysis of Time zero, POC and AOC treated Explants 61
Figure 3-4: Expressed genes in POC and AOC explants 63
Figure 4-1: Cytokines and Endotoxin effect on Placental Histamine Production 77
Figure 4-2: Effects of Cytokines and Endotoxin on Placental histamine production after 24 hours of culture 78
Figure 5-1: Effects of Histamine and Cytokine Synthesis and Production 87
Figure 5-2: Ratio of INF-γ and IL-10 Expression in the Placenta 88
Figure 5-3: Plots of differentially expressed among EHM and Control Samples 96
Figure 5-4: Causal effect identification of Elevated Histamine on Pre-eclampsia 104
Figure 6-1: Histamine fits the missing link in Pre-eclampsia Pathogenesis 118

NB: All figures used or presented here are original, either produced specifically for this commentary or taken from one of the publications submitted here for the award.

List of Tables

Table 2-1: Deamination and Methylation Metabolic Rates in Pregnancy 33
Table 2-2: Deamination to Methylation Ratio 34
Table 2-3: Clinical Symptoms of High Blood Histamine and Pre-eclampsia 37
Table 2-4: Normal Histamine and Pregnancy Histamine Levels 38
Table 3-1: Characteristics of Placentae 44
Table 5-1: Differentially Expressed Genes in NP and PE Placentae 90
Table 5-2: Histamine Specific Genes in PE Ontologies 100
Table 5-3: Histamine Regulated Genes Performing 80% of Histamine Mediated Functions in PE 101
Table 5-4: Histamine Mediated Pathways with Leading Edge Genes 102
Table 6-1: Summary of My Contributory 119
Table A-1: Maternal Blood Histamine Levels 144
Table A-2: Maternal Blood Histamine Trimester Multiple Comparisons 144
Table A-3: Maternal Urine Histamine after Aminoguanidine Treatment 145

List of Abbreviations

The following table is a list of abbreviations commonly used in the thesis. Gene symbols are not included in this list but in the respective gene ID tables.

Abbreviation	Meaning
AA	Ascorbic Acid
AGE	Absolute Gene Expression
ANOVA	Analysis of variance
AOC	Atmospheric Oxygen Concentration
BP	Biological Process
BSA	Bovine serum albumin
CADM2	cell adhesion molecule 2
CC	Cellular Component
cDNA	complementary deoxyribonucleic acid
CHE	Consistent High Expression
CLE	Consistent Low Expression
C/S	Caesarean Section
Ct	cycle threshold
CTAP-III,	Connective tissue-activating peptide III
DAG	Directed Acyclic Graph
DAO	Diamine Oxidase
dH2O	Distilled Water
dHDA	Defective Histamine-DAO-Axis
DiAG	Diacylglycerol
DLK1	delta-like 1 homolog (Drosophila)
DMEM	Dulbecco's modified eagle's medium
DMR	Deamination to Methylation Ratio
DMSO	Dimethyl sulphoxide
ECL	Enhanced chemiluminescence
ECM	Extracellular matrix
EDTA	Ethylene diamine tetraacetic acid
EHM	Elevated Histamine model
ESTs	Expressed sequence tags
EtOH	Ethanol
EVT	Extravillous trophoblasts
FDR	False discovery rate
FFPE	Formalin-fixed paraffin-embedded
FMI	Foeto-maternal Interface
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GDA	guanine deaminase
GEP	Gene expression profile
GO	Gene Ontology
GSA	Gene Set Analysis
GSEA	Gene Set Enrichment Analysis
H&E	Haematoxylin and eosin
H1R	Histamine Receptor 1
H_2O_2	Hydrogen peroxide
H2R	Histamine Receptor 2

H3R	Histamine Receptor 3
H4R	Histamine Receptor 4
HA	Histamine
hCG	Human Chorionic Gonadotrophine hormone
HDACs	Histone deactylases
HDC	Histidine Decarboxylase
5(S)HPETE	5(S)-Hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid
HG	Hyperemesis Gravidarum
HMT	Histamine n-methyl transferase
HrHRF	Human Recombinant Histamine-Releasing Factor
HRP	Horseradish peroxidase
HSPE	Histamine Specific Genes (expressed) in Pre-eclamptic Placentae (PE)
HUVEC	Human Vascular Endothelial Cell
IHC	Immunohistochemistry
ImAA	Imidazole Acetic Acid
ISH	In situ hybridisation
KEGG	Kyoto Encyclopedia of Genes and Genomes
LS KS	Fisher (LS) and the Kolmogorov-Smirnov (KS)
MCP-1,	Monocyte chemoattractant protein-1
MCS	multiple cloning sites
MeHA	Methyl-histamine
MF	Molecular Function
MIAA	Methyl-imidazole acetic acid
MIP-1 a,	Macrophage inflammatory protein 1 alpha
mRNA	Messenger Ribonucleic Acid
MSigDB	Molecular Signature Database
NGF	Nerve growth factor
NP	Normal Placenta
NVD	Normal Vaginal Delivery
NVP	Nausea and Vomiting in Pregnancy
OMH ratio	oxidized histamine/methylated histamine
LPS	Lipopolysaccharide
PAA	Phenotype Average Analysis
PBS	Phosphate-buffered saline
PE	Pre-eclampsia
PLC	Phospholipase-C
POC	Physiological Oxygen Concentration
PLP (P5P)	Pyridoxal phosphate (pyridoxal-5'-phosphate)
PMA	Phorbol myristate acetate
QC	Quality Control
RANTES	regulated upon activation, normal T cell expressed and secreted
RGE	Relative Gene Exression
RMA	Robust Multiarray Average
rRNA	Ribosomal Ribonucleic Acid
RT-PCR	Reverse Transcription - Polymersase Chain Reaction
SEM	Standard Error of the Mean
TFT	Transcription Factor Targets
WebGestalt	WEB-based GEne SeT AnaLysis Toolkit

Chapter 1

Introduction

1.1 Background

Pre-eclampsia (PE) is a major cause of perinatal mortality and it complicates up to 8% of all pregnancies in Western countries (Khan et al. 2006, Duley 2009, WHO 2014). It is one of the top 4 causes of maternal mortality and morbidity worldwide, causing 10 to 15% of maternal deaths (Duley 2009, Say et al. 2014, WHO 2014). PE is characterised by new hypertension (blood pressure of ≥140/90 mmHg) on two separate readings at least 6 hours apart presenting after 20 weeks' gestation in conjunction with clinically relevant proteinuria (≥300mg) per 24 hours and/or with symptoms, and/or biochemical and/or haematological impairment (Tranquilli et al. 2014, NICE 2017).

The symptoms, biochemical and haematological impairment are related to maternal organ dysfunction including renal insufficiency (creatinine >90 μmol/L; 1.02 mg/ dL); liver involvement (elevated transaminases – at least twice upper limit of normal ± right upper quadrant or epigastric abdominal pain); neurological complications (examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, and persistent visual scotomata); haematological complications (thrombocytopenia – platelet count below 150,000/dL, DIC, haemolysis) (Tranquilli et al. 2014).

PE is a multifactorial disease, and while there is a cautious acceptance of links between familial concordance and maternal polymorphism in the pathogenesis of the disorder (Sutherland et al. 1981, Chesley & Cooper 1986, Arngrimsson et al. 1990, Cincotta & Brennecke 1998, Esplin et al. 2001, Nilsson et al. 2004, Buurma et al. 2013, Sitras et al. 2015), the placenta is suggested as the primary cause of PE (Myatt 2002, Matsuo et al. 2007).

The notion denoting the placenta as the main cause of PE is embedded in the fact that the delivery of the placenta is the only known intervention that cures PE (Matsuo et al. 2007). Therefore, the links between PE and placentation is well investigated. Placentation is basically the process of formation and growth of the placenta and it involves tight regulation of trophoblast proliferation, differentiation and invasion. It is initiated shortly after implantation, when the blastocyst makes contact with the epithelial lining of the uterus (Damsky et al. 1997, Genbacev & Miller 2000). The process enables the trophoblast cells to breach and colonise uterine epithelia, the endometrium and surrounding vasculature to allow nutrient supply to the developing foetus (Pijnenborg 1988, Castellucci et al. 1990, Genbacev & Miller 2000, Reynolds et al. 2010).

The invasion process involves differentiation of the villous cytotrophoblast. Placental villi consisting of inner mesenchymal cells surrounded by a monolayer of mononuclear villous cytotrophoblast stem cells develop (Fisher & Damsky 1993, Meekins et al. 1994, Lyall 2006, Salamonsen et al. 2009). The cytotrophoblast layer differentiates by either fusing to form the overlying multinucleated syncytiotrophoblast or develops into extravillous trophoblasts (EVT) in anchoring villi

(Nelson et al. 1986, Irving et al. 1995, Damsky et al. 1997, James et al. 2005, Baczyk et al. 2006). The EVT which migrates away from the placenta differentiates further to form invasive extravillous trophoblast (iEVT) (Fisher & Damsky 1993, Zhou et al. 1997, Norwitz et al. 2001).

It seems that a major outcome of invading EVT is the adaptation of the decidua to sustain pregnancy through remodeling of uterine spiral arteries in readiness for increased blood supply required in the second and third trimesters, and sequestration of this blood supply from maternal vasoconstriction (Pijnenborg 1988, Meekins et al. 1994, Naicker et al. 2003, James et al. 2006, Pringle et al. 2010). The invading EVT thus remodel spiral arterioles by replacing maternal endothelium (Meekins et al. 1994, Lyall 2003, Pijnenborg et al. 2006a, Pijnenborg et al. 2006b, Harris et al. 2010, Pringle et al. 2010), and this morphogenesis is suggested to increase the diameter and compliance of the spiral arteries to permit a 12-fold increase in uterine blood flow to meet the demands of the growing foetus in later gestations (Martin 1965, Pringle et al. 2010). It is during the process of placentation that it is believed defective histamine regulation could lead to a cascade of events that trigger PE and some trophoblast related complications of human pregnancy.

1.2 Theories of Pre-eclampsia Pathophysiology and the Role of Histamine

Several theoretical concepts (Figure 1-1A) including abnormal angiogenic and anti-angiogenic factors, poor placentation, defective immunologic response, metabolic disorders, inflammation, and endothelial dysfunction have been proposed to explain the pathogenesis of PE (Mol et al. 2016). The conceptualisation of these theories as proposed to explain the pathophysiology of PE is complex (Redman 2014). Thus to

help with the understanding of the pathophysiology of PE, a two-stage model has been advocated (Redman 1991, Sargent et al. 2006, Gammill & Roberts 2007). Stage 1 speculates that the initiating perturbation of PE is a relative deficient placentation. The deficient placentation when established is suggested to lead to the precipitation of systemic inflammatory response that consequently results in the maternal pathophysiology of PE (Stage 2) (Redman et al. 1999, Roberts & Hubel 2009).

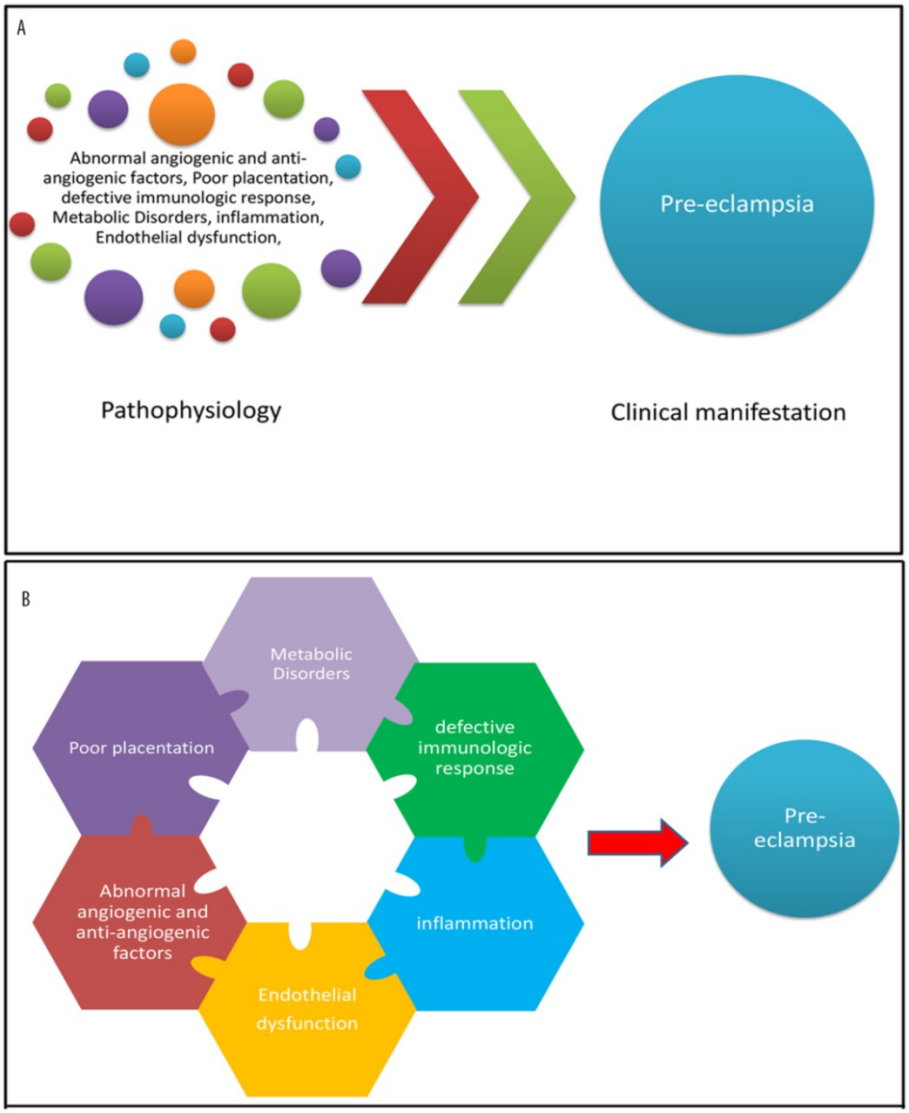


Figure 1-1: Theoretical Concepts for Pre-eclampsia Pathogenesis

Figure A: This is a theoretical conceptualisation of the pathophysiological changes associated with pre-eclampsia (PE); Figure B depicts the missing link in the pathophysiology of PE. Figure A is based on Redman 1991, Redman et al. 1999, Sargent et al. 2006, Gammill & Roberts 2007, Roberts & Hubel 2009, Redman 2014, Ilekis et al. 2016, Mol et al. 2016. Figure B is based on my re-interpretation of Figure A.

While it is accepted that the pathogenesis of PE is multifactorial and defective placentation plays critical role in the origins of PE (Ilekis et al. 2016), the changes or factors that link the pathophysiological presentations in the placenta to the clinical manifestation of PE have not been fully understood. In other words, it is acknowledged that stage 1 events are not sufficient to cause the stage 2 downstream maternal syndrome (Roberts & Hubel 2009), and that there must be a missing link with plausible functions to interact with both placental and maternal events (genetic, morphological changes, physiological adaptation, behavioural, environmental) to precipitate the clinical manifestation of PE (Figure 1-1B). It was upon the basis of this proposition that it was hypothesised histamine could be the missing link in the pathophysiology of PE, and therefore the ensuing studies commenced.

1.3 How could Histamine be the missing link in Pre-eclampsia Pathogenesis?

It was observed that trophoblast related complications of human pregnancy including pre-eclampsia, preterm labour (PL), spontaneous abortion (SA) and *hyperemesis gravidarum* (HG) present with elevated blood histamine and perilous but varying pregnancy and maternal outcomes (Kapeller-Adler 1941, Kapeller-Adler 1949, Southren et al. 1963, Beaven et al. 1975, Dubois et al. 1977, Minagawa et al. 1999, Redman et al. 1999). Histamine has a well-characterised role in the pathogenesis of emesis (Brunton 1996), and experimental elevation of histamine in humans causes nausea and vomiting with a clear relationship between dosage of infused histamine and severity of nausea and vomiting (Kapeller-Adler 1941, Ind et al. 1982). In addition to emesis, intravenous infusion of histamine dose dependently causes rise in heart (pulse) rate, temperature and flushing, or fall in diastolic pressure, and feeling of constriction in the head, headache (minor and severe depending on dose)

with or without visual disturbances, cyanosis, inspiratory dyspnoea, convulsions and unconsciousness (Kapeller-Adler 1941, Ind et al. 1982, Kaliner et al. 1982). These symptoms are common in HG and PE, however no further commentary will be provided on the links between histamine and HG. Similarly, pre-term labour presents with elevated histamine (Kirkel' et al. 1983, Caldwell et al. 1988, Riedel et al. 1989, Donahue et al. 1995, Kramer et al. 1995, Wen et al. 2001). While the pathophysiology of pre-term labour involves inflammatory processes similar to PE, the aetiology of pre-term labour is different from PE (Bytautiene et al. 2004a, Steel et al. 2005, Romero et al. 2014), and therefore no further consideration is also given to it in this commentary. Indeed, these complications of human pregnancy have in common precipitation of histamine-like inflammatory response as underlying pathogenesis (Regan 1992, Sacks et al. 1998, Minagawa et al. 1999, Sacks et al. 1999), nonetheless, due to major differences in the respective aetiology further discussion is herewith focused on histamine and PE.

Histamine is a pleiotropic amine, which appears in tissues of most vertebrates and invertebrates, and the histamine forming capacity of tissues with a net whole blood histamine flow within 0.1μM range (plasma equivalent in 2.0nM range) is associated with various physiological functions such as gastric acid secretion, immuno-modulation, cell proliferation and differentiation, tissue growth and wound healing (Beaven 1982, Falus & Meretey 1992, Kahlson et al. 1960a, Rivera et al. 2000, Tetlow & Woolley 2003). In humans, the normal blood histamine follows a bio-rhythmic change (Rehn et al. 1987), which is maintained by a balanced (Section 2.2.2 below) between the activities of the synthesising enzyme histidine decarboxylase (HDC 4.1.1.22), and the metabolising agents including the copper

containing diamine amine oxidase (DAO: EC 1.4.3.6), histamine N-methyltransferase (HNMT: EC 2.1.1.8) and plasma ascorbic acid (Beaven 1982, Clemetson 1980, Kahlson & Rosengren 1971, Wantke et al. 1998) (section 2.2.3 below).

The elevated blood histamine presents with both clinical symptoms and pathological effects including: oedema, hypertension, proteinuria, nausea & vomiting, headaches, platelet coagulation, endothelial damage, oxidative stress, dysregulation of glucose and lipid metabolism and immune reaction (summarised in Figure 1-2) (Horakova et al. 1977, Beaven 1978, el-Gendi et al. 1988, Shterental' et al. 1991, Thomas et al. 1991, Ebeigbe & Talabi 2014). These histamine effects are akin to maternal clinical manifestation or symptoms of PE.

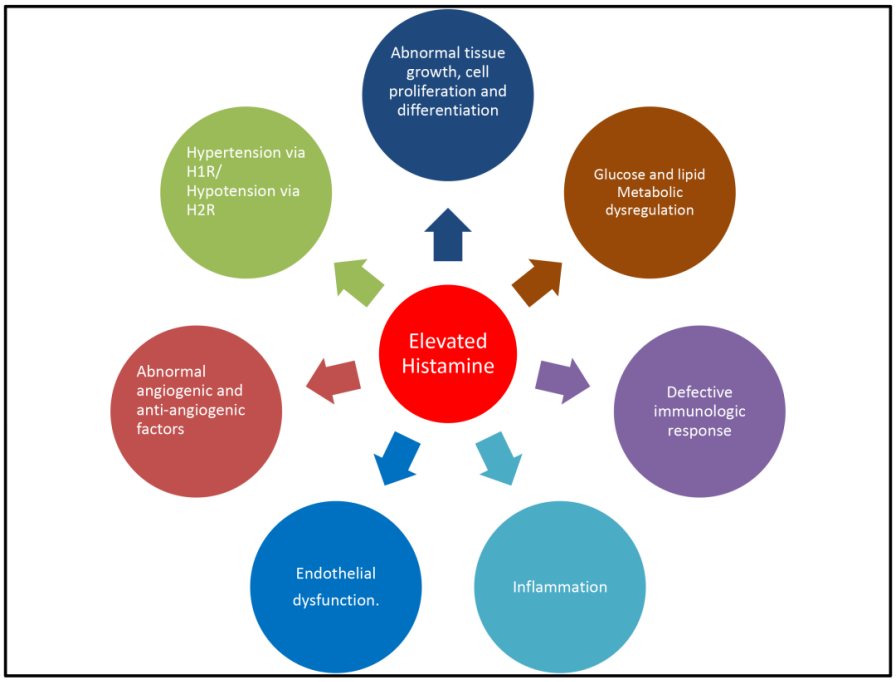


Figure 1-2: Summary of Systemic Effects of Elevated Histamine
Figure shows known effects of elevated histamine in human tissues

Also, randomised clinical trials of histamine augmentation confirmed that elevated histamine prevents inhibition of T cells and natural killer cells by monocyte-derived reactive oxygen metabolites and synergizes with IL-2 to up-regulates Th1 cell

responses in patients with melanoma (Asemissen et al. 2005). This report was revelatory, because it not only confirmed that ligand overload triggers dose dependent histamine post-receptor cascade of immunological responses but also supported a previous argument that elevated histamine up-regulates *in vivo,* thymic and pro-inflammatory cytokines as key pathological pathways (Falus & Meretey 1992, Arad et al. 1996, Panina-Bordignon et al. 1997, van der Pouw Kraan et al. 1998, Darmochwal-Kolarz et al. 1999, Hellstrand et al. 2000, Jutel et al. 2001).

Considering that Natural Killer (NK) and extrathymic T cells are abundant in the human placenta, and numerical changes and functional activation of NK and T cells are more prominent in the blood and urine of patients with PE than in normal pregnant women (Regan 1992, Sacks et al. 1998, Darmochwal-Kolarz et al. 1999, Minagawa et al. 1999, Redman et al. 1999, Saito et al. 1999); and that the activation of granulocytes, NK cells, and extrathymic T cells are suggested as essential for the maintenance of pregnancy but the over-activation thereof may be responsible for the onset of pregnancy disorders (Wegmann et al. 1993, Minagawa et al. 1999, Saito et al. 1999, Weetman 1999, Saito & Sakai 2003). It was asked whether the elevated histamine in PE could have effects, similar to that observed in peripheral tissues (Figure 1-2) and thus plausibly function as the missing link in the PE pathophysiology.

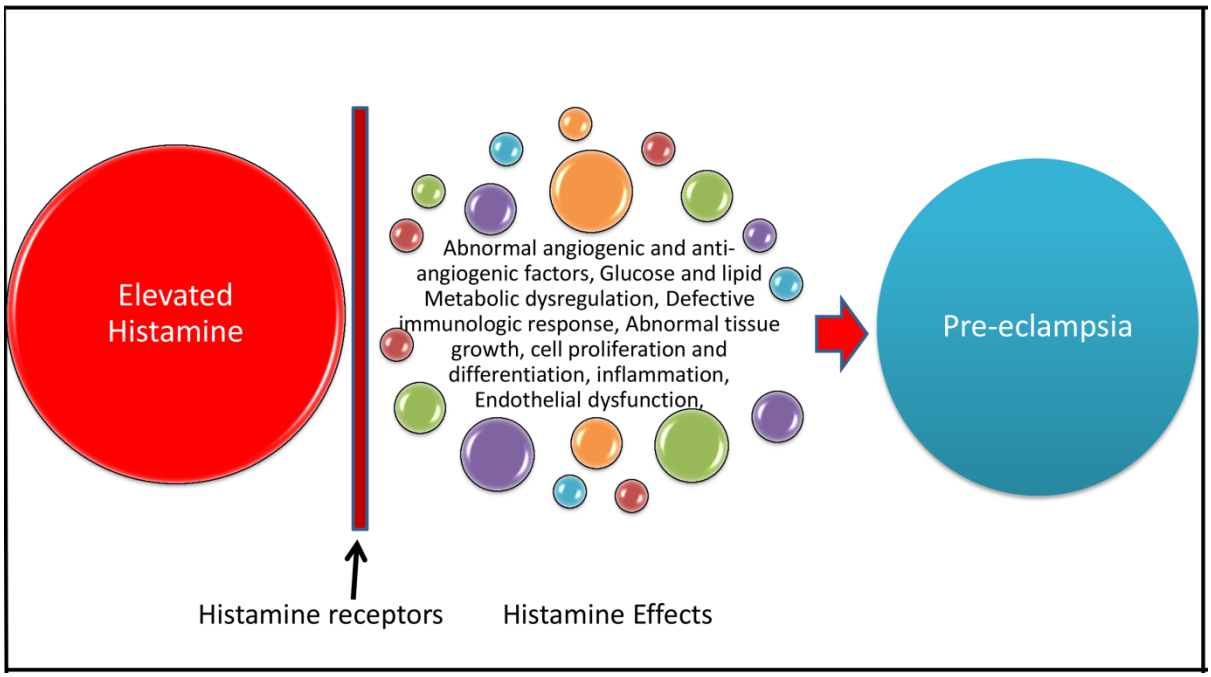


Figure 1-3: Conceptual Framework for Histamine in Pre-eclampsia Pathogenesis
Figure depicts the theoretical framework linking histamine to PE pathophysiology

To answer this question, it was hypothesised that for histamine to have effects in the placenta, histamine receptors should be expressed at the foeto-maternal interface, and the histamine receptors thus expressed would regulate placental gene expression (Figure 1-3). It was further hypothesised that for histamine to be established as the missing link in the pathophysiology of PE, elevated histamine would regulate specific genes that are also expressed in pre-eclamptic placentae, and the ontologies of these genes would be associated with pathophysiological changes implicated in pathogenesis of pre-eclampsia.

1.4 Academic Biography

With over 29 years' experience in healthcare and higher education (HE) including at least 16 years in senior positions in HE, I have made notable contributions to healthcare workforce development through both undergraduate and postgraduate biomedical science, nursing and medical workforce training. I studied Human Physiology and Pharmacology at the University Hertfordshire and later, Human Reproductive Biology at the Imperial College London. Clinically, I undertook training in nursing, midwifery and clinical andrology, and worked for over 10 years in both public and private healthcare institutions in London including St Samaritan's Hospital for women, London, Edgware General Hospital London, St Mary's Hospital Paddington, London and the NHS Direct.

Except for a brief period of time working as a visiting researcher at the then Institute of Obstetrics and Gynaecology of the former Royal Postgraduate Medical School in Queen Charlotte's and Chelsea Hospital, I have over the past 20 years worked sorely in HEI, mainly at the University of West London with a focus on teaching and research. My early career in HE started as basic science tutor for pre-registration nurses, focusing on teaching Physics and Chemistry as applied to Nursing and later as a lecturer in Life and Applied Sciences at Wolfson Institute of Health and Human Science. In later years, my teaching is mainly focused on precision and personalised medicine, genomics, bioinformatics, service design and improvement in healthcare,

I focus my technical expertise in two predominant areas: in the identification of novel diagnostic biomarkers and therapeutic targets for complications of human pregnancy and in development of high quality pedagogy to provide accessible world class

education. In these areas, I have authored other research and scholarly articles including technical reports, research, systematic reviews, and conference proceedings; some of which are presented here for the award of PhD by publication.

1.5 Portfolio of Publications

The following papers were therefore published from the investigations undertaken to answer the questions above and they form the basis for the award of PhD by Published work. The publications fall under the discipline of Genomics and Bioinformatics. The citations are numbered in the order in which they appear in the commentary rather than by year of publication.

1. **Brew O**, Sullivan MH. (2006) The links between maternal histamine levels and complications of human pregnancy. J Reprod Immunol. Dec;72(1-2):94-107
2. **Brew, O**., Sullivan, M.H. & Woodman, A. (2016) Comparison of Normal and Pre-Eclamptic Placental Gene Expression: A Systematic Review with Meta-Analysis. PloS One, 11(8), p.e0161504.
3. **Brew, O**., Nikolopoulou, E., Hughes, A., Christian, M., Lee, Y., Oduwole, O., Sullivan, M.H.F. & Woodman, A. (2016) Quality of placental RNA: Effects of explant size and culture duration. *Placenta*, 46, p.45-48.
4. **Brew, O.** & Sullivan, M.H.F. (2017) Oxygen and tissue culture affect placental gene expression. *Placenta*, 55, p.13-20.
5. **Brew O**, Lakasing L, Sullivan M. (2007) Differential Activity of Histidine Decarboxylase in Normal and Pre-eclamptic Placentae. *Placenta*. May-Jun;28(5-6):585-7.
6. **Brew O**, Sullivan, M. H., Roller S (2005), "Regulatory loops between cytokines and histamine in the human placenta", *Placenta*, vol. 26, no. 8-9, p. A.52
7. **Brew, OB** & Sullivan MH (2001) Localisation of mRNAs for diamine oxidase and histamine receptors H1 and H2, at the foeto-maternal interface of human pregnancy, *Inflammation Research,* 50(9): 449-452
8. **Brew, O**. & Sullivan, M.H.F. (2007) Histamine regulates placental diamine oxidase mrna expression - Evidence for a feedback loop decreasing histamine production in pregnancy? Placenta, 28(8 - 9), p.A71.
9. **Brew O**, Sullivan MHF (2015) Placental Gene Expression in Response to Histamine and Oxygen, *Gene Expression Omnibus*. Series accession number GSE74446 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74446)
10. **Brew O**, Sullivan M. (2017) Elevated Histamine Model: A Protocol for an ex vivo model for in vitro study of histamine effect on placenta. *Protocols.io* dx.doi.org/10.17504/protocols.io.jigckbw
11. **Brew O**, Sullivan MHF. Comparison of elevated histamine model and pre-eclamptic placental gene expression, *Unpublished*

Rationale for the inclusion of Publication items #6, #8 and #11

All articles submitted here are currently published as original research except items #6, #8, and #11. Items #6 and #8 were originally published as conference proceedings respectively at the International Federation of Placental Association (IFPA) in 2005 Glasgow, UK, and 2007, Kingston, Ontario, Canada. The abstracts from these conference proceedings are published as indicated in the citations. In this document, the findings from these studies are discussed in more detail to provide context to the work assembled herein. Particularly, item #6 supports publications items #5 and #7. Publication item #5 reported on the findings that HDC activity is increased in PE relative to normal placentae. Item #7 on the other hand shows that histamine receptors are expressed in the placenta. Studies reported in item #6 were conducted to test whether elevated histamine has effect in human placenta. The findings are thus discussed in detail in this context to provide unequivocal evidence that elevated histamine regulates the expression of key cytokines implicated in PE. Similarly, findings from item #8 are discussed below in this context of publications items #9 and #10 to collaboratively demonstrate the evidence that elevated histamine in the placenta not only affect the regulation of key placental proteins but also perturb the expression DAO genes. Item #11 is currently undergoing revision following submission for publication, and the content of this paper is discussed to provide depth and breadth, and contextualisation of the evidence herewith indicated.

1.6 Themes for Commentary

Commentary is hereby provided with four themes to show the links and coherence between the published works. The themes provide an account for originality of the works at the time of each project or publication, and identify my contributions to the subject of placental genomics, the regulatory roles of histamine and implications in pre-eclampsia. The commentary also provides reappraisal of the originality of the work and reflection about my professional development as a research practitioner and suggestions for future developments. The themes for the commentary are:

1. **Histamine links with Pre-eclampsia: Evidence from Systematic Reviews**
2. **Methodological Considerations- Model for studying *ex vivo* placental gene expression in response to histamine**
3. **Understanding causes of elevated Histamine in Pregnancy and Pre-eclampsia**
4. **Effects of histamine on placental genes expression: Implications for pre-eclampsia**

Chapter 2

Theme 1: Histamine links with Pre-eclampsia: Evidence from Systematic Reviews

In this chapter:

- The commentary is based on the findings from publication item #1:

  Brew O, Sullivan MH. (2006), The links between maternal histamine levels and complications of human pregnancy. J Reprod Immunol. Dec;72(1-2):94-107

- The results from systematic review of evidence that clarified our understanding of modalities of histamine production and regulation in human pregnancy are discussed.

Highlights:

- The placenta is the major source of maternal blood histamine during pregnancy.
- Maternal blood histamine level in normal early pregnancy (up to gestational week 8) is similar to pre-pregnancy levels.
- Direct oxidative deamination metabolic pathway by DAO, indirect deamination involving HNMT and DAO and hydroxylation involving ascorbic acid are the active histamine metabolic pathways during pregnancy.
- Histamine oxidative deamination by DAO is the dominant metabolic pathway in pregnant compared to non-pregnant women.
- In normal pregnancy maternal plasma DAO activity exponentially increase from gestational week 8, and peaks at mid-trimester (~gestational week 24). Concurrently, maternal blood histamine levels progressively decline also from gestational week 8 with a mid-trimester (~gestational week 24) nadir, and this forms a normal Histamine-DAO-Axis (HDA).
- In PE, there is an apparent failure of the HDA and this is termed defective Histamine-DAO-Axis (dHDA).
- dHDA presents with a failure of maternal plasma DAO activity to exponentially rise from gestation week 8, leading to diminished DAO activity and elevated maternal blood histamine.
- Regression analysis of maternal blood histamine in PE suggests first and second trimesters histamine levels are significantly higher in PE compared to levels in normal pregnancy.
- The ratio of histamine oxidative deamination to methylation metabolites concentrations in maternal blood or urine has predictive value for early identification of PE.

2.1 Introduction

Account of originality at the time of publication

In 1910, Dale and Laidlaw published their seminal work on the effect of histamine on pregnancy outcome (Dale & Laidlaw 1910). This work showed for the first time that elevated histamine in uterine tissues: in this case, subcutaneous injection of histamine into cats in late pregnancy causes strong periodic uterine contractions of the tonic type with no expulsive value but strong enough to cause premature placental separation and intrauterine death of litters (Dale & Laidlaw 1910). Several publications since this seminal work showed associations between increasing maternal blood histamine and specific pregnancy complications such as PE.

Phylogenetically, histamine appears in tissues of most vertebrates and invertebrates, and the histamine forming capacity of tissues seemingly correlates with various physiological functions such as gastric acid secretion, immuno-modulation, cell proliferation and differentiation, tissue growth and wound healing (Kahlson et al. 1960a, Tasaka 1991, Falus & Meretey 1992, Rivera et al. 2000). At the point of initiating the systematic reviews, evidence that histamine was produced during pregnancy was not disputed. However, the sources and significance of the amine in human pregnancy was unclear. There was also no previous systematic review of the evidence to demonstrate coherent links between the varying histamine levels and specific complications of human pregnancy. Therefore, a series of systematic reviews with narrative synthesis and or meta-analysis were conducted. Databases (Medline, Pubmed, EMBASE) were searched with keywords histamine, diamine oxidase, pregnancy, pre-eclampsia and their variants in Jan 2005 and repeated in April 2006. A total of 208 articles were reviewed to examine the level and extent of

associations between maternal blood histamine and specific pregnancy outcomes to answer the following questions:

1. During pregnancy, does elevated maternal blood histamine originate from the maternal issues as compared to foetal tissues?
2. In pregnant women, does elevated blood histamine cause clinical symptoms?
3. Does blood histamine levels and metabolism during pregnancy differ between women with normal pregnancy and those who develop PE?

2.2 Contributions made by the reviews to the understanding of the impact of Maternal Blood Histamine on pre-eclamptic pregnancy outcome

2.2.1 The Roles of Pre-formed and De Novo Histamine in Pregnancy

The first set of reviews was conducted to determine the sources and the type, i.e., pre-formed and de novo histamine in human pregnancy. It was observed that the nature of histamine production in both vertebrates and invertebrates indicates two main sources for histamine appearance in tissues: pre-formed histamine from storage cells e.g., mast cells, basophils, and platelets; and nascent histamine or histamine produced de novo by induction of histidine decarboxylase (HDC) activity (Beaven 1982, Kahlson & Rosengren 1971). In both cases the pyridoxal phosphate dependent HDC enzyme metabolises histidine to produce histamine (Reite 1972, Beaven 1982), and its activity mostly serves as a rate-limiting step for histamine production (Kahlson et al. 1958a, Nilsson et al. 1959). It was also clear that diamine oxidase (DAO), histamine methyl transferase (HMT), Pyridoxal phosphate (PLP), and ascorbic acid (AA) were established as important metabolising agents for both nascent and pre-formed histamine (Cooper & Schayer 1956, Schayer & Karjala 1956, Nandi et al. 1974, Chatterjee et al. 1975).

Using thematic analysis, the review revealed that the pre-formed histamine functions more as a stereoadaptive-defence agent, where in both vertebrates and invertebrates it is mainly involved with immediate defence response attack as in antigeneic or predatory invasion (Reite 1972, Beaven 1982). In these instances the review further showed that pre-formed histamine commonly occurs in secretory glands and in their secretions, e.g. in many poisonous glands; in gastric mucosa, gastric glands and gastric secretions of most eutherians and ancestral vertebrates of the cyclostomes and cartilaginous fish types, like hagfish and dogfish respectively; and in salivary glands of octopus and squid (Reite 1972). These initial findings prompted a subsequent review to establish the most common type of histamine produced during human pregnancy.

The subsequent results showed that: (1) Pre-formed histamine is produced and stored in mast cells in both male and female reproductive apparatus e.g. human testes, seminal vesicles, prostate glands, uterine tissues, placenta and ovaries (Wicksell 1949, Purcell & Hanahoe 1991, Purcell 1992, Szelag et al. 2002a, Szelag et al. 2002b); (2) That the rate of turnover of pre-formed histamine in maternal uterus and the placenta is quite slow and therefore suggests that pre-formed histamine may have minor physiological roles in early pregnancy or during human pregnancy (Beaven 1982); (3) That *de novo* synthesised histamine characterised by HDC activity is prominent in pregnancy and is localised in cleaving embryo, fallopian tube and at implantation sites (Blanco et al. 1970, Bronson & Wallach 1977, Shrivastav et al. 1988, Cocchiara et al. 1987, Cocchiara et al. 1992, Menezo et al. 1993, Cocchiara et al. 1996, Sawin et al. 1997, Jankovic et al. 1998); (4) While the pre-

formed histamine in uterine and endometrial tissues has been associated with muscle contraction and immuno-protection of uterine or endometrial milieu (Bytautiene et al. 2003, Bytautiene et al. 2004b, Purcell & Hanahoe 1991, Thomson et al. 2000, Wicksell 1949, King et al. 2003), *de novo* histamine synthesised by endometrial epithelia is shown in animal models and human cell lines to initiate implantation, functions in anabolic processes like regenerative growth, cell differentiation and proliferation, imuno-modulation, placentation, and foetal development (Kahlson et al. 1958a, Kahlson et al. 1958b, Kahlson et al. 1960b, Connolly et al. 1962, Johnson & Dey 1980, Dey & Hubbard 1981, Hine et al. 1985, Cocchiara et al. 1987, Rivera et al. 2000, Zhao et al. 2000, Dey et al. 2004, Liu et al. 2004), and hence could have similar roles in human pregnancy.

2.2.2 The Sources of Histamine during Pregnancy

It became clear that histamine thus observed in human pregnancy could originate from the uterus, foetal membranes and or from the placenta. Therefore, a further review question was conducted to clarify the source of increasing histamine in human pregnancy. For this review, attention was focused on human studies which have investigated *in vivo* metabolism of ^{14}C-histamine, labelled at the 2nd position of the imidazole ring in women including during pregnancy.

Narrative synthesis was used to summarise the findings from the included studies (Schayer 1952b, Cooper & Schayer 1956, Nilsson et al. 1959, Schayer 1959, Lindberg 1963b, Lindberg 1963a, Lindberg et al. 1963a, Lindberg et al. 1963b, Lindberg & Tornqvist 1966). The synthesis showed that (1) when labelled histamine was injected directly into maternal cubital vein, less than 5% of the histamine was

detected in umbilical vein; (2) when labelled histamine was injected into umbilical artery, less than 0.3% of the histamine was detected in maternal urine; (3) when the labelled histamine was injected into the uterus of pregnant and non-pregnant women, 49% as compared to 36% of histamine was metabolised during uterine passage into maternal blood respectively in pregnant and non-pregnant women; and (4) that M-methyl imidazole acetic acid (MIAA) resulting from methylation of the nitrogen on the imdazole ring remote from the amine side chain was secreted into maternal circulation from the placenta. These findings therefore suggested that the placenta, and not the uterus or the foetus is the major source of increasing histamine in maternal blood during pregnancy, and thus focused our attention on the placenta as the potential medium where histamine could exert effect on pregnancy outcome.

2.2.3 Histamine Metabolism in Pre-eclampsia

After establishing that the placenta is the main source of elevated blood histamine during human pregnancy, a critical question about what causes the elevated histamine in PE pregnancy however remained unclear. Therefore, a further review was undertaken to determine if there was any association between histamine metabolism and pre-eclamptic pregnancy outcome.

Generally, histamine is metabolised via 2 major pathways: methylation and deamination pathways. In the methylation pathway histamine is metabolised via methylation at the imidazole nitrogen furthest from the ethylamine side chain (termed tele-N or Nt) by the enzyme histamine-N-methyltransferase (HNMT) to N-Methyl-histamine (NMH), and monoamine oxidase (MAO) then metabolises the NMH to M-methyl imidazole acetic acid (MIAA) (Schayer 1952b, Cooper & Schayer 1956,

Schayer 1956, Schayer & Karjala 1956, Rothschild & Schayer 1958, Schayer & Reilly 1975). In the deamination pathway, DAO metabolises histamine to imidazole acetic acid (ImAA) (Schayer 1952b).

Using narrative synthesis, the review showed that the methylation and deamination of histamine pathways accounts for about 80% of histamine metabolism during human pregnancy, and hydroxylation of histamine into hydantoin propionic acid by ascorbic acid (Figure 2-1) could account for between 10 -20% (Ahlmark 1944, Wicksell 1949, Kapeller-Adler 1952a, Kapeller-Adler 1952b, Cooper & Schayer 1956, Schayer & Karjala 1956, Lindberg 1963a, Bjuro et al. 1964, Kapeller-Adler 1965, Nandi et al. 1974, Chatterjee et al. 1975).

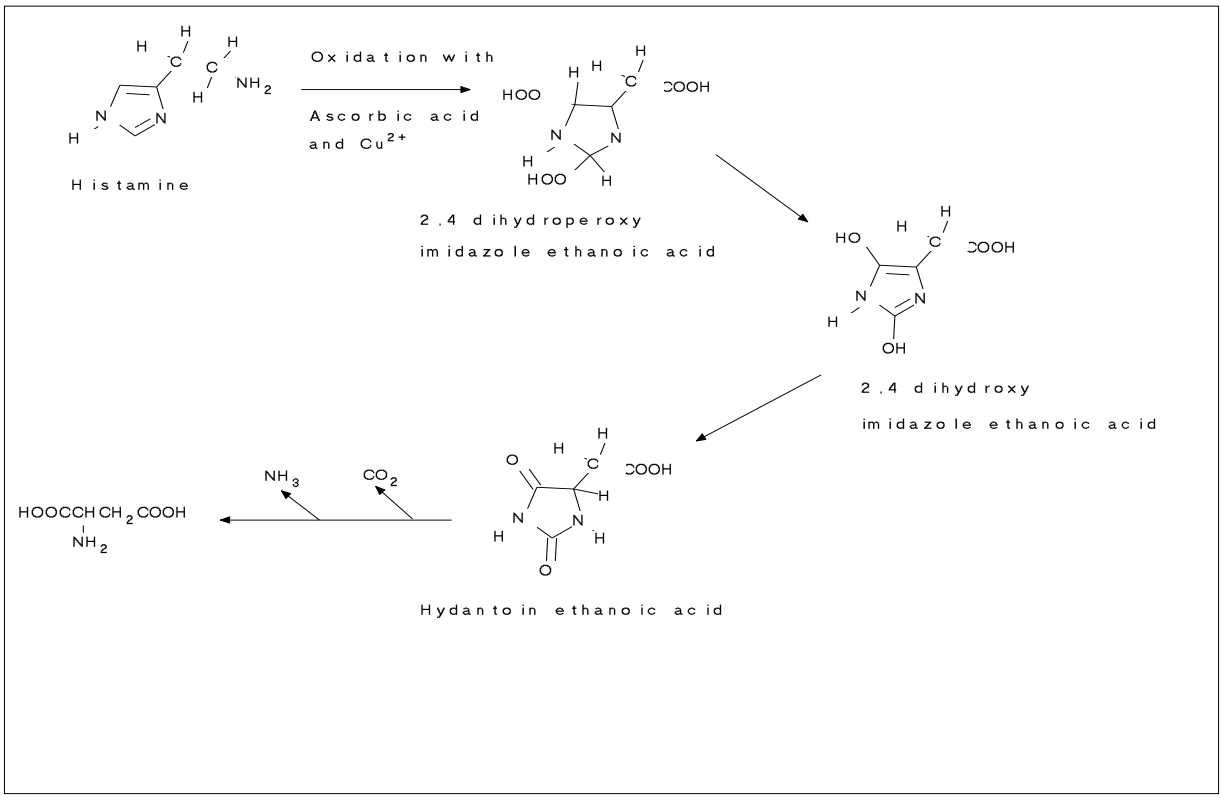


Figure 2-1: Metabolism of histamine by ascorbic acid

Figure 2-1 was written in 2006 based on the systematic review of metabolic effect of ascorbic acid on histamine.

The results also showed that the relative metabolism differed between maternal and non-pregnant women. Table 2-1 summarises the relative concentrations of histamine metabolites from the deamination and methylation pathways. The data (Table 2-1) shows an approximate 33% increase in histamine metabolism via direct deamination by DAO and 58% decrease in histamine metabolism via HNMT methylation during pregnancy compared to non-pregnant women, thus confirming that DAO is the dominant histamine metabolic pathway during pregnancy.

Table 2-1: Deamination and Methylation Metabolic Rates in Pregnancy

Histamine Metabolites	Non-pregnant women levels	Pregnant women levels	Average Change in Pregnancy
MIAA	32 – 59%	20 – 41%	-57.69%
ImAA	25 – 37%	26 – 53%,	32.7%

(Schayer 1952b, Nilsson et al. 1959, Lindberg 1963a); MIAA = M-methyl imidazole acetic acid; ImAA = imidazole acetic acid.

Somehow, the confirmation that DAO is the major histamine metabolising enzyme during human pregnancy was pedestrian. Nonetheless, a regression analysis revealed that the deamination to methylation ratio (DMR) of histamine metabolites in maternal blood has predictive value for pregnancy outcome, and this was novel (Table 2-2). Using a comparative analysis of the metabolites from pregnant and non-pregnant women, it was observed that the ratio of deaminated histamine metabolites (imidazole acetic acid) and the methylated metabolites (n-methyl-histamine, and methyl-imidazole acetic acid) were 4 and 2 folds respectively in pregnant and non-pregnant women arterial or venous blood (Table 2-2), and this was highly informative

of maternal blood capacity to control histamine within range associated with good pregnancy outcome prognosis.

Table 2-2: Deamination to Methylation Ratio

Relative abundance of Histamine Metabolites in Maternal Blood and Urine from Diamine Oxidase and Histamine N-Methyltransferase Activities					
Radio-labelled histamine metabolite	Sampling and radio-labelled histamine metabolite analysis count (cpm/ml)				
	Brachial Artery	Cubital Vein	Uterine Vein	Umbilical Vein	Urine
	Values in blood sample taken from 6 pregnant women (a)				From 4 pregnant women (b)
Ratio of ImAA ÷ (NMH + MIAA)	2.21	1.98	2.28	1.90	1.31
	Values in blood samples from 4 non-pregnant women (a)				From 3 non-pregnant women (b)
Ratio of ImAA ÷ (NMH + MIAA)	0.51	0.71	0.34	-	0.75
cpm = Counts per minutes; N-Methyl-histamine = NMH, Methyl-imidazole acetic acid = MIAA, Imidazole Acetic Acid = ImAA, (a) from (Lindberg 1963a), (b) from (Nilsson, LINDELL, Schayer, & Westling 1959)					

The analysis further showed that whilst the measurement of individual histamine metabolites in urine was erratic and non-predictive of pregnancy outcomes, the assessment of the ratio of the metabolites in urine or blood could provide a measure or competence of maternal blood to control histamine levels during pregnancy, and thus could predict plausible variations associated with PE as a plausible non-invasive model. Further work using prospective longitudinal studies however, is needed to test this emerging hypothesis.

In effect, these outputs suggested that there are three instead of two major histamine metabolic pathways during human pregnancy: (a) Direct DAO oxidative deamination metabolic pathway (b) indirect deamination pathway involving HNMT and DAO and

(c) hydroxylation pathway involving ascorbic acid. The work also suggested that a further review to examine the variations between maternal histamine metabolism in normal and complicated pregnancies could provide additional information to contribute to explaining the association between maternal blood histamine levels and PE. Therefore a further review was conducted to examine the mechanisms of histamine regulation during normal and complicated human pregnancies.

2.2.4 Association of diminished maternal DAO activity and increasing blood histamine with Pre-eclampsia

A further review using comparative analysis was conducted to examine the characteristics and profiles of maternal blood histamine and plasma DAO activity during normal and PE pregnancy. Twelve studies met the inclusion criteria for this analysis (Connolly et al. 1962, Southren et al. 1966a, Tryding & Willert 1968, Achari et al. 1971, Vanina et al. 1971, Beaven et al. 1975, Dubois et al. 1977, Sharma et al. 1981, Morgan & Hytten 1984, Sharma et al. 1984, Hine et al. 1985, Caldwell et al. 1988). The data synthesis was separated into animal and human profiles. The review showed that during normal pregnancy, maternal blood histamine levels gradually decrease below values found in healthy non-pregnant women (Figure 2-2), and this characteristic decline of gestational blood histamine was conserved in rodent models (Figure 2-3). In human normal pregnancy, the progressive decrease in maternal blood histamine (Figure 2-2) reaches a nadir at mid-gestation (weeks 20 and 24) and this was associated with a concurrent exponential rise in maternal plasma DAO activity from gestational week 8 onwards in normal pregnancies with a peak also at mid-gestational (weeks 20 and 24) (Southren et al. 1966a, Tryding & Willert 1968, Achari et al. 1971, Beaven et al. 1975, Dubois et al. 1977).

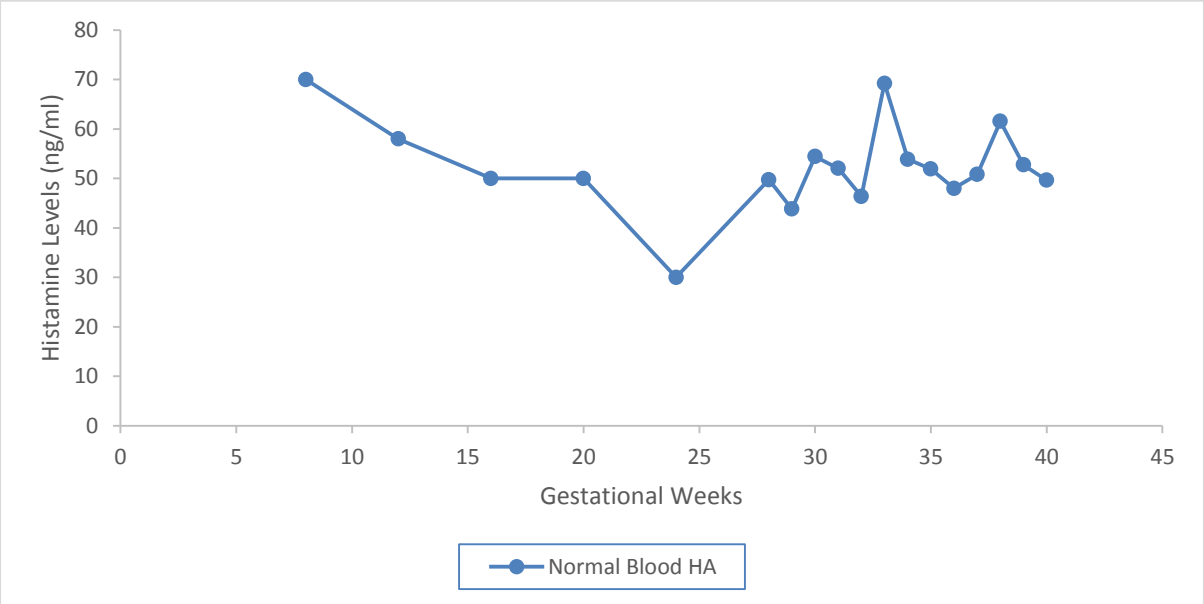


Figure 2-2: Mean plot of normal maternal blood histamine levels

(Southren et al. 1966a, Tryding & Willert 1968, Achari et al. 1971, Beaven et al. 1975, Dubois et al. 1977, Sharma et al. 1981, Sharma et al. 1984, Caldwell et al. 1988)

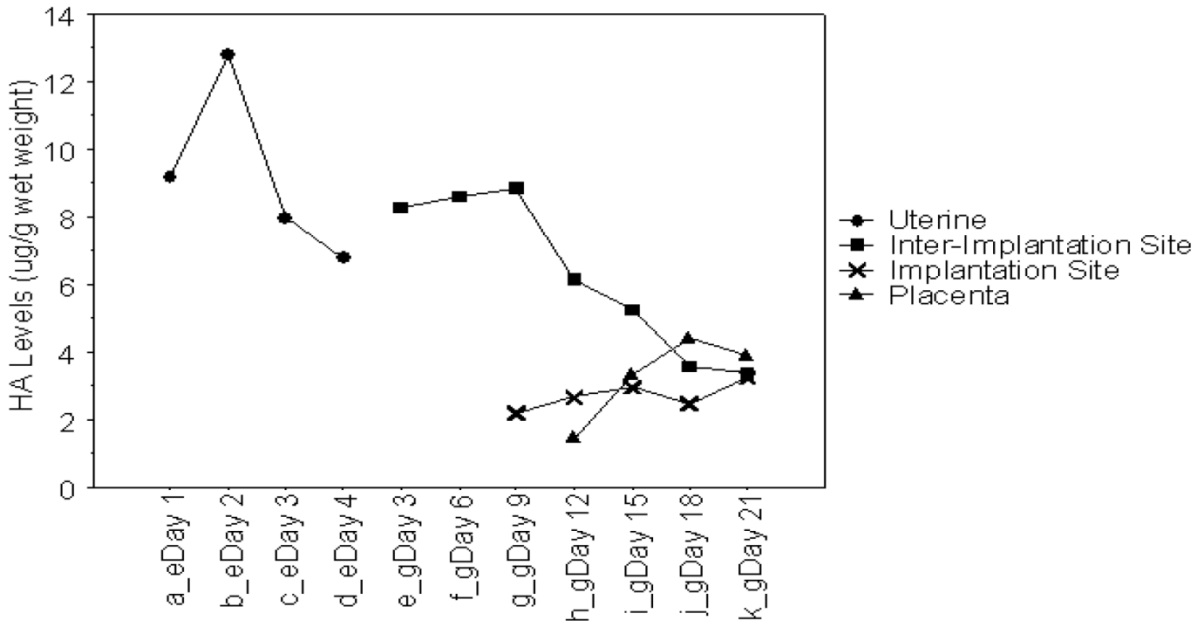


Figure 2-3: Rodent Oestrous and Gestational Histamine

Figure shows the profile of histamine production in rodent reproductive tissues across the oestrous cycle (eDay) and gestation (gDay). Data is mean plot of histamine levels pooled from rodent studies (Connolly et al. 1962, Hine et al. 1985).

The significance of the low histamine levels in mid-gestation is unclear; however the coincidence with DAO peak activity and the period of uterine tranquillity (Morgan & Hytten 1984) appears to support the hypothesis that tight control of maternal blood histamine is required for normal pregnancy outcome. Therefore, the review further examined the links between hyper-histaminemia occurring in specific gestational complications including preeclampsia, spontaneous abortion, preterm labour and *hyperemesis gravidarum.* In the case of PE, it was revealed that the complication presents with clinical symptoms similar to those observed in experimentally induced elevation of blood histamine in humans (Table 2-3).

Table 2-3: Clinical Symptoms of High Blood Histamine and Pre-eclampsia

Symptoms of Experimentally Induced High Blood Histamine in normal healthy human volunteers	Common Symptoms of PE
Dizziness Light-headedness Fainting Tachycardia Pyrexia Palpitation Continuous headache Severe headache Nervousness Convulsions (seizures) Difficulty breathing Flushing or redness of face Blue discoloration of face Blurred vision Chest discomfort or pain Hypotension Hypertension Diarrhoea Nausea Vomiting Metallic taste Oedema Abdominal or stomach spasm or cramps Proteinuria	Severe headaches Blurred vision Seeing flashing lights Severe heartburn Upper abdominal pain just below the ribs Nausea Vomiting Excessive weight gain Decreased urine output (fluid retention) Feeling very unwell Oedema Proteinuria Light sensitivity Decreased levels of platelets in your blood (thrombocytopenia) Impaired liver function Shortness of breath,

(Ind et al. 1982, Kaliner et al. 1982, Ind et al. 1983, Pollock et al. 1991, Thomas et al. 1991, Schmetterer et al. 1997, Akerman et al. 2002, Lassen et al. 2003, NICE 2008, NICE 2011)

Interestingly, the review also showed that the average maternal blood histamine levels in early normal gestation is similar to levels found in non-pregnant women (Table 2-4), but the levels in normal pregnancy decrease from gestational week 8 onwards to values below that found in healthy non-pregnant women (Figure 2-4 – Figure 2-6). In contrast, a logarithm regression of 3rd trimester maternal blood histamine level in PE suggests a significantly higher early maternal blood histamine levels compared to normal pregnancy for matched gestational ages (Figure 2-4). The average difference between NP and PE 3rd trimester blood histamine was 18.42%. Table A-1 & Table A-2 (appendix 1) provide further information on the differences and significance level between normal pregnancy and PE maternal blood histamine. Similar level of magnitude of change in histamine levels was observed in maternal urine histamine concentration after reanalysis of experimental inhibition of DAO activity with aminoguanidine data (Table A-3; Appendix 1). In this instance, the percentage rise in trimester 1 maternal urine histamine after inhibition of DAO with aminoguanidine was 18.73%.

Table 2-4: **Normal Histamine and Pregnancy Histamine Levels**

Histamine levels in Pregnancy and PE			
Condition	**Histamine** levels **Range (ng/ml)**	**Histamine** levels **(ng/ml) (Mean ± SEM)**	Reference
Normal Blood	25 – 65.0	56.7 ± 1.85	[1,2]
Normal Pregnancy:			
Trimester 1	58 – 70.0	64.0 ± 6.0	[3]
Trimester 2	30 – 50.0	43.3 ± 6.7	[3,4]
Trimester 3	35 – 74.3	52.9 ± 1.4	[3,5-11]
Pre-eclampsia: Trimester 3	49 – 94.3	69.7 ± 1.9	[8,10]

[1]: (Pfeiffer et al. 1970); [2]:(Lorenz et al. 1972); [3]:(Dubois et al. 1977); [4]: (Clemetson & Cafaro 1981); [5]: Effkemann & Werle 1940, [6]: Kapeller-Adler 1949; [7]: (Gunther & Glick 1967); [8]: (Achari et al. 1971); [9]: (Sharma et al. 1981), [10]: (Sharma et al. 1984); [11]: (Sharma et al. 1985).

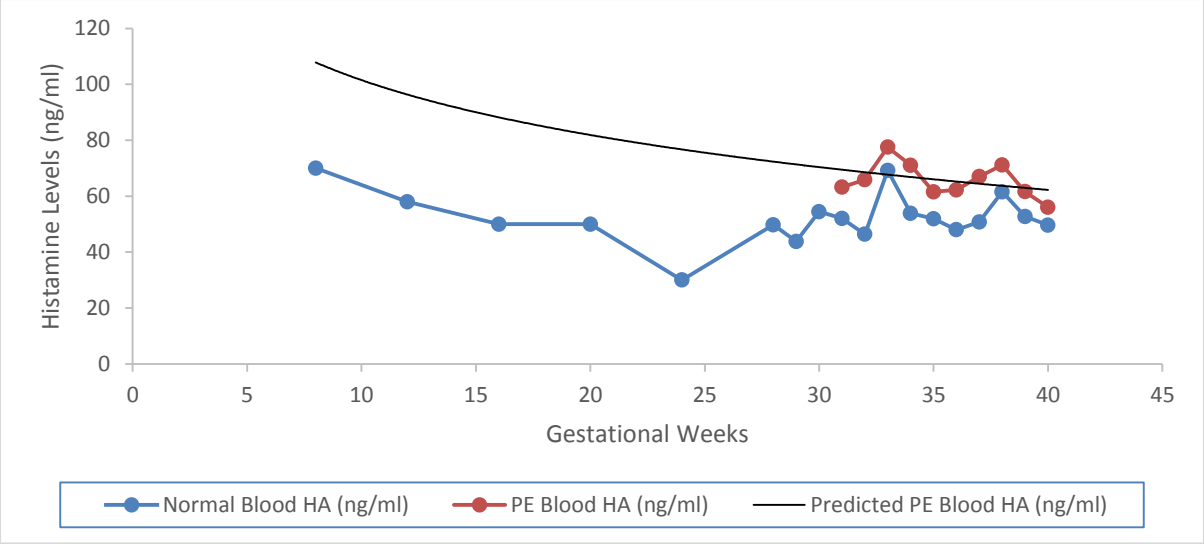


Figure 2-4: Normal and PE Maternal Blood Histamine levels superimposed
Figure is a plot of grand mean of blood histamine levels to depict the pattern of maternal blood histamine in normal (NP) and pre-eclampsia (PE) pregnancies. Black line shows predicted levels of early pregnancy maternal blood histamine in mother who later developed PE. Average rise of early pregnancy blood histamine in PE is projected to be 18.42% above normal. The pattern of gestational dependent variation in blood histamine is similar in both normal and PE pregnancies. (Southren et al. 1966a, Tryding & Willert 1968, Achari et al. 1971, Beaven et al. 1975, Dubois et al. 1977, Sharma et al. 1981, Sharma et al. 1984, Caldwell et al. 1988)

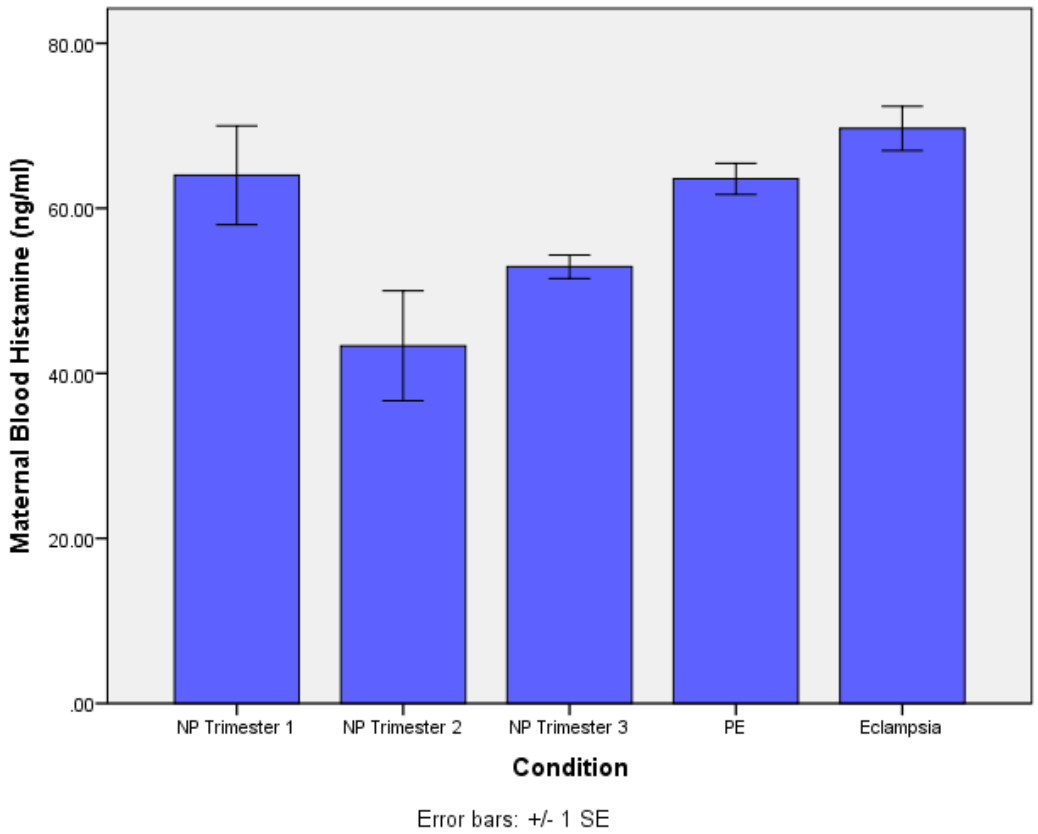


Figure 2-5: Normal Trimesters and Pre-eclamptic Maternal Blood Histamine

NP = Normal Pregnancy, PE = Pre-eclampsia

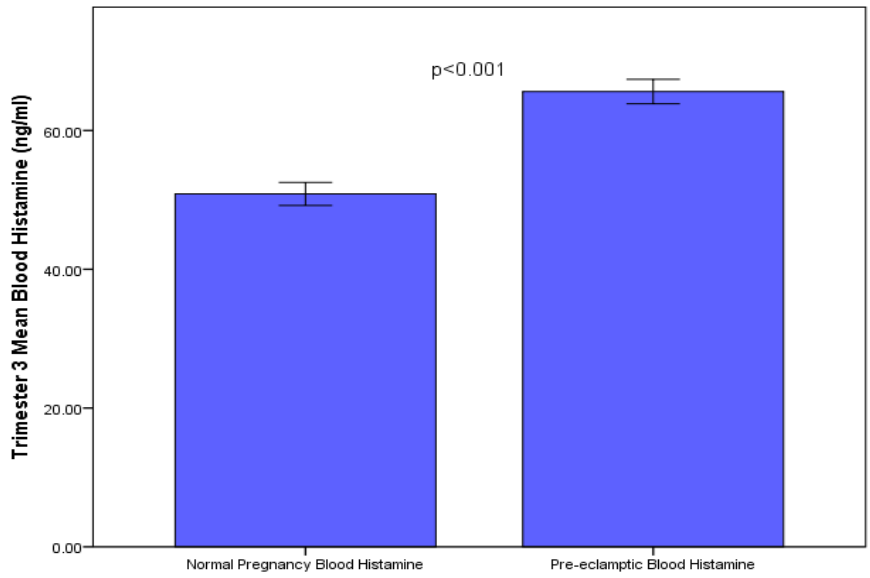


Figure 2-6: Normal and PE Maternal Blood Histamine levels Compared
Provide pooled analysis blood histamine means based on: (Achari et al. 1971, Dubois et al. 1977, Sharma et al. 1981, Sharma et al. 1984, Caldwell et al. 1988)

These findings showed that although the placenta is a major source of histamine, it also releases active diamine amine oxidase into the maternal circulation, which metabolises histamine (Purcell & Hanahoe 1991). The placental derived DAO once deported into maternal blood breaks down the histamine released from the placenta in normal pregnancy, leading to physiological levels of histamine in normal pregnancy. Clinically, the activity of the deported placental DAO in maternal blood increases a 1000 fold by mid-gestation in normal pregnancy to form a normal histamine-DAO-axis (Southren et al. 1966b, Beaven et al. 1975, Dubois et al. 1977). In PE, the DAO activity from the placenta is decreased thus, forming a defective histamine-DAO-axis (dHDA). This results in less histamine metabolism in the maternal circulation and higher systemic histamine levels in PE (Achari et al. 1971, Vanina et al. 1971, Beaven et al. 1975). Thus, normal Histamine-DAO-Axis (HDA) in pregnancy was defined as the concurrent exponential rise of maternal plasma DAO activity levels from gestational week 8 onwards with a peak at mid-trimester

(~gestational week 24), and progressive decline of maternal blood histamine levels below pre-pregnancy levels from gestational week 8 onwards with a mid-trimester (~gestational week 24) nadir. Defective Histamine-DAO-Axis (dHDA) in pregnancy on the other hand was defined as the failure of maternal plasma DAO activity to exponentially rise from gestation week 8, leading to diminished DAO activity and elevated maternal blood histamine akin to pre-pregnancy levels.

2.3 Conclusion

The series of reviews provided comprehensive knowledge and in-depth understanding of the links between histamine and PE. In summary, the studies broadened our understanding on the sources of elevated histamine in pregnancy by revealing that the placenta and not the uterus or the foetus is the major source of increasing histamine in maternal blood during pregnancy. The evidence reviewed was conclusive that methylation and deamination pathways accounts for about 80% of histamine metabolism during pregnancy, and hydroxylation of histamine into hydantoin propionic acid by ascorbic acid accounts for between 10 -20%. The work also affirmed that relative metabolic pathways differed between maternal and non-pregnant women, and DAO is the dominant histamine metabolic pathway during pregnancy. The work further showed that maternal blood histamine levels in early gestation are similar to levels found in non-pregnant women, and the levels in normal pregnancy decrease below values found in healthy non-pregnant women from around gestational week 8 onwards. However, in specific complication of pregnancy such as PE, DAO activity levels diminish after gestational week 8 and histamine levels remain elevated at levels similar to pre-gestational levels, thus forming a defective histamine-DAO-axis (dHDA).

Chapter 3

Theme 2: Methodological Considerations- Model for studying *ex vivo* placental gene expression in response to histamine

In this chapter:

- The commentary is based on methods used to investigate the works that underpin publication items # 2 – 11.
- The commentary discusses the rationale and challenges for using specific methodologies rather than a detailed discussion or report of the methods in general.

Highlights:

- Placental explant size (micro or macro) and duration of culture affect explant RNA quality.
- Micro explants (~50 mg) in optimum long-term culture (have undergone syncytiotrophoblast regeneration) at 8% oxygen produced RNA with quality akin to pre-culture explants.
- Quality of RNA from explants in early cultures (48 hours), presumably undergoing syncytiotrophoblast degeneration is compromised.
- Macro explants (~200 mg) after culture produced poor quality RNA and should be avoided.
- Gene expression patterns differ between pre-culture and cultured explants
- Atmospheric Oxygen Concentration up-regulates HIF1A transcription target gene set
- Atmospheric Oxygen Concentration regulated genes that favour apoptosis and inflammation
- Tissue culture up-regulates apoptosis and response to stress genes in placenta
- Tissue culture per se induces expression of genes with preponderance towards apoptosis, inflammation and tissue response to stress irrespective of culture oxygen concentration.
- Explant culture at Atmospheric Oxygen Concentration exacerbates tissue expression of stress and programmed cell death genes in placenta.
- Oxygen and tissue culture generally precipitate down-regulation of placental genes.

3.1 Introduction:

It is widely accepted that prudent evaluation and informed selection of choiced research methods are the most appropriate and cost effective approach to answer a research question. The selection of methods and the implementation thereof, are however influenced not only by the research question but also by practical considerations, such as the availability of resources, including the type of data available and the knowledge and skills of the persons undertaking the research, access to funds and time constraints. This chapter likewise, discusses key factors that impacted on decisions, selection, applications and implementation of methods used to investigate the genomic impact of histamine in human placenta. The discussions in this chapter are therefore based on rationales for: (1) Patients and the Placental choices; (2) Transition from Single Gene Analysis to Multi-gene Analysis; (3) Microarrays: Study Design Considerations and Rationale, and (4) Bioinformatics analyses. This chapter is therefore organised into three sub-sections as follows:

- In sub-section 1, the commentary focuses on addressing specific questions as: What was the need to be selective about patients? Why use placental explants instead of cell lines? What was the need for sampling tissues? Was the use of short term explant culture appropriate model for *ex vivo* investigation of histamine effect on the placenta? Blocking of DAO with aminoguanidine disulphate: what was the need?
- In sub-section 2, the focus shifts onto addressing the impact of Technological advancement on the research agenda. Specifically, the section addresses questions related to the need to reshape the research approach in line with evolution of technology from single gene expression analysis to multiplex, and then microarray.
- In sub-section 3, issues related to explant viability after culture and selection of an appropriate baseline control are discussed to show the respective contribution to the knowledge and understanding of placental explant culture.

3.2 Sub-section (1): Contributions made to the knowledge and understanding of Patients, the Placenta and Tissue Culture (#2 - #11)

3.2.1 The Patient

Human pregnancy has multiple outcomes including spontaneous abortion, PE, and pre-term labour. Therefore, defining the patients involved in the investigations was important to embed the findings in the context of specific complications of human pregnancy. Placental samples were taken from women whose pregnancy outcomes were classified as a first trimester social abortion, normal term, or PE (Table 3-1). Clinically diagnosed PE was defined in terms of WHO criteria (updated in 2014) as new hypertension (raised diastolic pressure to $\geq$90 mmHg on two occasions, 1–4 hours apart) presenting after 20 weeks with proteinuria ($\geq$30mg/dL or +1 or more on a single sample).

Table 3-1: Characteristics of Placentae

Clinical Condition of Placenta	Gestation (weeks)	Number of placenta	Delivery Method	Foetal birth weight
Normal	8 – 10	3	SA	NA
Normal	38 – 40	40	C/S	≥2.5kg ≤ 4.0kg
Pre-eclampsia	38 – 39	10	C/S	≥2.5kg ≤ 4.0kg

C/S = Caesarean Section; SA = Social Abortion

Thus, all placentae from normal pregnancies had no history of gestational hypertension or pre-existing hypertension, proteinuria, infection, glucosuria and other pregnancy related complications such as intra-uterine growth restriction.

3.2.2 Justification for using Placental Explants

The placenta is implicated in specific complications of human pregnancy such as PE (Myatt 2002, Matsuo et al. 2007). The mature human placenta is a discoid organ 20-25 cm in diameter, 3 cm thick and weighing 400-600g. Internally, it consists of a foetal villous tree bathed directly by maternal blood (Castellucci & Kaufmann 1982). The cells making up the internal of the mature placenta consists of several cell types including cytotrophoblast, syncytiotrophoblast, stromal, endothelial cells, pericytes, smooth muscle cells, erythrocytes and macrophages (Hofbauer cells) (Castellucci & Kaufmann 1982).

Various *in vitro* models including: choriocarcinoma cell lines such as BeWo, Jeg-3 and Jar cells; isolated primary trophoblast cells, placental mesenchymal stromal cell lines, and villous parenchyma (placental) explants have been used to investigate placental physiology, pharmacology and other functions (Miller et al. 2005, Bode et al. 2006, Orendi et al. 2011, Daoud et al. 2016, Qin et al. 2016). Of all the models however, placental explant is reported to closely mimic *in vivo* conditions to support *ex vivo* study of placental functions including cellular uptake, production and release of secretory components, cell interactions, proliferation, growth and differentiation, gene delivery, pharmacology, toxicology, and disease processes (Miller et al. 2005, Orendi et al. 2011).

Of particular importance to histamine effects, caesarean delivered explants were the first choice of tissue for investigating the roles of histamine in the placenta. This was principally important because, it is observed that placentae with uterine contractile activity produce varying levels of histamine: less in decidua from maternal side, and

high levels in tissues from foetal side (Szukiewicz et al. 1995). It is also established that tissue damage and stress destabilise histamine production (Schayer 1952a, Norn 1968, Neugebauer & Lorenz 1982, Sooranna et al. 2004, Mohan et al. 2007): thereby, making the study of histamine in placentae that have undergone uterine contraction less suitable for mimicking *in vivo* conditions. Furthermore, the cross-talk between the trophectoderm derived (trophoblasts) and mesenchymal derived cells in the placenta (Lewis et al. 1996) is difficult to mimic or practically absent in cell line models. Also, the cell line models generically show increased baseline expression of pro-inflammatory receptors such as Toll-like receptors and cytokines (Kauma et al. 1992, Lonsdale et al. 1996, Gierman et al. 2015). Thus, rendering cell lines as less attractive model in favour of placental villous tissue explants to investigate the genomic response of the placenta to histamine.

3.2.3 Tissue sampling

Fresh placental samples were collected from singleton pregnancies. Placentae were collected from the Imperial College NHS Trust following informed consent from mothers. Ethics permission was granted by the Hammersmith and Queen Charlotte's & Chelsea Hospitals Research Ethics Committee. Samples were taken from non-smoking mothers without gestational diabetes or other complications (except PE), who were delivered by elective Caesarean section. Each placenta was visually inspected for signs of excessive tears, necrosis, and infarction, and samples taken from healthy looking areas about 5 cm away from the placental cord with sterile sharp scissors. Approximately 2 cm^3 of the placental tissue were randomly cut with sterile scissors from three different sampling sites. Each sample was carefully excised to include an intact chorionic plate, villous parenchyma, basal plate and

decidua. All samples were collected from the maternity operating theatre, washed in phosphate-buffered saline (PBS) solution (pH 7.4) containing 10% penicillin, streptomycin and L-glutamine (Sigma) to remove excess blood and blood clots and transported in 150 ml sterile pots containing warm PBS with 10% penicillin, streptomycin and L-glutamine immediately to the laboratory. The placental explants were then aseptically dissected from the villous parenchyma of each sample, fragmented with scissors and incubated within 30 minutes of delivery.

3.2.4 Effects of Culture Duration, Explant Size and Oxygen on Explant Morphology (Publication #3)

The use of placental explants as model to investigate placental physiology is complicated by intrinsic variances related to the viability of the tissues in organ culture, duration of the organ culture, oxygen concentration of the culture media and the size of the explants (Miller et al. 2005). Concerning placental culture oxygen, different concentrations (atmospheric oxygen concentration (AOC) and Physiological oxygen concentrations (POC)) have generally been used during culture of term placentae. It has been argued that POC (8%) reflects *in vivo* physiology, and provides optimal culture conditions for term placental villous explants than AOC (20%) (Damsky et al. 1993, Genbacev et al. 1996, Caniggia et al. 2000, Huppertz et al. 2003, Miller et al. 2005, Burton et al. 2006).

Some studies have also reported increased deterioration of placental explant viability within 24 hours of culture (Palmer et al. 1997, Sooranna et al. 1999) while others have demonstrated its viability for more than 7 days in organ culture (Tao & Hertig 1965, Taylor & Hancock 1973, Faye et al. 2005). Moreover, placental explant

syncytiotrophoblast (STB) cell population are completely regenerated in the course of days in organ culture (Taylor & Hancock 1973, Palmer et al. 1997, Siman et al. 2001). Therefore, the establishment of an appropriate duration and condition for explant culture was crucial, as was determining the baseline control samples for reasons related to the plausible impact of syncytial degeneration on explant RNA quality. Thus, it was aimed to determine whether syncytial degeneration and regeneration could affect the interpretation of results, and also to establish an appropriate explant size, duration of culture and optimum oxygen concentration for the study of histamine effect on human placenta.

In order to evaluate the impact of placental explant size, culture method, culture oxygen concentration and duration of culture on explant viability and quality of RNA placental micro and macro explant in different culture settings were compared. Placental macro explants (approximately 200 mg wet weight) and micro explants (<50 mg wet weight) were cultured to time 0h, 24h, 48h, 72h, 96h, 120h and 144h (publication #3). Microcapillary electrophoretic RNA separation and analysis of automatically generated signal measurements was performed on RNA extracted from placental explants incubated at Physiological Oxygen Concentration (8%) or AOC in standard or optimum culture (publication #3). Explant viability was indirectly assessed with immunohistochemistry for the STB integrity.

Previous studies have established very clearly that placental tissue, particularly placental explants (Taylor & Hancock 1973, Watson et al. 1995, Tao & Hertig 1965) show loss of the STB layer during initial culture (2-4 days), followed by regeneration of the syncytium over longer culture periods (5 – 11 days) (Palmer et al. 1997, Siman et al. 2001). Therefore, STB integrity was measured by the extent of degeneration

and regeneration of the STB layer. STB degeneration was defined as the presence of small, dense and rounded nuclei, irregular syncytial layer with disintegration of the integrity of the apical membrane; detachment and sloughing off of the original syncytial layer in culture (Tao & Hertig 1965, Palmer et al. 1997). STB regeneration was on the other hand, defined according Siman et al (2001) as the progressive replacement of the original STB by a newly formed layer, evidenced by gradual thickening of the new STB as the original syncytium is sloughed off and lost.

The findings (publication #3) showed that integrity of RNA in the macro samples irrespective of oxygen concentration and micro explants cultured in AOC was poor throughout the culture period, whereas in the micro explants cultured in optimal media in POC, the RNA quality was transiently poor, and then improved by day 6 of culture. Thus, suggesting that micro explants cultured at POC have the best RNA quality and tissue structure whereas, macro explants were less viable after long-term culture. The findings also showed that placental explants undergoing syncytial degeneration produced poor quality RNA and should be avoided. The findings further showed that the RNA quality generically declined in tandem with STB degeneration, suggesting also that the STB viability could be a proxy measure of explant quality for high throughput experiments. In view of these, short term culture, macro explants and explants cultured in AOC were avoided in favour of micro explants in POC cultured at the gas interface to 6 days.

3.2.5 Modelling Defective Histamine-DAO-Axis (publications #9 - #11)

Ex-vivo defective histamine – DAO – axis (dHDA) also referred to Elevated Histamine Model (EHM) was modelled in human placental explants to identify genes that are affected by elevated histamine in the placenta and examined their functions in PE. Diamine oxidase (DAO; EC 1.4.3.22) also known as copper-containing-amine oxidase; histaminase; amiloride binding protein-1, was first discovered as a histamine-inactivating enzyme (Best 1929). This enzyme catalyzes the oxidative deamination of histamine to an aldehyde, ammonia, and hydrogen peroxide (Zeller 1938, Schayer 1959). DAO is mostly expressed in tissues (e.g., intestines, kidney, thymus, kidney cortex, seminal vesicles, placenta, and in the plasma of pregnant women), that transport large quantities of histamine (Marcou et al. 1938, Gunther & Glick 1967, Tryding & Willert 1968, Beaven & Shaff 1975). And in publication #7, it was demonstrated that DAO is localized densely at the foeto-maternal interface to the cross borders between maternal (decidual) and foetal (trophoblastic) tissues, thereby confirming previous postulates about the topological orientation of the enzyme in human placenta (Buffoni 1966, Gunther & Glick 1967, Tornqvist 1969).

In normal human pregnancy, the parallel increase in DAO activity in the placenta and in maternal blood along with gestational age creates an effective histamine-DAO-axis (HDA), with DAO activity present throughout the course of pregnancy. In the pregnancies with normal HDA, maternal plasma DAO activity levels spontaneously rise exponentially to a 1000 fold from gestational week 8 and peaks at gestational weeks 20 and 24 (Ahlmark 1944, Southren et al. 1966b, Gunther & Glick 1967, Weingold & Southren 1968, Tufvesson 1978, Beaven et al. 1975, Dubois et al. 1977). In contrast, the spontaneous exponential rise of DAO activity is abrogated from gestational week 8 onwards thus, leading to a defective histamine-DAO-axis in

pregnancies such as PE that present with high blood histamine (Southren et al. 1966b, Achari et al. 1971, Beaven et al. 1975, Legge & Duff 1981).

Thus, modelling the EHM was crucial to deciphering the functions of increasing histamine in PE. This was achieved by inhibiting DAO enzyme activity in regenerated syncytiotrophoblast placental explants with aminoguanidine to mimic *in vivo* condition. The explants were incubated at the liquid-gas interface in 8% oxygen, 37°C in basic media for 5 days (the culture media were changed at days 2 and 4) to allow for syncytiotrophoblast degeneration and regeneration. This was followed by a final media change on day 5, containing 100nM histamine in the presence of aminoguanidine (10^{-4} M final concentration) for 24 hours histamine treatment, or basic media without aminoguanidine for control samples. Endogenous placental DAO metabolises histamine in 1g of placental explant at a rate of 45.54 µg/hr (Kapeller-Adler 1952b). Therefore, to be able to observe the effect of the exogenous histamine, DAO activity was blocked with aminoguanidine in the treatment samples. Aminoguanidine is a specific inhibitor of DAO enzyme activity (Tamura et al. 1989). This culture method therefore enabled us to create an *ex vivo* elevated histamine model (EHM) with some parallel features of an *in vivo* defective histamine-DAO-axis system.

3.3 Sub-section 2: Justification for evolution of methodological techniques and contributions knowledge: - **Transition from Single Gene to Multi-gene Analysis**

Detailed analyses of biological processes that underpin health are identified by most UK research funding bodies (e.g., MRC) as key step in the search for new ways of treating disease. Thus post-genome research focus is shifted to the accurate annotation of genomic sequences and the interplay between genes and proteins, and high throughput analysis of genetic variability between patients and susceptibility to disease or failed therapy. In tandem, methodological approach for the study of histamine role in placental gene expression evolved from a pre-genome approach focusing on single index genes using Polymerase Chain Reaction (PCR) technique through real time multiplex PCR to post genome high throughput techniques such as microarrays.

The changes to the technical approaches were necessary because of the multiple foci of the research agenda. First, a major focus of the study was to investigate the interplay between genes and proteins. Databases containing large numbers of genetic sequences and polymorphisms were available to facilitate the investigations however the method of investigation significantly influenced the rate of progress and dissemination. Similarly, to be able to significantly contribute towards faculty research output required maximisation of limited research time to decipher parallel gene expression profiles of normal and disease tissues in different clinical states. Thus, there was an inherent need to maximise potentials and resources through technological advancement.

Cost effective analysis of the project in year Jan 2005 showed that it was extremely laborious, costly and time consuming to use low throughput techniques to investigate effect of histamine on placental gene expression. For example, in the year 2000, using conventional PCR technique, working 11 hours per day, five days a week for 6 months, enabled the study of the expression characteristic of 4 genes only. In contrast, the expression characteristics of 6 genes were studied over 3 weeks (7 hours per day; 3 days per week) in December 2004, with real-time multiplex qPCR technology at a fraction of the consumable cost for the year 2000 work. Moreover, the evolution of new high throughput technologies to quantify the expression of specific genes eliminated the need for some time-consuming and hazardous steps such as radio-labelling, scintillation counting, and post PCR analysis using carcinogenic chemicals such as ethidium bromide in gel electrophoresis. Thus on the grounds of cost effectiveness, expediency and safety, it was justified that application of high throughput technique to study histamine effects on placental gene expression was the best value for money option to significantly enhance the rate of data turnover and discoveries.

3.4 Sub-section 3: Microarrays and Bioinformatics: Considerations, Rationale and Contributions to Knowledge of Placental Gene Expression

3.4.1 Microarray Hybridisation (Publications #4, #9 and #11)

In publications #4, #9 and #11, placental RNA hybridised onto Affymetrix microarrays were used to investigate the effects of oxygen and histamine in human placentae. In brief, RNA was extracted from micro explants with TRIZOL (Invitrogen, UK) and quality assessed. Total RNA was processed into labelled cDNA with NuGEN™ Ovation™ RNA Amplification System V2 and FL-Ovation™ cDNA Biotin Module V2

(Nugen). The resultant fragmented and labelled cDNA was added to the hybridisation cocktail in accordance with the NuGEN™ guidelines for microarray hybridisation onto Affymetrix GeneChip® Human Genome U133 Plus 2.0 arrays: one sample per array, (Figure 3-1) in Affymetrix GeneChip® Hybridisation Oven 640 for 18 hours at 45°C. Features that retained bound labelled cRNA after washing were visualized using the GeneChip® Scanner 3000 (Affymetrix).

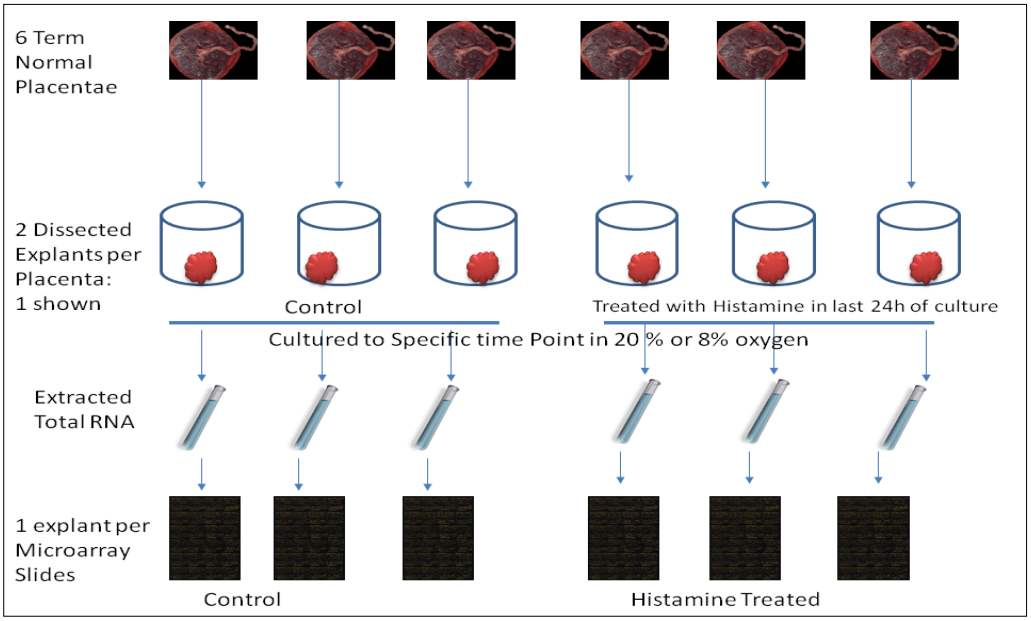


Figure 3-1: Microarray Experiment Culture flowchart

Total RNA from term placental explants were hybridised to Affymetrix Human Genome U133 Plus 2.0 Array. Explants were incubated for 6 days in POC or AOC. Half of the explants RNA under each of the ambient oxygen were treated with histamine and aminoguanidine in the last 24 hours of organ culture. Non-histamine & aminoguanidine treated samples served as the respective control samples.

3.4.2 Rationale for using Loop microarray design (Publications #4, #9 and #11)

A critical component of microarray studies is to design the experiment to make the analysis of the data and the interpretation of the results as uncomplicated but as powerful as possible, given the purpose of the experiment and the constraints of the experimental material (Yang et al. 2002, Yang & Speed 2002). Thus, two main microarray experiment designs: single factor or one-way experiment, and multi-factorial experimental designs (Yang & Speed 2002, Peng & Stromberg 2003) were

considered for the study to investigate the effects of histamine and oxygen in placental explants. Single factor designs typically include studies that investigate the differences in expression between two or more time points following treatment (time-course experiments), or different regions or subtypes of tissues such as normal and pre-eclamptic placentae (Kerr & Churchill 2001, Simon et al. 2002, Yang & Speed 2002, Blalock 2003). The microarray experimental designs that have two or more factors to be considered jointly, where each factor in turn may have two or more levels are normally considered multi-factorial (Yang & Speed 2002, Wit & McClure 2004).

Within the multi-factorial array designs, there are at least two basic types: a design with common reference or loop baselines (controls) (Kerr *et al.*, 2000; Kerr & Churchill, 2001b; Yang & Speed, 2002; Wit & McClure, 2004; Kerr & Churchill, 2007). A loop design (Figure 3-2a) describes the method where samples are directly compared by direct pairing of sample array slides, usually based on scientific aim or biological interest of the study, whereas a common reference design describes the method where all samples are indirectly compared by referring to a single reference sample (Figure 3-2b) usually a pre-culture time-zero sample (Kerr & Churchill, 2001a; Yang & Speed, 2002; Wit & McClure, 2004). In effect, loop design uses direct comparison between slides without a common reference so that the choice of a baseline control depends on the experimental question.

a: Loop Design

b: Common Reference Design

A = slide hybridised with RNA from placental explants treated with 8% Oxygen only
B = slide hybridised with RNA from placental explants treated with 20% Oxygen only
AC = slide hybridised with RNA from placental explants treated with Histamine and 8% Oxygen
BC = slide hybridised with RNA from placental explants treated with Histamine and 20% Oxygen
R = slide hybridised with RNA from placental explants not treated with neither Histamine nor Oxygen

Figure 3-2: Microarray Experiment Design

Previous comparative work reporting on the appropriateness of loop design (Yang & Speed 2002) showed that loop design gave the smallest confounding variance for estimating differential expression between the main effects, whereas common reference usually derived from time zero samples (e.g., Figure 3-2b), by far gave the worst variance for estimating the interaction in both 2 X 2 factorial and single factor time-course experiments (Yang & Speed 2002). Thus, in our loop design (Figure 3-2a), samples were compared with each other in a loop order according the biological or experimental questions. Our direct loop order was driven by 3 main scientific questions aimed at determining whether: (1) gene expression between explants cultured for 6 days in POC or AOC differ, (2) the effect of histamine on placental explants gene expression differ between those cultured in POC and AOC; and (3) gene expression in cultured explants with diminished DAO activity but treated with histamine differ from cultured explants without diminished DAO and histamine treatment. Therefore the loop design allowed for direct comparison of time equivalent controls with treated sample. For example, in order to determine relative

changes in gene expression between explants cultured in POC and AOC, slide A was compared with B where slide A was made the control (Figure 3-2A). Therefore, based on the biological interests posed by the experimental questions a loop design was deemed more appropriate to enhance the biological relevance of the results.

3.4.3 Dealing with the impacts of variances (Publications #4, #9 and #11)

While it is well demonstrated that one of the best designs to limit confounding variance in multi-factorial microarray experiment is loop design instead of common reference design (Kerr *et al.*, 2000; Kerr & Churchill, 2001b; Yang & Speed, 2002; Wit & McClure, 2004; Kerr & Churchill, 2007), there are constraints associated with this type of experimental designs. Often, the constraints are linked to strategies to deal with inherent variances that may affect data interpretation. In this instance, the documented sources of variance have broadly been classified either as biological or technical variance (Blalock 2003). At the beginning of the studies, it was clear that both biological and technical variances in microarray studies could be effectively contained with judicious statistically based experimental design to account for four basic factors: control, randomisation, replicates and balance (Peng & Stromberg 2003, Schena 2003, Wit & McClure 2004).

Replicates in microarray studies are deemed highly important in creating a degree of variation in measurements to provide independence of data, and to offer the benefits of averaging log expressions to infer external validity (Lee et al. 2000, Yang & Speed 2002, Peng & Stromberg 2003). The two types of replicates (biological and technical) described in microarray experiments appear to have different degrees of impact on variance in gene expression. The technical replicate refers to replication in which the

target mRNA is from the same pool or extraction, whereas biological replicate refers to hybridizations that involve mRNA from different extractions or different samples of cells from a particular cell line or tissue; or from target mRNA, which comes from different individuals or different versions of a cell line (Lee et al. 2000, Yang & Speed 2002).

Of these, it is suggested that technical replicates generally involve a smaller degree of variation in measurements than the biological replicates, and therefore do not provide a greater deal of impact on external validity (Lee et al. 2000, Yang & Speed 2002, Peng & Stromberg 2003). In contrast, the literature suggests that external validity of microarray findings can be improved with meticulous prediction of biological replicates to ascertain appropriate sample size to detect statistical differences in gene expression along with appropriate use of control samples to establish baseline expression (Yang & Speed 2002, Wright & Simon 2003, Dobbin & Simon 2005). Analysis of variance distribution incorporating random variance model to estimate the within-class variance for each gene in the human genome to predict sample size (Wright & Simon 2003, Dobbin & Simon 2005) was therefore performed in Chipster v1.4.7. Consequently, biological replicates of 6 per class was predicted to identify differentially expressed genes at Type 1 error = 0.05, (Power = 70%, hypothesized mean difference in $\log_2$ expression between classes = 1).

3.4.4 Validation of the Microarray Design: Biological Relevance of Pre-culture explant as baseline control for histamine treated cultured explants (Publication #3 & #4)

To validate the loop design, the effects of tissue culture and oxygen on placental gene expression were examined to ascertain the biological relevance of studying histamine effects in POC or AOC, and also for using pre-culture explants as control for the cultured explants. It was argued that pre-culture (time zero) placental explant expression was needed as a common reference baseline control to put the cultured explant microarray expression into perspective as opposed to using cultured non-treated explants as baseline for the cultured treated samples. Therefore, the hypotheses that (1) *entropy* of gene expression of pre-culture time zero explant and explants that have been cultured and undergone STB regeneration were mutually meaningful and biologically relevant; (2) oxygen concentration and tissue culture per se do not affect the explant gene expression and the interpretations thereof were tested.

3.4.4.1 Biological Relevance between Pre-culture and cultured Explants for RGE (#4, 9, 11)

To answer the question of using time zero explants as common reference baseline for the cultured explants, biological relevance network analysis was performed with placental micro explants cultured for 6 days in POC (4 biological replicate arrays), in AOC (6 biological replicate arrays) and pre-culture time zero explant (5 biological replicates) microarrays. All placentae were delivered by elective caesarean sections.

The biological relevant networks analysis was performed by computing the *entropy* of gene expression patterns between the microarray samples to provide mutual information (information content) for RNA expression patterns from each hybridised explant sample (Butte & Kohane, 2000). The correlation coefficient between explants was measured by comparing the expression pattern of genes on each slide to that of

every other gene. Minimum Pearson correlation (R^2) threshold was set as 0.7700514, and maximum Pearson correlation (R^2) threshold at 1.0. To observe all true relevance networks, highest entropy filter was also set to 100%.

The analysis showed 55 biologically relevant clusters or links (Figure 3-3), with two mutually exclusive relevance subnets. Subnet 1 contained 10 experiments entirely from explants cultured for 6 days, while subnet 2 contained 5 experiments exclusively from pre-culture (time zero) explants. The percentages of biologically relevant networks in each subnet were respectively 67% and 33% for subnet 1 and 2. The explants were represented as nodes in the network and lines were drawn between the nodes (Figure 3-3) to show the link was biologically relevant when the respective correlation coefficient was R^2 >0.77. All gene expression entropies for the 15 microarrays were linked through comprehensive pair-wise mutual information analysis, but no biologically relevant network was identified between pre-culture time zero explants and 6 days cultured explants. The findings suggested that the links between gene expression in pre-culture time-zero and explants cultured to 6 days were not biologically relevant to support direct comparison. Thus, indicating that the use of pre-culture time-zero explants as a baseline control for 6 days culture time-point placental microarray experiment could complicate interpretation of results.

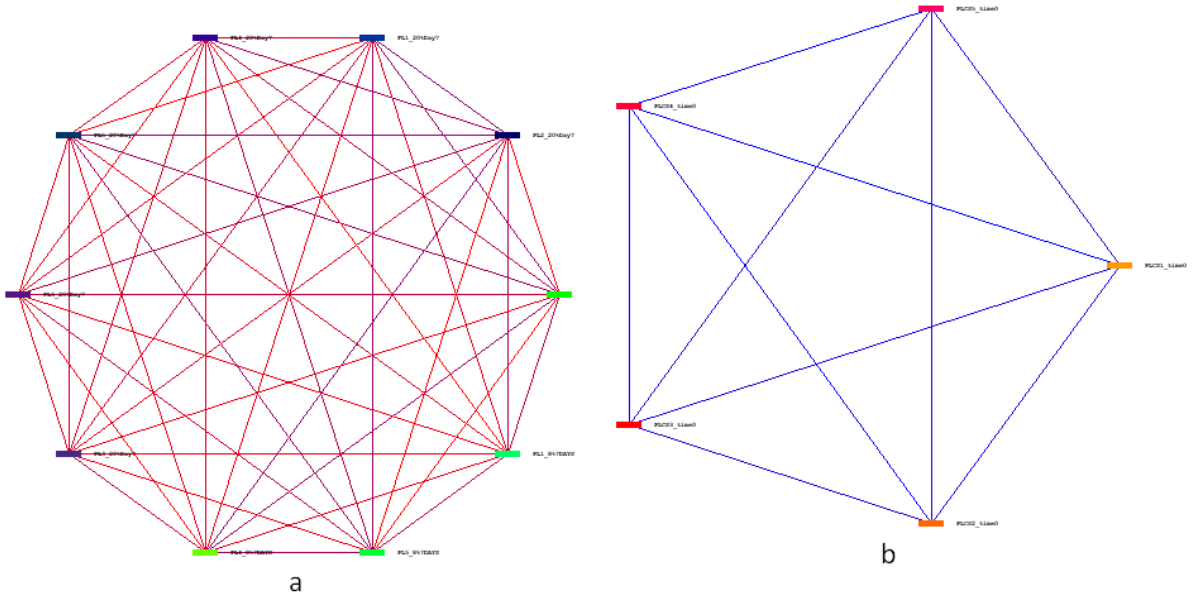


Figure 3-3: Biological Relevance Network Analysis of Time zero, POC and AOC treated Explants

Fifteen placental explant microarrays from 11 elective caesarean normal placentae (pre-culture explants = red nodes, n = 5; explants cultured in POC for 6 days = green nodes, n = 4; explants cultured in AOC for 6 days = blue nodes, n = 6) were analysed to identify biologically relevant networks (BRN) between each pair of samples. A total of 55 BRNs were identified. (a) = true links between cultured explants (green nodes = POC explants; blue nodes = AOC explants); (b) true links between pre-culture explants (orange nodes). No BRN were found between pre-culture and cultured explants at minimum Pearson Correlation $R^2 = 0.77$. Networks (lines) coloured in red represent elements that are positively correlated while networks coloured in blue represent elements that are negatively correlated.

3.4.4.2 Impact of Tissue Culture and Oxygen on Explant Gene Expression (#4)

To further examine the implication of the lack of biologically relevant links between pre-culture and 6 days cultured explants on the interpretation of results, the differences in placental transcriptomic changes in response to tissue culture and oxygen, and the relevance of genetic alterations and pathways associated with POC and AOC culture were investigated. Placental explants were collected from normal term (38-39 weeks of gestation) placentae with no previous uterine contractile activity. Placental gene expression profile was evaluated with analysis of GeneChip® Human Genome U133 Plus 2.0 arrays (Affymetrix).

Absolute gene expression (AGE) analysis was performed using One Class RankProd statistics to identify 'consistently high expressed' (CHE) and 'consistently low expressed' (CLE) significant genes respectively in Time Zero (pre-culture) explants (designated as T0), and explants cultured in POC and AOC for 6 days. FDR Confidence (1-alpha) was set at 99.9% (FDR <0.001). The analysis identified a total of 635, 1207 and 1760 significant genes that were consistently expressed in pre-culture (T0); POC and AOC cultured explants respectively (Figure 3-4). Of these, 224 genes were exclusively expressed in pre-culture samples. In contrast, 69 genes (1 CHE and 68 CLE) were exclusively expressed in POC and 574 genes (292 CHE, and 282 CLE) in AOC cultured explants only.

The analysis also identified a further 48 genes that were expressed in both pre-culture and AOC treated explants but not in POC treated explants. In addition, it was observed that the CHE genes appeared insensitive to POC, but rather to tissue culture and AOC. In that, all CHE genes (except *SIGLEC6*) expressed in POC explants were also expressed consistently at high levels in AOC explants. Furthermore, there was a core set of 770 significant genes that were expressed in both POC and AOC treated samples only, and appeared to be genes suggestively responding to tissue culture per se. No genes were exclusively expressed consistently between pre-culture and POC treated samples only. However, using class prediction modelling incorporating Leave-one-out cross-validation and ROC curves analysis, the study identified the expression of 134 genes that were significantly ($p<0.01$, AUC = 0.82(CCP), 0.81(DLDA), 0.81(BCCP)) associated with prolonged explant culture at AOC.

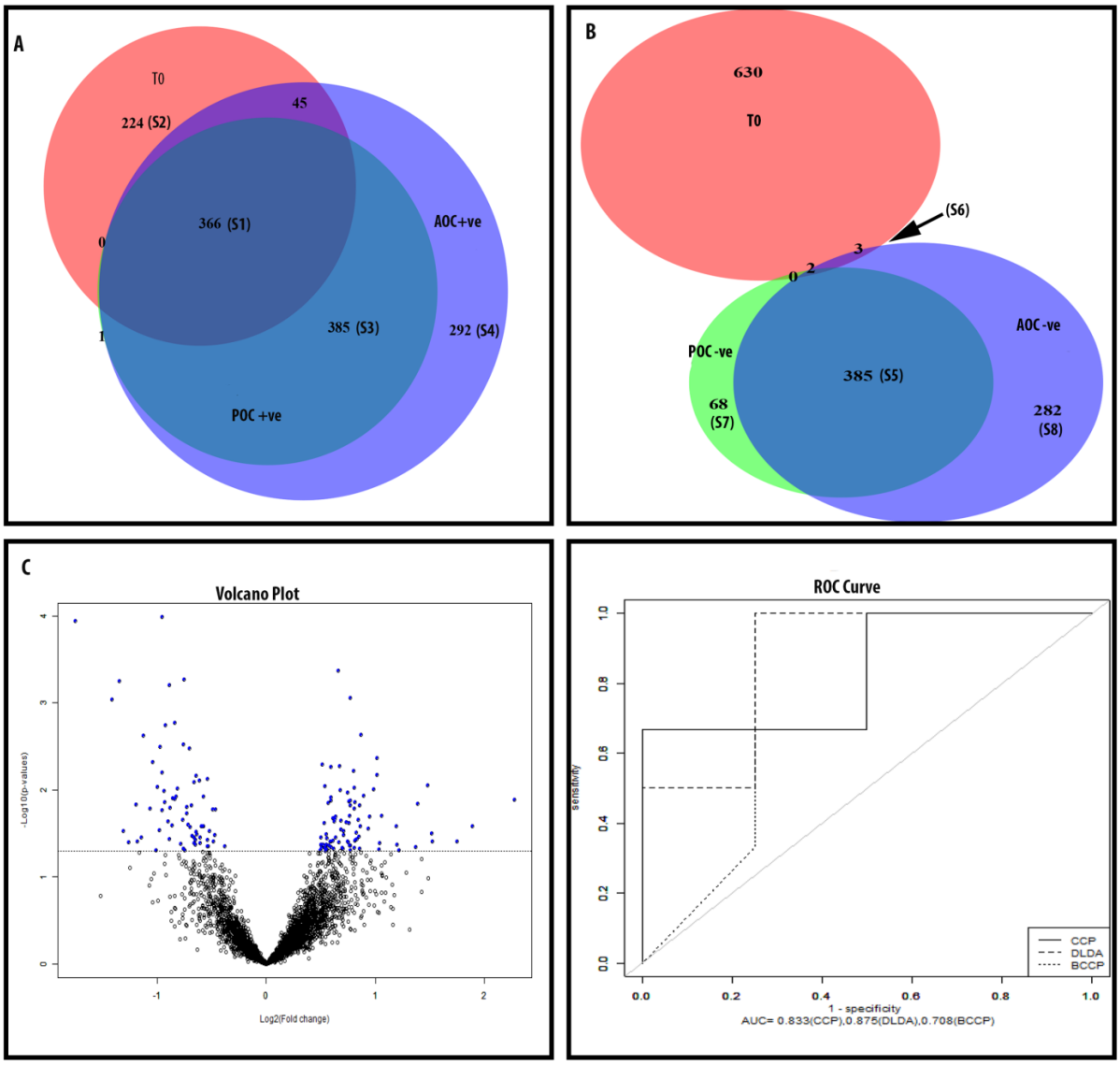


Figure 3-4: Expressed genes in POC and AOC explants
Figure A shows overlap of consistent high level expressed (CHE) genes between pre-culture, and explants culture at AOC and POC. Figure B shows overlap between pre-culture CHE genes and cultured explants consistent low expressed (CLE) genes. Figure C shows a volcano plot of 157 significant genes (blue dots). Random variance model parameters at a= 1.31652, b= 17.03489, Kolmogorov-Smirnov statistic= 0.01 and a nominal significance level (dotted line) of each univariate test at p< 0.05 (210 exact permutations). Figure D shows the ROC curve from the Bayesian Compound Covariate Predictor for AOC associated genes. S1 – S8 = Gene Sub-sets 1 – 8; T0 = Pre-culture CHE genes; AOC = Atmospheric Oxygen Concentration; POC = Physiologic Oxygen Concentration; +ve = CHE; -ve = CLE.

Subsequently, through analysis of the gene ontologies and pathways enriched by the pre-culture, POC and AOC significant gene sets it was shown that the preserved set of genes that appeared to respond to tissue culture irrespective of oxygen concentration were significantly involved in programmed cell death, cell death, death, stress response, protein metabolic process, electron transport activity, RNA

translation and oxidoreductase activity, regulation of cytosol, cytoplasmic and organelle parts of the placental cells. These therefore suggested that tissue culture per se induces the expression of genes that regulate a cluster of cathartic functions in the placenta.

The work was thus extended to examine the effects of culture oxygen concentration on Transcription Factor Target gene sets (TFTs) expression. It was observed that culture at AOC down-regulated specific TFTs including REL, ETV4, ATF3, STAT1, JUND and STAT5B. Through text mining analysis, these TFTs were identified to be involved in cell growth, proliferation, invasion, regeneration, differentiation, transformation, tissue viability, protection from apoptosis, and glands development. Conversely, AOC up-regulated TFTs such as HIF1A, PPARA, CEBPD, STAT3, and CEBPE, that are associated with oxygen regulation, immune and inflammation responses; leptin mediated response; lipid metabolism; suppression and mis-regulation of cell growth and proliferation; cell morphogenesis; induction of apoptosis; and oxygen regulation. These findings collectively suggested that:

1. Gene expression patterns differ between pre-culture and cultured explants
2. Placental explants' responses to tissue culture per se induce expression of genes with preponderance towards apoptosis, inflammation and tissue response to stress irrespective of culture oxygen concentration.
3. The magnitude of the tissue response to stress and programmed cell death in placenta is sensitive to AOC.
4. Explants culture at AOC exacerbates tissue response to stress and programmed cell death in placenta.

5. AOC up-regulates HIF1A transcription target gene set
6. Oxygen and tissue culture generally precipitate down-regulation of a sub-set of placental genes

Overall, the findings suggested that the lack of biologically relevant links between pre-culture and 6 days cultured explants was underpinned by critical biological variance, and that the use of pre-culture time zero explants as baseline for 6 days cultured explants could complicate the interpretation rather than offer meaningful baseline. The findings also confirmed previous reports (Thompson et al. 2007, Fajardy et al. 2009) that culture per se affects explant gene expression. It further showed that irrespective of oxygen concentration, tissue culture per se down-regulates more genes but the effect is exacerbated in explants cultured under AOC. Therefore, it was acknowledged that in a time point (not time series) experiment variances introduced by explant culture including syncytial degeneration and regeneration and differential gene expression would complicate the interpretation of gene expression induced by histamine after long-term culture, and hence pre-culture explant was deemed unsuitable as baseline control for relative gene expression analysis (RGE).

3.4.5 Rationale for Bioinformatics methodologies (Publications #2, #4 and #11)

Bioinformatics analyses were performed to transform gene expression data into biological information. R Bioconductor statistics were used within R Studio (RStudio 2015), BRB ArrayTools (Simon et al 2007), Gene Ontology Tree Machine (GOTM, (Zhang et al. 2004)), "**WEB**-based **GE**ne **S**e**T** **A**na**L**ysis **T**oolkit (WebGestalt, (Wang et al. 2013)), MEV4 (Saeed et al. 2006), Reactome (Fabregat et al. 2016), Chipster (Tuimala et al. 2008), INMEX (Xia et al. 2013) and NetworkAnalyst (Xia et al. 2015)

to process microarrays and also to probe the Gene Ontology, Kyoto Encyclopedia of Genes and Genomes (KEGG), BIOCARTA and Molecular Signature Database (MSigDB) data bases to assess the biological relevance and significance of genes according to similarity in function, participation in signalling pathways, and protein domains.

3.4.5.1 Gene Findings: AGE and RGE (#2, #4, #11)

The usual approach to finding significantly expressed genes from microarray experiments relies on relative gene expression (RGE), which is defined as the relative quantitation of the differences in the expression level of a gene between the case and control samples (Xia et al. 2013). Traditionally, this approach is suggested as highly suitable for candidate gene discovery or class prediction studies, but limited in its ability to find genes that are significantly expressed consistently at low or high levels in the same class or phenotype (Schena et al. 1995, Dudley et al. 2002, Simon et al. 2002). To find such regulated genes would require absolute gene expression (AGE) analysis, but existing methods for microarray AGE relied on common reference methods (Seita et al. 2012). In the common reference method, microarray data were normalized individually with the common reference and averaged. These averaged values were then mapped onto the probeset meta-profile in order to obtain the population's percentile and expression value for each probeset (Seita et al. 2012). While this methodology was helpful if the objective was to compare a probeset value against existing values, the value thus computed was relative and arbitrary; and subject to changes to the pooled value. It was therefore proposed that RankProd analysis method could be used to perform AGE without reliance on a common reference.

RankProd analysis is a non-parametric statistic originally modelled for RGE (Breitling et al. 2004). It combines the gene rank from the different arrays together instead of using actual expression data to select genes that are consistently ranked high or low (Breitling et al. 2004, Hong & Breitling 2008). The product ranks from all samples are then calculated as the test statistic in100X permutations with a nominated False Discovery Rate (FDR), for example, confidence at 1 – alpha = 95.0% or 99.9%. The RankProd analysis generates three pools of data classified as Negative Significant, Positive Significant and Non-significant. In the context of intra-class AGE, negative significant gene pool contains genes that are consistently expressed at low levels (CLE) across the libraries from the same class, and are thus classified as down-regulated (Saeed et al. 2003). Positive significant gene pool on the other hand contains genes that are consistently expressed at high levels (CHE) across all libraries from the same class (up-regulated) (Saeed et al. 2003). Genes that have very low expression below the present call or background threshold cut-off point, and genes that have inconsistent expression across all libraries from same class are classified as non-significant (Saeed et al. 2003).

In contrast, positive significant genes (up-regulated) identified from RGE are genes that are more highly expressed in treatment group relative to the expression in control class or libraries; and negative significant genes (down-regulated) are the genes that are more highly expressed in the control class relative to treatment class. Both RGE and AGE methods have been used at different stages in the work to answer different questions. For example, in paper #2, AGE methodology was for the first time used successfully to identify comprehensive gene-signature that included

genes with consistently low or high expression in both pre-eclamptic and normal placentae. The findings showed that the use of AGE analysis enables independent description of a comprehensive, globally and consistently expressed genes in the case and control samples. On the other hand, the findings showed that overt use of RGE analysis to the disadvantage of AGE could limit gene sets and our understanding of the real time and complexities of changes that could occur in a case or disease state.

The findings also confirmed earlier reports (Simon et al. 2003, Breitling et al. 2004) that RGE not only identifies limited candidate genes but could also exclude large proportion of genes that may be of relevance in characterising the molecular pathology of a disease including those with biologically relevant low level expression and genes with similar levels of expression in both the case and control samples. The findings further suggested that RGE could inherently identify genes whose expression patterns may be inconsistent but might have large differential expression between control and case samples.

3.4.5.2 Finding Biological Relevance of Genes (#2, #4 & #11)

For interpreting the expression results in terms of higher order of biological processes or molecular pathways, Gene Set Analysis methodology (GSA) method (Efron & Tibshirani 2007), Gene Set Enrichment Analysis ((GSEA), (Subramanian et al. 2005)), Over-representation analysis, Paroto Analysis for Vital Few Genes and Leading Edge Metagene ((LEM), (Subramanian et al. 2007)) analysis were used to identify over-represented, enriched and leading edge genes from Gene Ontology (GO) term, genetic pathways, transcription factor target gene sets.

3.4.5.3 GSA and GSEA (#2, #4 & #11)

GSEA is a computational method that determines whether *a priori* defined set of genes shows statistically significant, concordant differences between two biological states (e.g. phenotypes) (Subramanian et al. 2005). GSA was mostly used in the studies that focused on deciphering the differential enrichment of pathways between experimental and control samples. The Efron-Tibshirani's GSA uses the maxmean statistics, a modification of the GSEA procedure (Subramanian et al. 2005) for assessing significance and direction of regulation of pre-defined gene-sets. The maxmean statistic identifies the direction of regulation of the gene set by a measure of the absolute value of the positive or negative part of gene scores di in the gene set (Efron & Tibshirani 2007). In this analysis, all gene scores di in the gene set were taken to set all of the negative ones to zero. Average of the positive scores were then taken to give a positive part average = avpos. Similarly, gene 'scores di' in the gene set were taken to set all of the positive ones to zero and then average for the negative side were taken to give the negative part average avneg (Efron & Tibshirani 2007, Simon et al. 2007). Thus, the overall score for the gene set was avpos (up-regulated) if |avpos| > |avneg|, or otherwise it is avneg (down-regulated) (Simon et al. 2007). Consequently, transcription factor target gene sets and biochemical pathways analyses were performed.

3.4.5.4 Leading Edge Metagene (#11)

LEM is build-up of the GSEA to find the leading-edge subset in a gene set. Leading-edge subset in a gene set are those genes that appear in a ranked list at or before the point at which the running sum reaches its maximum deviation from zero (Subramanian et al. 2005). LEM is useful for identifying the core genes that account

for the gene set's enrichment signal. Therefore in the investigation of the effects of histamine on placental gene expression, downstream analysis of the gene set enrichment results with GSEA leading edge metagene (LEM) method was performed to identify groups of genes that were co-regulated by histamine, and are likely to form the core sub-signatures of genes that accounts for the gene set's enrichment signal to establish the distinct biological processes or pathways in PE placentae.

3.4.5.5 Pareto Analysis for Vital Few Genes (#11)

The Pareto Principle was applied to identify the "vital few" genes responsible for producing most of histamine effects. The principle as originally applied to quality improvement suggests that a great majority of effects (80%) are produced by a few key causes (20%) (Juran 1975). The importance of identifying the vital few histamine regulated genes lies in the plausibility that nothing of significance can happen in response to histamine unless these vital few genes are regulated. The analysis involved rank ordering of the frequency of involvement of a particular gene in multiple biological functions or ontologies, and applied the Lorenz (Lorenz 1905) cumulative curve to depict the distribution of the vital few genes.

3.5 Conclusion

There were many challenges for studying histamine effects on the placenta. The challenges ranged from using an appropriate *ex vivo* model that limited iatrogenic inflammation to tissues that are primed for or are already pro-inflammatory to maintaining maximal concentration of histamine introduced to experimental samples. These experimentations made substantial contributions to these areas of placental

research by showing that inflammatory effects can be controlled to investigate the effects of histamine on placenta that have undergone Syncytiotrophoblast degeneration and regeneration. These studies also showed that it is possible to obtain high quality RNA akin to pre-culture quality from micro explants cultured for 6 days at the liquid-gas interface. These studies also confirmed that placental explants are viable after 6 days culture but the explant size affects the viability and integrity after culture. These studies further showed that morphological assessment of the degree of STB degeneration is parallel to explant RIN and could be used as proxy measure for explant viability. These investigations further contributed to the knowledge of placental tissue culture by providing genomic evidence to confirm that placental explant culture at atmospheric oxygen was pathological and that culture per se triggered the expression of cathartic genes, and the effect is worsened by culture in atmospheric oxygen. In contrast, explant culture in an optimum hypoxic condition (8% oxygen) was physiological and optimum for term placental explant culture. The discussion of the rationale underpinning the bioinformatics approach that incorporated absolute gene expression modelling to uncover PE pathways and the complexity of histamine pathways in PE placentae demonstrated additions to knowledge about novel methods to architype intra-phenotype significant gene expressions.

Chapter 4

Theme 3: Understanding causes of elevated Histamine in Pregnancy and Pre-eclampsia

In this chapter:

The commentary is based on publication items, #5, #6 and #7:

- (#5) **Brew O**, Lakasing L, Sullivan M. (2007) Differential Activity of Histidine Decarboxylase in Normal and Pre-eclamptic Placentae. *Placenta*. May-Jun; 28(5-6):585-7.

- (#6) **Brew O**, Sullivan, M. H., & Roller S 2005, "Regulatory loops between cytokines and histamine in the human placenta", *Placenta*, vol. 26, no. 8-9, p. A.52

- (#7) **Brew, OB** & Sullivan MH (2001) Localisation of mRNAs for diamine oxidase and histamine receptors H1 and H2, at the foeto-maternal interface of human pregnancy, *Inflammation Research,* 50(9): 449-452

The focus of the commentary is to discuss the investigations that broadened our understanding of the causes of elevated histamine in pregnancy, with emphasis on histamine synthesis, production and metabolism in PE placenta.

Highlights:

- Histamine synthesis based on de novo HDC activity is higher in PE placentae than in normal placentae
- The cytokines IL-10, IL-1β and INF-γ time dependently regulated histamine production in human placenta, inducing both rapid (30 minute) and prolonged (24h) increases in histamine.
- Th1 (INF-γ) and Th2 (IL-10) like cytokines, pro-inflammatory (IL-1β) cytokines and endotoxins induce biphasic histamine increase in placenta. The responses are suggestive of both histamine release from storage and *de novo* histamine synthesis.
- Histamine receptors are expressed in human placenta.
- The co-expression of histamine receptors and DAO is consistent with a role for histamine at the foeto-maternal interface of human pregnancy
- There was a regulatory feedback loop between histamine, cytokines and DAO expression in human placenta.
- The expression of DAO in the decidua is widespread, indicating that the enzyme could also function to prevent prolonged exposure of decidual cells to bioactive histamine.

4.1 Introduction:

PE is a complex disorder of pregnancy that involves a systemic inflammatory response, endothelial activation within the maternal vascular system, increased maternal blood histamine and decreased histamine metabolism (Kapeller-Adler 1941, Redman et al. 1999, Chambers et al. 2001, Sargent et al. 2003). A number of the features of PE (hypertension, proteinuria, oedema, nausea, and headaches) as outlined in publication item #1 also mimic those of hyperhistaminemia (hypertension, proteinuria, oedema, nausea and vomiting, headaches, tachycardia, palpitation, convulsions, metallic taste). Normally, maternal plasma DAO levels increase up to 1000-fold during pregnancy (Southren et al. 1966a, Beaven et al. 1975), but this increase in DAO is attenuated in PE, with a resultant high blood histamine (Kapeller-Adler 1944, Achari et al. 1971).

The placenta is the primary source of histamine and DAO in pregnancy. However, the human placenta functions both to produce nutrition and remove waste products for the developing foetus (Lyall 2002), thus suggesting that histamine as found in the placenta with increased levels in PE placentae could be a mere by-product of foetal development and hence may not have functional roles in the placenta. Therefore it was hypothesised that (1) for histamine to have functional roles in the placenta it must express receptors at the foeto-maternal interface; (2) that placental HDC activity did differ between normal and PE tissues; and (3) that pro-inflammatory cytokines and endotoxins would increase histamine synthesis in the placenta.

Using DNA and RNA extraction, gene cloning, gene amplification and *in situ* hybridization technologies, the expression of mRNAs for histamine receptors H1 and

H2, and for diamine oxidase at the foeto-maternal interface were localised. Similarly, using enzyme assays including fluorometric and chemiluminescent methodologies and RNA amplification with reverse transcription polymerase chain reactions (RT-PCR) HDC activity and the effect of histamine on cytokines expression in the placenta were investigated. The commentary will critically reflect on the relative contribution made by these findings to the knowledge and understanding of molecular functions of histamine in human placenta.

4.2 HDC activity in PE placentae (Publication #5)

In theme 1, it was established that maternal histamine levels in PE increases above levels found in normal pregnancies, and the difference is significant (Figure 2-6). It was also established that the placenta is the source of the elevated histamine in human pregnancy and that nascent or *de novo* histamine rather than pre-formed histamine is the most likely source of the elevated histamine in PE. In order to increase our understanding of the source of the elevated histamine in PE, an enzyme assay study using fluorometric, ELISA, and chemiluminescent methodologies was conducted to test the hypothesis that placental HDC activity did not differ between normal and PE tissues and histamine.

The findings showed that HDC activity was present in all placental tissues tested, and normal placentae produced histamine at the rate of 4.77 ± 0.87 ng/g/hr, compared with 5.49 ± 0.52 ng/g/hr from the pre-eclamptic tissue ($p<0.05$, ANOVA). The work also showed that histamine production varied substantially between tissues, so the net rate of production for each placenta (Histamine level after 1 hour

of incubation – histamine level in corresponding control tissue) was compared using log ratios. In normal tissues this was -0.37 ± 0.26 ng/g/hr, compared with 1.86 ± 0.5 ng/g/hr from pre-eclamptic tissue (means ± s.e.m.), a significant difference ($p = 0.0003$ ANOVA). Thus confirming that histamine synthesis based on de novo HDC activity is higher in PE placentae than in normal placentae, and could contribute to the increased levels of histamine in maternal blood in pre-eclamptic pregnancies.

This finding did not identify whether the change in pre-eclamptic placental histamine is a cause of the disorder, or an effect of it, but it made it clear that the placenta can contribute to the pathology. Although this study showed that the rate of histamine synthesis is increased in PE placentae, it could not confirm previous report that histamine content may be increased in pre-eclamptic placentae (Szukiewicz *et al.*, 1999a). This observation was congruent because the study was intended to investigate synthesis rather than storage which may explain the difference. The findings however confirmed that the placenta contains histamine (Kapeller-Adler, 1952; Purcell, 1992), which would be produced by HDC. It was therefore suggested that in normal pregnancy histamine is stored in the placenta but the synthetic capacity may be relatively low compared to pre-eclamptic placentae.

4.3 Effects of Cytokines and Endotoxins on Histamine Production in the Placenta (Publication #6)

In attempts to investigate the causes of elevated histamine in PE, the effects of index cytokines for inflammation and endotoxins on histamine production in the placenta were examined. In this work, a total of six placental samples were collected from 6 different normal pregnancies. Three placental explants per sample were used to investigate the effect of key cytokines and endotoxins on histamine production in the placenta. Index cytokines Interleukin 1 beta (IL-1β), Interferon gamma (IFN-γ), Interleukin 10 (IL-10) respectively for pro-labour, Th-1, Th-2 cytokines families and endotoxin lipopolysaccharide (LPS) were tested.

Tissues in triplicates were stimulated with IL-1β (1 ng/ml), LPS (10 ng/ml), IL-10 (10 ng/ml), IFN-γ (100 U/ml) in the presence of aminoguanidine (10^{-4} M final conc.) and cultured to different time courses (30min, 1hr, 2hr, 4hr, 8hr & 24hr). Histamine was then measured with histamine ELISA (IBL, Hamburg) and corrected for tissue weight. The difference in histamine production in response to the stimulant over the specified time course were statistically analysed with ANOVA and Bonferroni/Dunn post hoc to determine significance difference. The analysis showed that histamine was produced with a mean baseline (time zero) value of 31.1± 4.4 nmol/L (mean ± SEM, n = 6), and IL-10, IL-1beta and INF-γ time dependently regulated histamine production in human placenta (Figure 4-1).

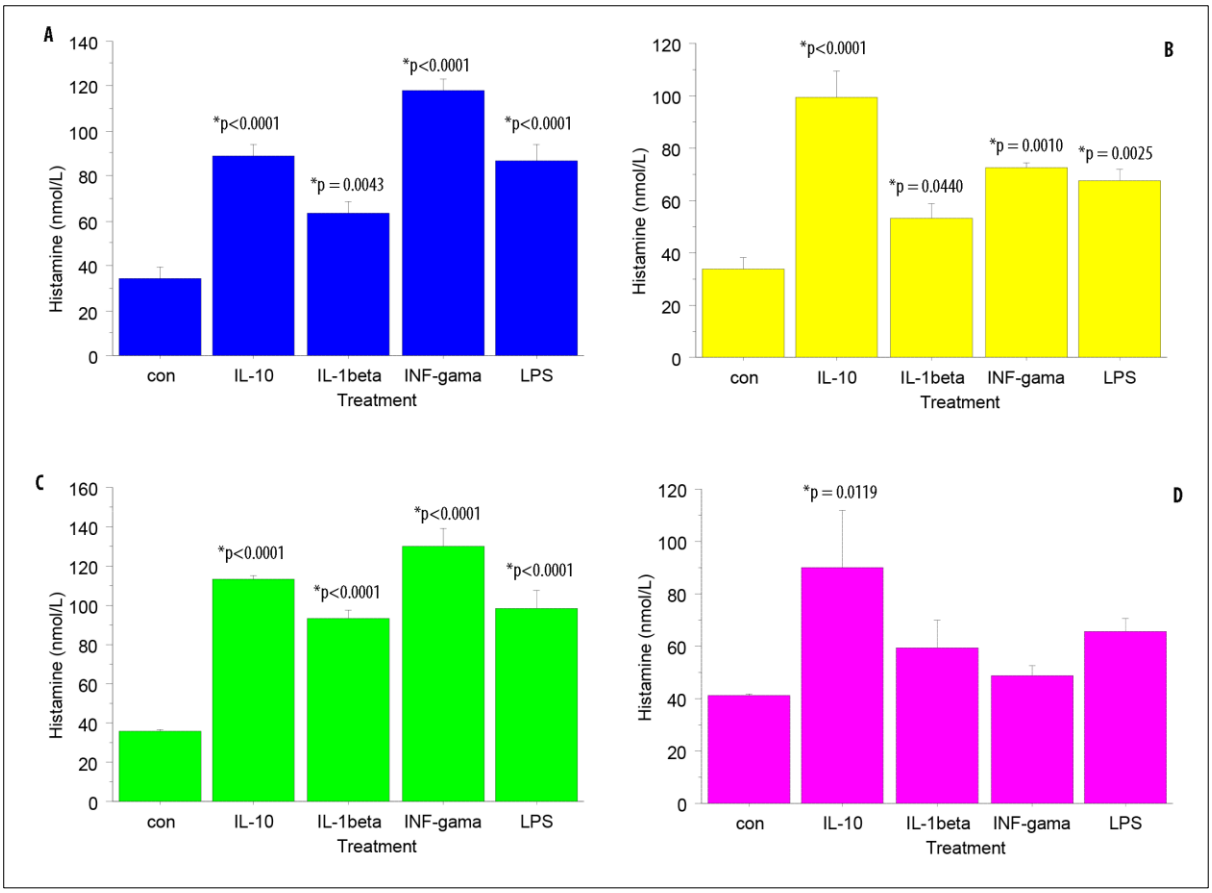


Figure 4-1: Cytokines and Endotoxin effect on Placental Histamine Production
Figures A – D show the effects of the index cytokines Interleukin 1 beta (IL-1β), Interferon gamma (IFN-γ = INF-gama), Interleukin 10 (IL-10) and Lipopolysaccharides (LPS) on histamine production in normal placenta respectively at times 30 mins, 1, hour, 2 hours and 4 hours. * = significant difference between control and stimulant induced histamine levels.

Figure 4-1A – D showed that IL-1β, IFN-γ, IL-10 and LPS up-regulated short term histamine production in human placenta. All stimulants used significantly (overall $p<0.0001$ Bonferroni/Dun test) increased histamine levels after 30 minutes of culture (Figure 4-1A). DAO activity was blocked during culture. This implies histamine levels would naturally rise during culture. Therefore stimulant induced histamine production was compared with time-matched control (non-treated) samples. While the effects of the endotoxin (LPS), pro-labour (IL-1β,) and Th-1 cytokines (IFN-γ) on histamine production were transient with maximal response at 2 hours (Figure 4-1C), the effect

of the Th-2 cytokine (IL-10) was sustained at the end of the 4 hours of culture (Figure 4-1D).

The transient effect observed at the end of 4 hours was reversed after 24 hours culture (Figure 4-2). All cytokines and the endotoxin tested significantly increased histamine levels after 24 hours culture (Figure 4-2). Thus, indicating that the index cytokines and endotoxin could both release histamine from storage (effect at 30 minutes) and induce histamine synthesis (effect after 24 hours).

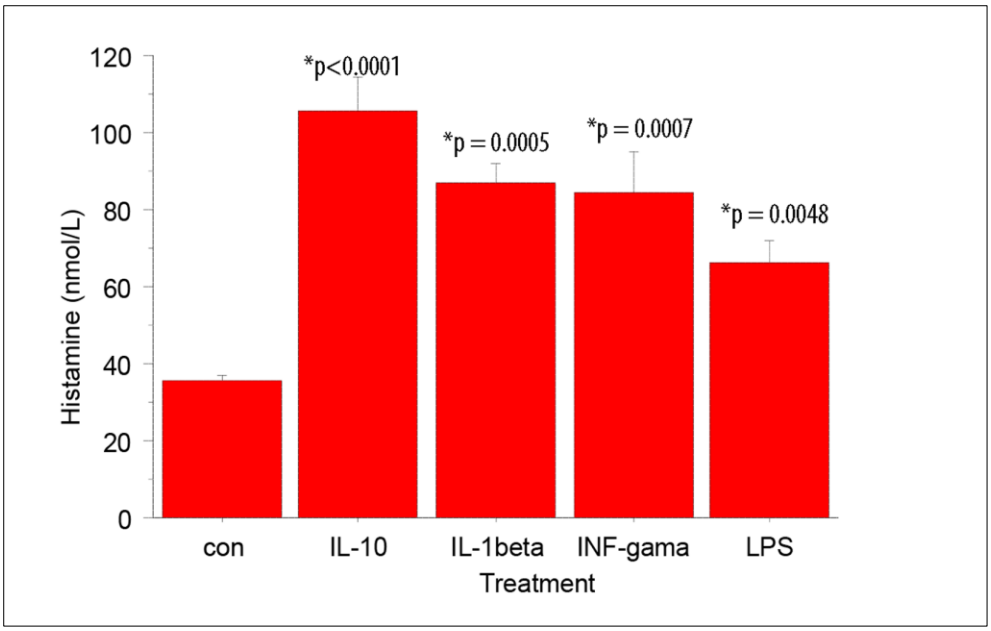


Figure 4-2: Effects of Cytokines and Endotoxin on Placental histamine production after 24 hours of culture

Figure shows the effects of the index cytokines Interleukin 1 beta (IL-1β), Interferon gamma (IFN-γ = INF-gama), Interleukin 10 (IL-10) and Lipopolysaccharides (LPS) on histamine production in normal placenta after 24 hours. * = significant difference between control and stimulant induced histamine levels.

These results showed that IL-1β, IL10 and LPS overall increased histamine levels at the 0.1 µmolar range, and that in the short term the pro-labour cytokines, Th-2 like cytokines and endotoxins could stimulate histamine production in the placenta at concentrations similar to levels expected to mediate physiological functions such as

such tissue remodelling, and cell proliferation while the INF γ (TH-1 cytokine index) produced histamine in the range normally associated with pathophysiology.

4.4 Histamine receptors and DAO mRNA are expressed in human placenta (Publication #7)

To determine whether the histamine thus produced in the placenta has functional roles in the placenta, DNA and RNA extraction, gene cloning, gene amplification and *in situ* hybridization technologies were used to examine and localise the expression of mRNAs for histamine receptors H1 and H2, and for diamine oxidase at the foeto-maternal interface. The results showed that co-expression of histamine receptors and DAO is consistent with a role for histamine at the foeto-maternal interface of human pregnancy.

These findings are important, not only for the understanding of possible role of histamine in human pregnancy but also showed for the first time that H1R, H2R and DAO mRNAs are expressed in juxtaposed positions in the placental and decidual components of the foeto-maternal interface of human pregnancy. The expression of mRNAs for histamine receptors H1 and H2 in human amnion, chorion, decidua and villous cytotrophoblast and stromal cells was novel. Previous studies at the time of publication had reported on finding the genes for histamine receptors 1 and 2 in human placenta tissues (Murakami et al. 1999) but not on the expression of the genes for the receptors in the specific tissues. Subsequent studies built upon this work to examine the expression of receptors in the syncytiotrophoblast cell layers (Matsuyama et al. 2004, Matsuyama et al. 2006).

The presence of mRNA for DAO in human placenta was documented previously (Zhang *et al.*, 1995; Zhang & McIntire, 1996), but the localisation of mRNA expression had not been previously reported at the time of going to press. Therefore this work was significant in that it extended the understanding and knowledge of the expression site of the enzyme in the syncytium. The work further provided insight into the plausible role of DAO as a metabolic barrier to prevent excessive entry of active histamine into the maternal or foetal circulations. Similarly, the widespread expression of the enzyme in the decidua showed that it could also function to prevent prolonged exposure of decidual cells to bioactive histamine.

4.5 Conclusion

As the expression of histamine receptors is generally required for any functional effects of the amine in tissues, the localisation of histamine receptors in human amnion and chorion as well as in decidua and villous trophoblast cells was a crucial discovery. The findings clearly showed that these tissues could be targets for the histamine thus produced *de novo* in the placenta, and therefore enhances our knowledge and understanding of the molecular functions of histamine in human placenta. These studies further showed that products of inflammation, tissue remodelling, cell proliferation as normal placentation processes could trigger increased production of histamine. The findings therefore demonstrate the contribution made to the knowledge and understanding of the mechanisms that may underlie the regulation of histamine production in human placenta; a breakdown of which could contribute to elevated histamine associated with pre-eclampsia.

Chapter 5

Theme 4: Effects of histamine on placental genes expression: Implications for pre-eclampsia

In this chapter:

The commentary is based on the publication items #2, #6, #8 - #11

- (#2) **Brew, O**., Sullivan, M.H. & Woodman, A. (2016) Comparison of Normal and Pre-Eclamptic Placental Gene Expression: A Systematic Review with Meta-Analysis. *PloS One*, 11(8), p.e0161504.
- (#6) **Brew O**, Sullivan, M. H., & Roller S (2005), "Regulatory loops between cytokines and histamine in the human placenta", *Placenta*, vol. 26, no. 8-9, p. A.52
- (#8) **Brew, O**. & Sullivan, M.H.F. (2007) Histamine regulates placental diamine oxidase mrna expression - Evidence for a feedback loop decreasing histamine production in pregnancy? *Placenta*, 28(8 - 9), p.A71.
- (#9) **Brew OB,** Sullivan MHF (2015) Placental Gene Expression in Response to Histamine and Oxygen, *Gene Expression Omnibus*. Series accession number GSE74446 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74446)
- (#10) **Brew O**, Sullivan M. (2017) Elevated Histamine Model: A Protocol for an ex vivo model for in vitro study of histamine effect on placenta. *Protocols.io* dx.doi.org/10.17504/protocols.io.jigckbw
- (#11) **Brew O**, Sullivan MHF. Comparison of elevated histamine model and pre-eclamptic placental gene expression,

Highlights:

- Histamine and DAO: two major biomarkers implicated in pre-eclampsia form a bi-directional regulatory negative feed-forward relationship in the placenta.
- Increasing histamine down-regulates DAO mRNA expression and this could lead to diminished DAO activity in the placenta.
- Histamine up-regulated IL-1b, IL-10 and INF-γ production term placentae and this was reflected in increased long-term changes in mRNA levels.
- The relative up-regulation of Th-1 cytokine INF-γ in response to prolonged exposure of high dose histamine is greater than that of IL-10 (Th-2 type cytokine).
- Pre-eclamptic (PE) placentae express unique set of genes that are not significantly expressed in Normal (NP), but retain its ability to express the genes associated with in NP.
- NP and PE placentae both share common biological pathways but additional pathways that are not significantly affected in NP are uniquely enriched in PE placentae.
- Multiple Over-Represented genetic pathways underpin the aetiology of PE.
- The natural process by which pre-eclampsia develops is affected by the amount of histamine in the maternal blood and placenta.
- The natural process by which pre-eclampsia develops is indirectly affected by histamine via covariate functional groups regulated by specific PE placental genes.

5.1 Introduction

Several studies have demonstrated that histamine signalling through HRH1 generally activates cGMP, Ca^{2+}fluxes, CaMKII, NFκB, Phospholipase A_2, and Phospholipase D (Hill 1990, Jutel et al. 2001, Bakker et al. 2004, Matsubara et al. 2006). In contrast, HRH2 activates cAMP, PKC-α PKC-β1 adenylate cyclase, c-Fos, c-Jun, and Ca^{2+} fluxes in the placenta (Beaven 1982, McMillan et al. 1985, Hill 1990, Ghosh et al. 2001, Jutel et al. 2001, Davio et al. 2002). These histamine signalling pathways have been linked to regulation of placental cytokines, trophoblast proliferation, differentiation, inflammation, placental growth and cervical remodelling (Falus & Meretey 1992, Hamano et al. 1998, Jutel et al. 2001, Ma et al. 2002, Liu et al. 2004, Cricco et al. 2006, Matsubara et al. 2006, Matsuyama et al. 2006, Fukuda et al. 2007). Nonetheless, knowledge on placental gene expression in response to histamine was limited.

With the advent of molecular and genomic data and complementary analytical tools, the possibility of going beyond the traditional index gene finding to a more complex investigation of the genomic architecture of the placenta was deemed feasible. Therefore, a comprehensive analysis of the placental gene expression in response to histamine was conducted with a combination of gene expression analysis technology including multiplex RT-qPCR and microarray technologies to provide better understanding of the effects of histamine in normal and pre-eclamptic placentae.

5.2 Histamine has regulatory feedback loop with Placental DAO (Publication #6 & #8)

A relationship between histamine concentration and diamine oxidase activity has been known for sometimes. It was reported as far back in the 60s that histamine in high concentration inhibits intestinal diamine oxidase activity in animal models (Mondovi et al. 1965, Sasiak et al. 1975). Yet, this relationship was not tested in human placenta even though there was increasing evidence that maternal plasma diminished DAO activity concur with increasing maternal blood histamine (Section 2.2.4). Perhaps, this scarcity of evidence could or may have been partly due to lack of clarity on the source of increasing blood histamine and DAO in maternal blood. In an attempt to increase knowledge and our understanding of the relationship between histamine and DAO in the placenta, the effect of histamine on DAO mRNA expression was examined.

The study was guided by the hypothesis that increasing histamine concentration had inhibitory effect on placental DAO mRNA expression in placental explants. Therefore in a time series experiment, placental explants were treated with histamine at varying concentrations 0.01μM, 1μM and 100μM and cultured for 24 hours. Total RNA was extracted from time zero controls, time course histamine treated and non-treated (negative controls) cultured placental explants and analysed with multiplex real time RT-qPCR for DAO mRNA expression. Fold change expression relative to time zero samples was determined with ΔCT method using beta-actin as reference gene.

The findings showed that histamine regulated DAO mRNA expression, and the response was time and concentration dependent. Overall, 0.01μM histamine

concentration induced exponential increase in DAO expression with highest response occurring after 24 hours. The findings also showed that prolonged exposure to 1μM histamine concentration up-regulated DAO but not as strong as 0.01μM, whereas response to 100μM histamine was biphasic. At the high concentration (100μM), there was an immediate strong up-regulation followed by complete down-regulation after 24 hours.

Interestingly, the findings confirmed the previously identified (Mondovi et al. 1965, Sasiak et al. 1975) dose dependent histamine inhibition of DAO activity and further showed that the effect could suggestively be intrinsically linked to histamine regulative effect on DAO gene expression in human placenta. Furthermore, the findings suggested that histamine appears to induce immediate concentration dependent increase in DAO mRNA expression, which if translated to protein would accelerate histamine metabolism. And while physiological levels of histamine (0.01μM) increase placental DAO expression, long term exposure of placental explants to increasing histamine concentration appears to diminish DAO mRNA expression. These findings also showed a bi-directional feed-forward loop between histamine and DAO: two major biomarkers implicated in pre-eclampsia. In this loop, increasing histamine levels appear to trigger DAO mRNA expression which could lead to increased DAO protein activity and subsequent deamination and removal of accumulating histamine in the placenta. The findings also showed that excess production of histamine could down-regulate DAO mRNA expression leading to diminished DAO activity and further elevation of histamine levels in the placenta.

5.3 Histamine regulates placental Th-1/Th-2 regulation (Publication #6)

Human CD4+ and CD8+ T lymphocytes, macrophages, stromal cells express HDC, H1R and H2R mRNA, and produce endogenously synthesised histamine (van der Pouw Kraan et al. 1998, Hellstrand et al. 2000). Thus, changes in histamine concentration have been linked to activation of human T cells synthesis of Th-2 type cytokines, and down regulation of Th-1 cytokines (van der Pouw Kraan et al. 1998). Typically, histamine through receptor H2 activation inhibits IL-12 mRNA synthesis, while up regulating IL-10 in monocytes or macrophages (van der Pouw Kraan et al. 1998, Packard & Khan 2003). Histamine also inhibits IL-2 and INF-γ synthesis in mice T lymphocytes (Kubo & Nakano 1999) and IL-1 production in mice macrophage (Falus & Meretey 1992, Kubo & Nakano 1999). It is also shown that specific cytokines including IL-3 and GM-CSF regulate T cell production of histamine (Falus & Meretey 1992, Kubo & Nakano 1999).

Relative synthesis of Th-1 and Th-2 type cytokines are implicated in the regulation of maternal immune response at the Foeto-Maternal Interface (FMI) (Wegmann et al. 1993). In this mechanism, it is suggested the foeto-placental unit redirects maternal immunity away from cell mediated immunity towards enhanced humoral responsiveness. In this case, it is observed that the balance between Th-1 and Th-2 cytokines is switched in favour of Th-2 cytokines (Wegmann et al. 1993) as activation of cell mediated immunity is usually harmful to the foetus (Rukavina & G 2000). CD4+ T helper Th-1 cells are known to secrete IL-2, IFNγ and TNF α, where IL-2 and TNF α in turn activates NK cells towards cell directed lysis (Rukavina & Vince G, 2000; El *et al.*, 2008; Laskarin *et al.*, 2008). Both murine and human pregnancies with increased expression of Th-1 cytokines are highly unsuccessful

(Lin *et al.*, 1993; Wegmann *et al.*, 1993). Conversely, successful pregnancies tend to have increased expression of Th-2 cytokines (IL-4, IL-5, IL-10 and IL-13) (Rukavina & Vince G, 2000).

Therefore, it was hypothesized that during normal pregnancy, histamine once produced could regulate Th- like cytokines in the placenta to modulate maternal immune response in favour of protecting the allogeneic foeto-placental allograft.

The effects of increasing histamine concentrations (10^{-7}, 10^{-5}, 10^{-3} M/L) respectively for low, medium and high dose on placental synthesis and production of cytokines were therefore evaluated. RNA was extracted from the control and histamine treated explants cultured for up to 24 hours and subsequently analysed with real time RT-qPCR. Gene expression was calculated with Pfaffl equation for the expression of IL1beta, IL-10 and INF-γ, with beta-actin as housekeeping gene. The respective proteins were assayed with ELISA. Figure 5-1 A, C and E show the effects of histamine on IL1beta, IL-10 and INF-γ mRNA expression respectively. The corresponding protein levels are shown in Figure 5-1 B, D and F respectively.

The results showed that overall, histamine in low dose up-regulated (Figure 5-1) the expression of IL-10, IL-1β and INF-γ mRNA in term placenta after prolonged exposure, although there was an immediate inhibitory effect within 2 hours of IL-10, and INF-γ gene expressions. In contrast, high dose histamine inhibited the mRNA expression of IL-10 and INF-γ transcriptomic expression after prolonged exposure. In the case of IL-1β, the low dose histamine had minimal effect on the mRNA

expression after 24 hours but high concentration transiently increased the expression levels with peak effect occurring after 12 hours.

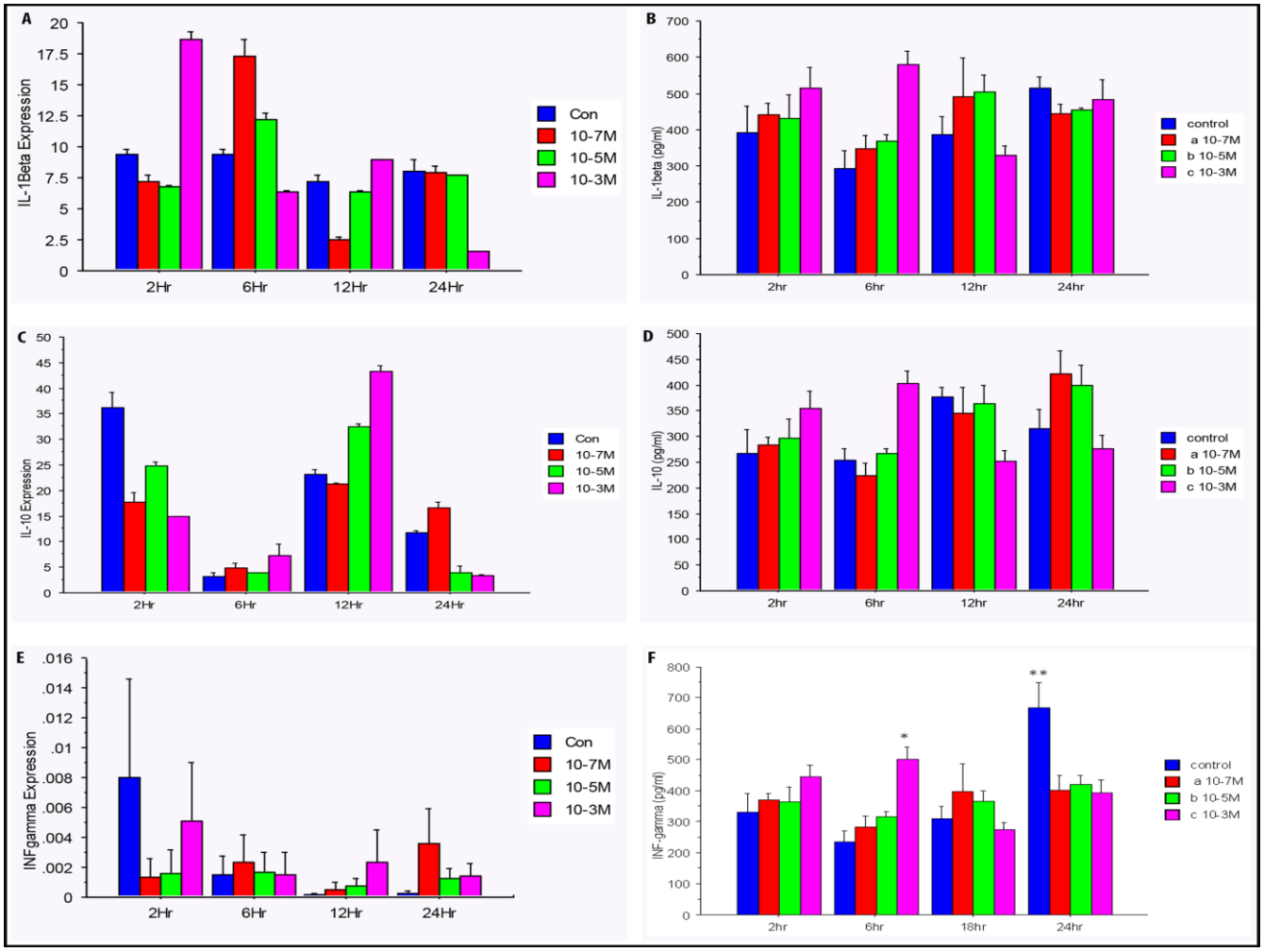


Figure 5-1: Effects of Histamine and Cytokine Synthesis and Production

Figures A, C and E represent effects of histamine on IL1beta, IL-10 and INF-γ mRNA expression respectively. Figures B, D and F show the respective protein synthesis for IL1beta, IL-10 and INF-γ after 24 hours. Expression and protein levels are baseline (time zero) normalized.

The changes in the transcriptomic expression were reflected in the message translation into protein. As shown in Figure 5-1 B, D and F, the most persistent change in response to low dose histamine was observed after 24 hours of culture: low dose histamine caused gradual, yet sustained increase in IL-1beta, IL-10 and

INF-γ levels while high dose transiently increased the protein levels with peak effect occurring after 6 hours.

Also, while the individual gene expression data appeared to suggest that the effect of histamine on the respective genes after 24 hours could be minimal, analysis of the relative expression of INF-γ to IL-10 representing TH-1/Th-2 index (Figure 5-2) revealed a contrasting outcome. These findings (Figure 5-2) showed that the relative up-regulation of the Th-1 cytokine INF-γ in response to prolonged exposure of high dose histamine was greater ($p < 0.001$) than that of IL-10 (Th-2 type cytokine). In contrast, the relative expression of INF-γ to IL-10 mRNA expression at low dose histamine was significantly ($p=0.018$) decreased (i.e., Th-1/Th-2 ratio), therefore skewing the balance of the Th-1/Th-2 ratio in favour of Th-2 response in low dose histamine.

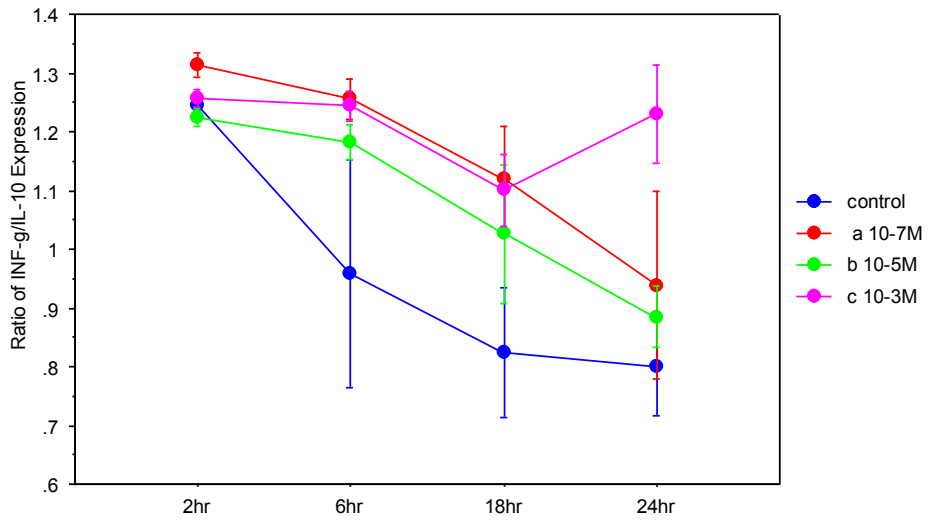


Figure 5-2: Ratio of INF-γ and IL-10 Expression in the Placenta

These findings suggested that low dose histamine relatively up-regulated both Th-1/Th-2 response in the placenta but favoured relative abundance of Th-2 response. These findings also confirmed previous reports that physiological levels of histamine support humoral immune response in the placenta, in favour of Th-2 up-regulation towards positive pregnancy outcome (Falus & Meretey 1992, Wegmann et al. 1993, van der Pouw Kraan et al. 1998, Weetman 1999, Lim et al. 2000, Pap et al. 2007).

5.4 Uncovering the complexities of PE genetic pathways (Publication #2)

Having established that there was a clear distinction between the levels of maternal blood histamine in PE and in normal pregnancy (Section 2.2.3); it was felt necessary to ascertain the extent to which placental gene expression differed between normal and pre-eclamptic placentae. A systematic review with meta-analysis of placental transcriptomic expression arrays was therefore conducted: first, to define the gene sets associated with normal and PE placentae; and secondly, to ascertain whether there was any relationship between the genes associated with PE and the clinical and or pathological presentation of the condition.

Using Absolute Gene Expression (AGE) methodology (section 3.4.5.1), significant genes defined as genes that were consistently up-regulated or down-regulated in PE and normal placentae (NP) were identified. Out of a total of 16,701, 5146 (31%) and 4394 (26%) placental genes were respectively identified as significantly down-regulated and up-regulated in the PE placentae (Table 5-1). In contrast, 846 and 1076 genes were respectively down-regulated and up-regulated in normal placentae (Table 5-1). The expression levels of 14779 and 7161 genes respectively in NP and

PE placentae were inconsistent and thus were classified as non-significant (Table 5-1).

Table 5-1: Differentially Expressed Genes in NP and PE Placentae

		Absolute PE only	Absolute NP only
Negatively Significant Genes	# of down-regulated Significant Genes	5146	846
	% of down-regulated Significant Genes	31%	5%
Positively Significant Genes	# of up-regulated Significant Genes	4394	1076
	% of up-regulated Significant Genes	26%	6%
Non-Significant Genes	# of Non-Significant Genes	7161	14779
	% of Non-Significant Genes	43%	88%

A total of 167 microarray samples (PE = 68; NP = 99) were meta-analysed as case-to-control matched samples with Fisher's method or as case (PE) only and control (NP) only with RankProd analysis. About 31% more genes were identified as differentially expressed (DE) in PE only than in case matched baseline (NP) subtraction PE. RankProd analysis Confidence at (1 - alpha): 95.0%; False Significant Proportion: 0.05 or less; p value threshold for fisher's metaP = 0.05. PE = Pre-eclampsia Placentae; NP = Normal Placentae.

Bioinformatics analyses of the significantly regulated genes showed that the genes were involved in 207 and 126 biological pathways respectively for PE and NP. Interestingly, all the 126 pathways enriched by the NP placental genes were also enriched in PE, but with higher enrichment ratios. A further 81 biological pathways were significantly enriched by PE placental genes only. Crucially, a number of pathways that have been associated with clinical manifestation of PE, including allograft rejection, apoptosis, VEGF signalling pathway, notch signalling pathway, and p53 signalling pathway, were significantly enriched in PE only. In addition, pathways such as histidine metabolism pathway and Fc epsilon RI-mediated signalling pathway were also significantly enriched in PE placentae only. These pathways have known histamine mediated functions. Thus, suggesting that histamine has some functional roles in the placenta and could be involved in the pathogenesis of PE.

For example, Fc epsilon RI-mediated signalling pathway is a pathway in mast cells. Mast cell concentration is significantly increased in PE placentae (Purcell 1992, Szewczyk et al. 2005). Fc epsilon RI-mediated signalling in mast cell is initiated by interaction of multivalent allogens with the extracellular domain of the alpha chain of Fc epsilon RI to release pre-formed histamines, proteoglycans (especially heparin), phospholipase A2 and subsequently, leukotrienes (LTC4, LTD4 and LTE4), prostaglandins (especially PDG2), and cytokines including TNF-alpha, IL-4 and IL-5 (Kambayashi & Koretzky 2007). These mast cell activated mediators and cytokines levels are increased in PE, and increasing levels of these products have been associated with inflammatory responses.

Inflammation pathways have been strongly linked to the pathophysiology of PE (Ilekis et al. 2016), and it has been suggested that the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway mediates excessive maternal intravascular inflammation that leads to endothelial dysfunction (Aban et al. 2004, Redman et al. 1999). In this context, it has been hypothesised that PE arises as a result of an excessive maternal intravascular inflammatory response to pregnancy, and that it involves the activation of both innate and the adaptive immune system, neutrophil, and the complement system pathways which involve both histamine and mast cell signalling (Greer et al. 1991, Falus & Meretey 1992, Prieto et al. 1997, Redman et al. 1999, Belo et al. 2003, Lynch et al. 2008).

Similarly, allograft rejection, graft vs. host disease, and primary immunodeficiency pathways were significantly enriched in PE. This observation is consistent with

previous opinions that heightened immune responses in PE pregnancies could be a consequence of chronic foeto-allograft rejection reaction (Martinez-Varea et al. 2014). Accordingly, PE shares similarities with graft rejection linked to over activation of immune pathways (Wegmann et al. 1993, Weetman 1999, Raghupathy 2001, Saito & Sakai 2003, Dong et al. 2005, Wilczynski 2006). Integral to this is the argument that disequilibrium of Th-1/Th-2 cytokine balance in favour of Th-1 (IL-2, IL-12, IL-15, IL-18, IFNgamma, TNFalpha vs. IL-4, IL-10, TGFbeta); precipitation of subsets of immunocompetent cells (T CD4, suppressor gammadeltaT, cytotoxic T CD8, Treg, Tr1, uterine NK cells); innate immunity (NK cytotoxic cells, macrophages, neutrophils and complement); adhesion molecules; fgl2 prothrombinase activation (Wegmann et al. 1993, Weetman 1999, Raghupathy 2001, Saito & Sakai 2003, Dong et al. 2005, Wilczynski 2006) and under-expression of Heme oxygenase-1 (HO-1) (Linzke et al. 2014) underpin the development of PE.

These findings not only confirmed the previous reports that inflammation and immunologic pathways are involved in PE pathophysiology, but also made major contributions to the knowledge and understanding of PE by providing evidence for a comprehensive database of gene sets with impacts on biomarker synthesis and early screening for PE. The PE immuno-inflammatory paradigm is however not universally supported as a recent review by Ahmed and Ramma (Ahmed & Ramma 2015) appears to down-play the roles of inflammatory, hypoxia and immunologic pathways in favour of angiogenic response as the cause of PE.

Ahmed and Ramma (Ahmed & Ramma 2015) argued that recent work supports the hypothesis that PE arises because of the loss of vascular endothelial growth factor (VEGF) activity, which in turn is caused by increase in the levels of endogenous soluble fms-like tyrosine kinase-1 (sFlt-1), an anti-angiogenic factor (Ahmed & Ramma 2015). SFlt-1 binds and reduces free circulating levels of the pro-angiogenic factor VEGF, and thus inhibits the beneficial effects mediated by flt-1 (also known as vascular endothelial growth factor receptor 1 (VEGFR-1)) on maternal endothelium, with consequential maternal hypertension and proteinuria (Andraweera et al. 2012, Ahmed et al. 1997).

It is further argued that altered balance of circulating pro-angiogenic/anti-angiogenic factors such sFlt-1, soluble endoglin, and placenta growth factor (PlGF) are unique to PE (Andraweera et al. 2012, Ahmed et al. 1995, Ramma et al. 2012, Ahmed & Ramma 2015, Ahmed et al. 1997). Interestingly, we also found evidence to support this view: for it was identified that VEGF signalling pathway was significantly enriched only by the PE placental genes. This therefore led to a conclusion that biological pathways affected in PE are complex, and instead of the uni-lateral conclusion drawn by Ahmed and Ramma (Ahmed & Ramma 2015), our findings offered more evidence to support the more global view that multiple and concurrent dysregulated pathways underpin the aetiology of PE (Ilekis et al. 2016), and no single pathway could be associated with the origins of PE. Thus, this work made major contributions to the knowledge and understanding of the molecular pathology or the genomic basis of PE, and provided further evidence for the links between histamine and PE.

5.5 Histamine regulates specific genes in pre-eclamptic placenta (Publications #9, 10 & #11)

While it is widely accepted that the placenta is a major source of elevated histamine in the blood of women with PE (section 2.2.2), the placenta concurrently releases highly active diamine amine oxidase (DAO) enzymes into the maternal circulation to metabolise the histamine, thus forming histamine-DAO-Axis (HDA) (section 2.2.3). However, in contrast to the effective clearance of histamine in normal pregnancy, the histamine-DAO-axis in PE is defective (section 2.2.4). Yet, our understanding of the impact of the mechanistic failure of the HDA and the consequential effects of elevated histamine on placental gene expression in PE was tenuous.

Therefore, an *ex vivo* elevated histamine model (EHM) in human placental explants with some parallel characteristic to *in vivo* defective histamine – DAO – axis (dHDA) system was developed (section 3.2.5 above) and used to examine the effects of elevated histamine on the placental gene expression. The work was supported by the hypotheses that (1) genes regulated by histamine in elevated histamine placental model would show similar changes in expression to PE specific placental genes and (2) genes regulated by elevated histamine in an EHM would have known functions that could be biologically relevant in PE placentae.

5.5.1 Global Changes in EHM Gene Expression

First, the gene expression patterns among histamine treated (also known as EHM) and control explants were compared to determine if the two classes differ with regard to expression profiles and whether the differences were significant. The BRB ArrayTools platform (Section 3.4.5) was used for the analyses. As reported in publications #3 and #4, quality control (QC) of the microarray raw data was

assessed with Expression Console (Affymetrix) for .CEL files integrity. Probes with unusual signal patterns or signal strength and arrays showing low correlation between hybridization controls thus failed this initial QC measures were excluded from further analysis. The .CEL raw data were imported and processed with Robust Multi-array Average (RMA) into BRB-Array Tools version 4.5.1 – Stable (Simon et al. 2007), and further processed using the R bionconductor packages (including Affy, annotate, annaffy, gcrma, simpleaffy) into log2-transformed and quantile-normalised linear model expression matrix. Genes showing minimal variation across the set of arrays were excluded from the analysis. Genes whose expression differed by at least 1.5 fold from the median in at least 20% of the arrays were retained. After reducing multiple probeset to singles using the most variable probeset measured by IQR across arrays, 20233 genes were used for further analyses.

The changes in gene expression in response to histamine treatment relative to the control under Physiologic oxygen concentration (8% O_2) were established. Increases were determined for mRNA that were considered present or absent in the control and present in the histamine treated sample. Similarly, decreases were determined for mRNA that were present in the control sample and present or absent in the histamine treated sample. Scatterplot of Phenotype Averages (SPA) and Class Comparison analyses (CCA) (Simon et al. 2007) were used to establish the difference in gene expression.

For the SPA analysis, average log-ratios within one phenotype (control samples), were plotted on the x-axis against the average log-ratio within the other phenotype (histamine treated samples), on the y-axis in a scatterplot. These averages were then taken on a gene-by-gene basis, and each gene was represented by a single point in the resulting scatterplot. The differentially expressed genes among the two

phenotypes were determined as genes whose fold-change between the geometric mean of the expression ratios within each of the two classes was greater than a fixed threshold (1.5-fold change). Fold-change was defined as the ratio of geometric mean of intensities in class 1 to geometric mean of intensities in class 2, where class 1 was the treatment group and class 2, the control group. Thus, fold-change of less than 1 was regarded as down-regulation, and that greater than 1 as up-regulated. The SPA analysis identified (Figure 5-3A) a total of 494 differentially expressed genes (315 up-regulated and 179 down-regulated) between the EHM and Control samples.

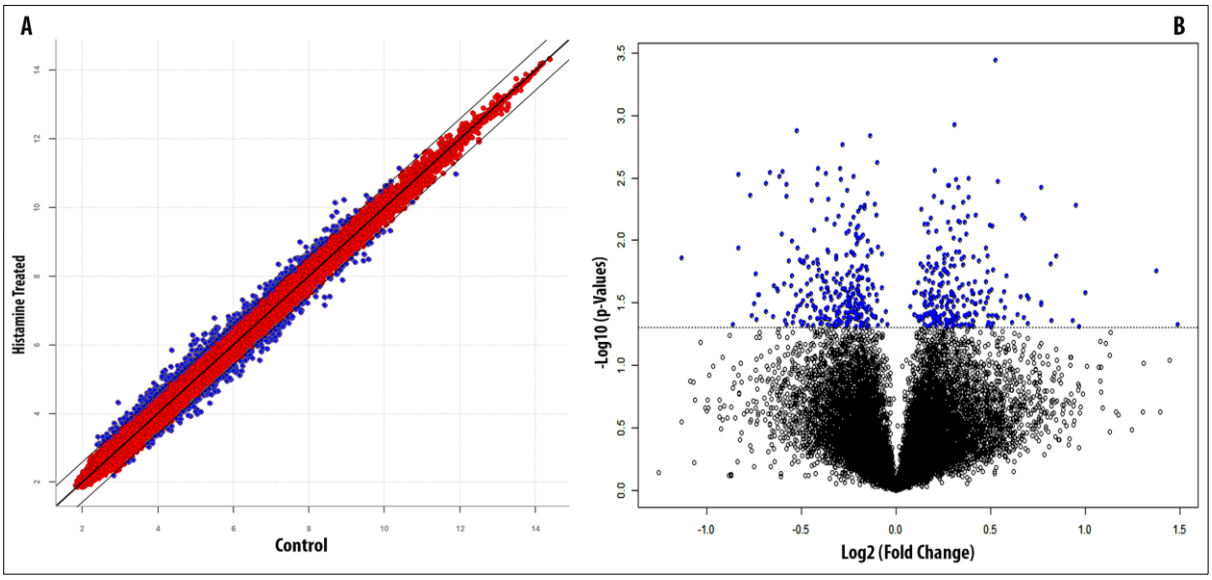


Figure 5-3: Plots of differentially expressed among EHM and Control Samples
Figure A shows the Scatterplot of differentially expressed genes with 1.5-fold change among elevated histamine model (EHM) control samples. Figure B shows a volcano plot of significant ($p<0.05$) differentially genes among EHM and control classes, identified with Class Comparison analysis. EHM = histamine treated samples. Appendix 3, 4 & 5 contain gene lists for both figures A and B. Expression patterns of the genes were confirmed by performing RT-qPCR on three randomly selected genes (AKR1C1, IGFBP5, and EREG relative to L19; Appendix 9).

To determine whether the differences in expression observed among the EHM (histamine treated) and control classes were significant, class comparisons analysis using a random-variance t-test was performed (Figure 5-3B). Figure 5-3B shows the

results of the Class comparison analysis as a Volcano Plot of the significantly expressed genes among EHM and the controls.

In the Class comparison analysis, the purpose was to establish a general trend in the class differences with regard to expression profiles therefore a modest p value of less the 0.05 was deemed sufficient (Simon et al. 2007). The Class Comparison analysis was used because it is a powerful model that permits as was intended, the sharing of information among genes about within-class variation without assuming that all genes have the same variance (Wright & Simon, 2003).

Three key facts were learnt from these analyses:

1. That elevated histamine alters placental gene expression
2. That the relative differences in expression of specific genes between elevated histamine model and control sample are significant.
3. While the analyses identified specific genes with significant differential expression between the EHM and control samples, the information revealed very little insight into the plausible roles or effects of the elevated histamine regulated significant genes in the pathophysiology of PE placentae.

Therefore further innovative bioinformatics analyses were undertaken to decipher the plausible effects of elevated histamine on the placenta in the pathophysiology of PE

5.5.2 Determination of the plausible effects of histamine in PE Placentae

In the preceding section, the point was raised that the mere comparison of differential gene expression between EHM and control samples provided limited insight into the plausible role of placental histamine in PE pathophysiology. This view was drawn from the fact that in addition to PE, defective histamine – DAO – Axis

leading to elevated histamine occurs in other trophoblast related complications of human pregnancy such as pre-term labour and spontaneous abortion. Therefore, in order to determine the effects or roles of elevated histamine in PE placentae, innovative yet robust down-stream analyses were performed. The purpose of these down-stream analyses was to identify specific elevated histamine regulated placental genes relevant to PE.

To achieve this, intra-class absolute gene expression analysis (Section 3.4.5.1) and *In Vivo* Validation of *In vitro* Expression protocols were developed. PE placental genes were identified from the pool of genes regulated in EHM. The *In Vivo* validation of *In vitro* Expression is a complex bioinformatics workflow involving a systematic review process, meta-analysis, comparison analysis and confusion matrix analysis.

First, GEO and ArrayExpress databases were systematically searched for case control pre-eclampsia OR histamine AND Placenta Homo sapiens RNA array assays. Multiple RNA array assays for PE matched with NP placentae were retrieved. Intra-class Absolute Gene Expression analysis using RankProd statistics was applied to identify significant genes in EHM model, PE placentae and normal placentae respectively. The PE genes were defined as a set of specific genes that are consistently expressed exclusively in PE placentae but not in normal placenta. A variant definition for PE genes in EHM model was given as the set of consistently expressed genes in response to elevated histamine in EHM that mapped to genes consistently expressed in PE placentae but not to consistent significantly expressed genes in normal placentae, or to cultured control sample.

The analyses identified 270 genes that were consistently expressed in the elevated histamine model and mapped to PE placental specific genes. That is, these genes were not consistently expressed in normal pregnancy placentae or cultured explants without histamine treatment, and thus was classified as histamine regulated specific genes in PE (HSPE). The overall concordance between the expression pattern of the HSPE genes and the PE specific genes, measured as accuracy and intra-class correlation coefficient were 92%, Kappa = 0.836 respectively. Also, 94.3% of the down-regulated HSPE genes were down-regulated in PE placentae. Similarly, 90% of the up-regulated HSPE genes were up-regulated in PE placentae. These suggested that the pattern of expression of the HSPE genes closely mimicked gene expression pattern of the *in vivo* PE placentae.

With this clarity, the biological functions of the HSPE genes were examined with in-depth bioinformatics pipeline including Over-representation of Gene Ontologies, Pareto Analysis for vital few genes and GSEA Leading Edge Metagene (LEM) analyses of biological pathways for leading edge genes. The analyses uncovered complex gene ontologies (Table 5-2) that offered stronger elucidation of plausible histamine functions in PE placentae. Analysis for the vital few genes revealed that 51 (vital few) of the 270 HSPE genes (Table 5-3) were involved in 80% of the associated biological functions. Downstream analysis with leading edge meta-genes analysis further identified 24 enriched pathways (Table 5-4) from 4 databases (KEGG, Reactome, Wikipathways and Panther) that histamine specific consistent regulated genes could play significant roles.

Table 5-2: Histamine Specific Genes in PE Ontologies

GO Category	GO Term ID	Name	C	O	E	R	Pvalue
MF	GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	87	9	1.17	7.71	2.46E-06
MF	GO:0002064	epithelial cell development	195	12	2.61	4.59	1.30E-05
MF	GO:0060684	epithelial-mesenchymal cell signaling	7	3	0.09	31.96	8.00E-05
MF	GO:0060710	chorio-allantoic fusion	7	3	0.09	31.96	8.00E-05
MF	GO:0048002	antigen processing and presentation of peptide antigen	173	10	2.32	4.31	1.16E-04
MF	GO:0006397	mRNA processing	463	17	6.21	2.74	1.75E-04
MF	GO:0003334	keratinocyte development	11	3	0.15	20.34	3.62E-04
MF	GO:0010608	posttranscriptional regulation of gene expression	454	16	6.09	2.63	4.26E-04
MF	GO:0001738	morphogenesis of a polarized epithelium	134	8	1.8	4.45	4.51E-04
MF	GO:0071456	cellular response to hypoxia	136	8	1.82	4.39	4.98E-04
CC	GO:0005913	cell-cell adherens junction	318	16	2.9	5.51	3.65E-08
CC	GO:0015629	actin cytoskeleton	456	16	4.16	3.84	4.46E-06
CC	GO:0044297	cell body	472	16	4.31	3.71	6.89E-06
CC	GO:0005925	focal adhesion	390	14	3.56	3.93	1.41E-05
CC	GO:0005924	cell-substrate adherens junction	393	14	3.59	3.9	1.53E-05
CC	GO:0030055	cell-substrate junction	398	14	3.63	3.85	1.76E-05
CC	GO:0043292	contractile fiber	209	10	1.91	5.24	2.26E-05
CC	GO:0098858	actin-based cell projection	180	9	1.64	5.47	4.16E-05
CC	GO:0043025	neuronal cell body	411	13	3.75	3.46	1.05E-04
CC	GO:0005681	spliceosomal complex	173	8	1.58	5.06	1.92E-04
MF	GO:0098641	cadherin binding involved in cell-cell adhesion	277	14	3.24	4.32	4.85E-06
MF	GO:0098632	protein binding involved in cell-cell adhesion	288	14	3.37	4.16	7.58E-06
MF	GO:0098631	protein binding involved in cell adhesion	293	14	3.42	4.09	9.22E-06
MF	GO:0045296	cadherin binding	294	14	3.44	4.07	9.59E-06
MF	GO:0045505	dynein intermediate chain binding	7	3	0.08	36.66	5.32E-05
MF	GO:0003779	actin binding	390	15	4.56	3.29	5.60E-05
MF	GO:0050839	cell adhesion molecule binding	445	16	5.2	3.08	7.02E-05
MF	GO:0005520	insulin-like growth factor binding	28	4	0.33	12.22	2.97E-04
MF	GO:0031994	insulin-like growth factor I binding	12	3	0.14	21.39	3.20E-04
MF	GO:0010385	double-stranded methylated DNA binding	5	2	0.06	34.22	1.33E-03

C: the number of reference genes in the category; O: the number of genes in the user gene list and also in the category; E: The expected number in the category; R: ratio of enrichment; PValue: p value from hyergeometric test.

Table 5-3: **Histamine Regulated Genes Performing 80% of Histamine Mediated Functions in PE**

Rank	GeneSymbol	Name	Count of Functions	Sign
1	FLNB	filamin B, beta (actin binding protein 278)	14	(+ve)
2	ITGA6*	integrin, alpha 6	12	(+ve)
3	HSPA1A	heat shock 70kDa protein 1A	11	(+ve)
4	MPRIP	myosin phosphatase Rho interacting protein	11	(+ve)
5	CDC42	cell division cycle 42 (GTP binding protein, 25kDa)	10	(+ve)
6	PHLDB2	pleckstrin homology-like domain, family B, member 2	9	(+ve)
7	CALD1	caldesmon 1	9	(+ve)
8	PICALM	phosphatidylinositol binding clathrin assembly protein	8	(+ve)
9	RAN	RAN, member RAS oncogene family	8	(+ve)
10	PALLD	palladin, cytoskeletal associated protein	8	(+ve)
11	TJP1	tight junction protein 1 (zona occludens 1)	7	(+ve)
12	RAB1A	RAB1A, member RAS oncogene family	7	(+ve)
13	LARP1	La ribonucleoprotein domain family, member 1	7	(+ve)
14	KIF5B	kinesin family member 5B	6	(+ve)
15	GJA1	gap junction protein, alpha 1, 43kDa	6	(+ve)
16	TPM4	tropomyosin 4	6	(+ve)
17	PAFAH1B1	platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa	6	(+ve)
18	BZW1*	basic leucine zipper and W2 domains 1	6	(+ve)
19	OPRM1	opioid receptor, mu 1	5	(-ve)
20	ACTR3	ARP3 actin-related protein 3 homolog (yeast)	5	(+ve)
21	PDIA3*	protein disulfide isomerase family A, member 3	5	(+ve)
22	SEPT2	septin 2	5	(+ve)
23	ARF1	ADP-ribosylation factor 1	5	(+ve)
24	PSMD14	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14	4	(+ve)
25	PSMA2	proteasome (prosome, macropain) subunit, alpha type, 2	4	(+ve)
26	PSMD4	proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	4	(+ve)
27	PSME4	proteasome (prosome, macropain) activator subunit 4	4	(+ve)
28	ZFP36L1	zinc finger protein 36, C3H type-like 1	4	(+ve)
29	ABRA	actin-binding Rho activating protein	3	(-ve)
30	RALA	v-ral simian leukemia viral oncogene homolog A (ras related)	3	(+ve)
31	APP	amyloid beta (A4) precursor protein	3	(+ve)
32	IQGAP2**	IQ motif containing GTPase activating protein 2	3	(-ve)
33	TPM2	tropomyosin 2 (beta)	3	(+ve)
34	ADAR	adenosine deaminase, RNA-specific	3	(+ve)
35	FOXA1	forkhead box A1	3	(-ve)
36	TRPC5	transient receptor potential cation channel, subfamily C, member 5	3	(-ve)
37	GPM6A	glycoprotein M6A	3	(-ve)
38	MPL	myeloproliferative leukemia virus oncogene	3	(-ve)
39	CYR61	cysteine-rich, angiogenic inducer, 61	3	(+ve)
40	SYNCRIP	synaptotagmin binding, cytoplasmic RNA interacting protein	3	(+ve)
41	MTPN	myotrophin	2	(+ve)
42	ANXA3	annexin A3	2	(+ve)
43	HNRNPH3	heterogeneous nuclear ribonucleoprotein H3 (2H9)	2	(+ve)
44	MYO3B	myosin IIIB	2	(-ve)

45	GLRA3	glycine receptor, alpha 3	2	(-ve)
46	ARPC4	actin related protein 2/3 complex, subunit 4, 20kDa	2	(+ve)
47	SEC13	SEC13 homolog (S. cerevisiae)	2	(+ve)
48	DLG5	discs, large homolog 5 (Drosophila)	2	(+ve)
49	SLC5A7	solute carrier family 5 (choline transporter), member 7	2	(-ve)
50	DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6	2	(+ve)
51	KHDRBS1	KH domain containing, RNA binding, signal transduction associated 1	2	(+ve)

Count = number of GO terms gene is involved; (+ve) = consistently expressed at High levels; (-ve) = consistently expressed at low levels. Levels of expression of all genes matched the expression levels in PE placentae except where indicated: * signifies genes that are expressed at high levels in EHM but at low levels in PE placentae; ** genes expressed at low levels in EHM but at high levels in PE placentae.

Table 5-4: **Histamine Mediated Pathways with Leading Edge Genes**

ID	Name	Size	L	ES	NES
WP455	GPCRs, Class A Rhodopsin-like	8	8	-0.62	-1.71
hsa04080	Neuroactive ligand-receptor interaction - Homo sapiens (human)	8	8	-0.62	-1.84
hsa05200	Pathways in cancer - Homo sapiens (human)	9	8	0.61	1.4
R-HSA-500792	GPCR ligand binding	10	8	-0.32	-0.97
WP2431	Spinal Cord Injury	7	6	0.43	0.95
hsa04144	Endocytosis - Homo sapiens (human)	6	6	0.69	1.47
WP2377	Integrated Pancreatic Cancer Pathway	6	5	0.56	1.18
WP411	mRNA Processing	5	5	0.48	0.97
hsa04260	Cardiac muscle contraction - Homo sapiens (human)	5	5	0.75	1.54
hsa04932	Non-alcoholic fatty liver disease (NAFLD) - Homo sapiens (human)	5	5	0.81	1.64
hsa04072	Phospholipase D signaling pathway - Homo sapiens (human)	5	5	0.7	1.41
hsa04015	Rap1 signaling pathway - Homo sapiens (human)	6	5	0.64	1.35
hsa05132	Salmonella infection - Homo sapiens (human)	5	5	0.68	1.37
P00034	Integrin signalling pathway	6	5	0.56	1.19
R-HSA-425407	SLC-mediated transmembrane transport	5	5	-0.62	-1.42
R-HSA-418555	G alpha (s) signalling events	5	5	-0.56	-1.28
WP138	Androgen receptor signaling pathway	5	4	0.71	1.46
WP481	Insulin Signaling	6	4	0.69	1.48
WP306	Focal Adhesion	5	4	0.43	0.86
hsa05010	Alzheimer's disease - Homo sapiens (human)	6	4	0.73	1.57
hsa04022	cGMP-PKG signaling pathway - Homo sapiens (human)	5	4	0.78	1.59
hsa04010	MAPK signaling pathway - Homo sapiens (human)	5	4	0.64	1.29
P00016	Cytoskeletal regulation by Rho GTPase	5	4	0.43	0.89
WP2380	Brain-Derived Neurotrophic Factor (BDNF) signaling pathway	5	2	0.4	0.81

Table is sorted according to L. Size: the number of genes in the user gene list and also in the category; L: the number of leading edge genes; ES: Enrichment Score; NES: Normalized Enrichment Score; (-) = down-regulated pathway

This work showed a strong correlation between significantly enriched GOs (Table 5-2) and the pathways identified with leading edge meta-gene analysis (Table 5-4). For example, pathways including Insulin Signaling, Focal Adhesion, mRNA Processing, Salmonella infection and muscle contraction are very closely related to GO terms such as insulin-like growth factor I binding, focal adhesion, mRNA processing, antigen processing and presentation of peptide antigen, dynein intermediate chain binding, and actin binding. Thus, confirming that genes consistently regulated by elevated histamine in an EHM model had functional roles that were biologically relevant in PE placentae.

5.5.3 Identification of causal effect of histamine specific genes in PE

In order to determine plausible causal effect of the histamine regulated genes in PE, Directed acyclic graphs (DAGs) causal analysis was performed using dagitty.net (Textor et al. 2011). This study was aimed at (1) identifying the minimal sufficient adjustment sets of information from the GOs and biological pathways enriched by histamine regulated genes in PE; (2) to identify the Instruments and conditional variables, the adjustments necessary to estimate the total effect, and the minimal sufficient adjustment sets for estimating the direct effect of elevated histamine on pre-eclampsia to derive testable implications.

To achieve these, extensive literature review using thematic analysis was firstly conducted on the functions of the genes enriching the significant GOs and pathways to document the covariates' associations with the elevated histamine (exposure) and PE (outcome). DAG tool was then used to identify the minimal adjustment set of covariate to minimize the magnitude of bias (Figure 5-4A).

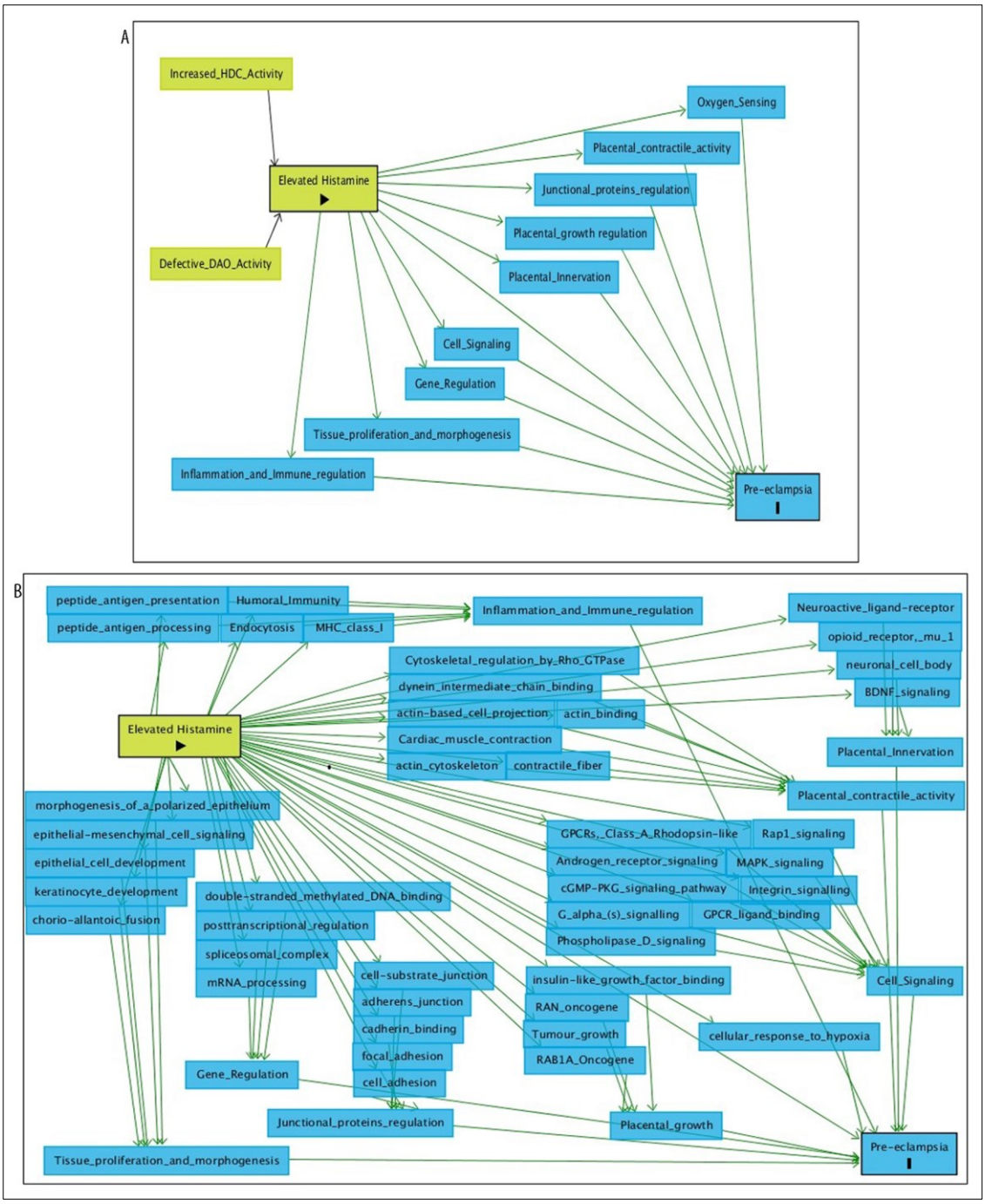


Figure 5-4: Causal effect identification of Elevated Histamine on Pre-eclampsia

DAG Direct Acrylic Graph for the 9 major functional groupings for histamine regulated genes in PE (Figure A). Figure B shows the DAG causal pathways between histamine and PE and regulated genes functions covariates. ▶ exposure; ▮ outcome; ancestor of exposure; ancestor of outcome; causal path

The analysis enabled the identification of 9 minimal sufficient adjustment functional group covariates (Figure 5-4A) with 54 sub-functional group covariates and 45

causal paths (Figure 5-4B) to estimate direct effect of ‘Elevated Histamine’ on Pre-eclampsia. The analysis also revealed that no adjustment to the minimum functional groups was necessary to estimate the total effect. Nonetheless, it clarified the roles of DAO and HDC in PE by predicting that defective DAO and increased HDC activities are instrumental and conditional variables for the estimating the causal effect of elevated histamine in PE.

Also, the analysis clarified that the likelihood that elevated histamine will directly cause PE is nominal but greatly through the regulation of specific genes that activate minimal sufficient functional covariate and sub-functional groups. The minimal but major covariate functional groups included: Cell Signalling, Gene Regulation, Inflammation and Immune regulation, Junctional proteins regulation, Oxygen Sensing, Placental Innervation, Placental contractile activity, Placental growth, Tissue proliferation and morphogenesis**.**

This study was very important to furthering our understanding of the relationship between histamine and PE. In that, in addition to generating 57 testable implications for future work, it refined our knowledge that (1) the natural process which determines the amount of histamine in the placental and maternal blood is affected by DAO and HDC activity; (2) the natural process by which pre-eclampsia develops is affected by the amount of histamine in the maternal blood and placenta; (3) the natural process by which pre-eclampsia develops is not affected by the DAO and HDC activities other than indirectly via the histamine deposit; and finally (4) the natural process by which pre-eclampsia develops is indirectly affected by histamine via functional group covariates, and causal paths.

5.6 Discussions and Conclusion

Data mining for the functions and relevance of the enriched biological pathways revealed a complex network of functions, yet a better understanding of the effects of elevated histamine in the placenta. While histamine is generally known to regulate several physiological processes, including neurotransmission, gastric acid secretion, inflammation, and smooth muscle tone, elevated histamine in the placenta through this cluster of studies have been shown to regulate the expression of vital genes with complex functions that are implicated in PE.

The study examining the relationship between histamine and DAO was the first to demonstrate that these biomarkers implicated in pre-eclampsia form a bi-directional regulatory negative feed-forward relationship in the placenta. The causal effect analysis clarified that the decreased activity of DAO and increased HDC activity are actually instrumental and conditional variables that are expected for elevated histamine to occur in PE placentae. Using a self-validating gene pool, our understanding of the effect of histamine in the pathophysiology of PE was further broadened. The study of the effect of elevated histamine on placental explants was the first to successfully model *ex vivo* defective histamine-DAO-axis to provide an *in vitro* culture model to investigate the effect of elevated histamine in parallel with *in vivo* PE placentae. From these works, it was also shown for the first time that PE placentae express unique set of significant genes in addition to the significant genes expressed in NP, and that elevated histamine regulated a subset of these PE specific genes *in vitro*.

This revelation provided further unique insight into the biological pathways through which elevated histamine would seem to exert effects in the pathophysiology of PE. For example, the identification of the functional group covariate 'Placental Innervation' consisted of leading edge genes that formed the sub-functional covariates including Neuroactive ligand-receptor interaction - Homo sapiens (human), Brain-Derived Neurotrophic Factor (BDNF) signalling pathway and Spinal Cord Injury regulating. The Placental contractile activity functional covariate group consisted of sub-groups including: muscle contraction including Cardiac muscle contraction - Homo sapiens (human) pathway, actin cytoskeleton, contractile fiber, actin-based cell projection, dynein intermediate chain binding, actin binding and Cytoskeletal regulation by Rho GTPase pathways.

These findings were fascinating, nonetheless unexpected because the placenta is currently recognised as not an innervated organ (Walker & McLean 1971, Khong et al. 1997, Marzioni et al. 2004). The findings therefore provided new opportunity to re-evaluate this long-held view that the placenta is de-innervated. The outcome of this re-evaluation was that currently, based on limited publications there is no evidence that the placenta is innervated. However, our work provided fresh evidence to justify further studies to examine whether elevated histamine levels in placentae destined to develop PE could regulate subsets of genes that might preserve innervation and contractility associated with PE placental vasculature (Reilly & Russell 1977, Szukiewicz et al. 1999, Pijnenborg et al. 2006b). Alternatively, the findings offered the opportunity to ask whether there is yet an un-identified pathway in PE placentae that mimics Neuroactive ligand-receptor interaction - Homo sapiens (human), Brain-Derived Neurotrophic Factor (BDNF) signaling pathway and Spinal Cord Injury

smooth muscle contraction pathways in the placenta, and thus warrant further investigation.

In addition to the generation of new theories about the functions of the placenta, the studies confirmed previous observations of the effects of histamine. It was previously reported that increasing histamine causes placental microvascular endothelial damage through redistribution of PECAM-1 and VE-cadherin molecules to non-junctional regions, thus inducing vascular permeability and exposure of the basement membrane and vascular muscles to vasoactive ligands (Leach et al. 1995, Leach & Firth 1997, Chambers et al. 2001). It is also established that endothelial cell activation and dysfunction are also strongly associated with PE (Roberts et al. 1989, Taylor et al. 1998). Our work not only confirmed these previous reports but provided further insight into the complex network of genetic pathways including cell-cell adherens junction, focal adhesion, cadherin binding involved in cell-cell adhesion, and protein binding involved in cell-cell adhesion through which elevated histamine could function to precipitate endothelial dysfunction in PE.

Notwithstanding the effect of histamine on inflammatory pathways in the placenta, the studies on the relationship between histamine and index cytokines provided both quantitative RNA and protein assays to confirm that histamine regulate pro-inflammatory cytokines including IL-1b, IL-10 and INF-γ in the placenta, and elevated histamine tended to favour PE linked Th-1 cytokine INF-γ response.

Taken together, the studies not only elaborated on the complexity of histamine's function in the placenta, but added to our knowledge and understanding of the

pathogenesis of PE by showing that elevated histamine could significantly precipitate PE through the regulation of key placental genes involved in inflammation and immune system regulation; tissue proliferation and morphogenesis; regulation of junctional proteins; maintenance of placental contractile activity; regulation of growth; impairment of cell signalling; and regulation of placental gene expression. Thus, confirming that histamine has significant mediating and or sustaining functions in the placenta, and that further studies to confirm causal effect of elevated histamine in PE pathophysiology is warranted.

Chapter 6
Final Conclusion

6.1 Reflection on contributions made by the publications to the knowledge and Science of Placental Genomics and Pre-eclampsia

In this commentary, I have critically reflected on the rationale for investigating the functional roles of histamine in human placenta with a focus on pre-eclampsia. I have also critically reflected on the suitability of the methods, results and the relative contribution made by the series of publications to the knowledge and understanding of placental genomics with reference to the effects of histamine in human placenta.

6.1.1 The placenta expresses histamine receptors, and histamine production and elimination in the PE placentae are impaired

Prior to the onset of my research, it was known that maternal blood histamine levels are elevated during PE (Figure 2-4). However, due to lack of evidence for histamine functionality in the placenta, very limited progress was made to advance our understanding on the causal effects of the amine on PE. Most importantly, knowledge about the topological distribution of histamine receptor expression in human placenta was extremely limited. Nonetheless, through the preceding discussions, I have shown that one of my publications (Section 4.4) was the first to report that histamine receptors H1R and H2R are expressed in juxtaposed positions with DAO message at the foeto-maternal interface in the villous parenchyma.

Prior to this discovery, it was known that histamine receptors H1R and H2R have both constitutive and inductive activities in other systems, and the constitutive activity was associated with a basal whole blood histamine concentration within the 0.1μM

range (plasma equivalent in 2.0 nM range) (Kahlson *et al.*, 1960b; Kahlson & Rosengren, 1971; Beaven, 1982; Bakker *et al.*, 2000; Alewijnse *et al.*, 2000). This view was postulated as a physiological switch for fine tuning specific cellular activities including tissue remodelling, immuno-modulation, cell proliferation and differentiation, tissue growth and wound healing (Kahlson, 1960; Kahlson *et al.*, 1960a; Kahlson & Rosengren, 1968; Kahlson & Rosengren, 1971; Kahlson & Rosengren, 1972; Beaven, 1978; Erlik *et al.*, 1979; Krishna *et al.*, 1986; Krishna *et al.*, 1989; Tetlow & Woolley, 2003). Nonetheless, the extent to which histamine modulates human placental tissue remodelling was unclear. Therefore, I undertook subsequent studies to generate new knowledge to enhance our understanding on the effects of histamine in human placenta and PE.

Importantly, with meticulous examination of the literature, I provided coherent knowledge and additional insight into the understanding of the profile and dynamics of maternal blood histamine levels in both normal and pre-eclamptic pregnancies (section 2.2). I thus contributed to further our understanding of the nature of maternal blood histamine level profiling that while maternal blood histamine levels in the course of normal pregnancy generally fall below pre-pregnancy levels (Figure 2-2), the levels remain elevated in PE (Figure 2-4). I have also contributed to clarifying that the elevated histamine levels in PE are due to a defective histamine-DAO-axis (section 2.2.4), which in turn could be caused by increased histamine synthesis (section 4.2) and diminished DAO activity (section 5.2).

I provided specific evidence to contribute to knowledge on this matter by examining factors that could affect histamine production and elimination in the placenta, and

thus impact on PE. I showed that placental histidine decarboxylase activity is increased in PE placentae, and cytokines and mitogens increase histamine production in the placenta. I also showed that histamine has a feedforward regulatory loop with DAO, where increasing histamine levels cause down-regulation of DAO gene expression.

6.1.2 Elevated histamine in the placenta has functional roles: it regulates production of Th-1/Th-2 like cytokines in the placenta

The immune-modulative effect of histamine was previously demonstrated in other systems to be dependent on the concentration of bioactive histamine. For example, increasing histamine concentration inhibited macrophage ROS production and thus reversed NK cells and CTL deactivation by macrophage (Mellqvist *et al.*, 2000; Hellstrand *et al.*, 2000b). This was consistent with the clinical observations where decreased or falling DAO activity led to increased bioactive histamine and resultant spontaneous abortions (Southren et al. 1966b).

Therefore, I examined and generated further knowledge on placental histamine physiology that provided evidence to support the claims that histamine dose dependently activates Th-1/Th-2 response in the placenta (Figure 5-2). Here, I demonstrated that low dose histamine up-regulates Th-2-type cytokine responses, and that this fine tuning could sustain successful foetal allograft and pregnancy (Wegmann *et al.*, 1993) while high dose histamine up-regulated Th-1-type cytokine responses with potential to cause foetal demise (Weetman 1999). This contribution first and foremost, confirmed previous reports of Th-1/Th-2 switches in human placenta (Darmochwal-Kolarz et al. 1999, Saito et al. 1999, Saito & Sakai 2003). The

work further showed that the observed histamine dose dependent regulation of Th-1/Th-2 response in peripheral tissues (Packard & Khan 2003) was also present in human placenta and that, varying concentrations of FMI circulating histamine does have key regulatory roles on the expression of the local Th-1/Th-2 cytokine genes.

6.1.3 Optimum culture condition is fundamental for appropriate investigation of histamine effect in human placenta and implications in PE

Having established that the placenta expresses histamine receptors and histamine has bi-directional regulatory effect with DAO and key index cytokines (sections 4.3 and 5.3), further investigations involving genome wide transcriptomic expression analyses were used to study the effects of histamine on the global expression of placental gene with a focus on PE. In the first instance, an ex vivo defective Histamine-DAO-axis also referred to elevated histamine model was developed (section 3.2.5). In course of this investigation, I provided new evidence to confirm that matching *in vitro* oxygen tension with *in vivo* conditions (which vary with gestational age) was a key component for optimal condition for placental explant culture (section 3.2.4). And that oxygen levels could have marked effects on for example, trophoblast apoptosis during the placental explant cultures (Damsky et al. 1993, Genbacev et al. 1996, Caniggia et al. 2000, Burton & Caniggia 2001, Huppertz et al. 2003). I contributed further knowledge to provide clarity on the extent to which tissue culture per se, culture oxygen concentration and culture duration affect placental gene expression. I provided genetic evidence to confirm previous morphological observations (Burton & Caniggia 2001, Burton & Jauniaux 2004, Burton et al. 2006) that high oxygen, i.e, atmospheric oxygen concentration (AOC) was not physiological for term placental culture. In this evidence, I showed that micro

explants were viable after 6 days culture in both AOC and Physiological Oxygen Concentration (POC). However, there was marked difference in tissue morphology between explants cultured in POC and AOC. I provided new evidence that while the explants were viable after 6 days culture, there was more syncytial detachment and loss in explants cultured in AOC; and that the tissue morphology and RNA quality in explants cultured in POC was akin to pre-culture explants. I also showed for the first time that placental RNA quality decline in tandem with STB degeneration and syncytial damage and loss. Hence, I provided new knowledge to further our understanding that micro explants cultured at POC have the best mRNA quality and tissue structure after long-term culture to support high throughput experiments.

Regarding knowledge and understanding of the effects of placental explant culture on placental gene expression, I provided comprehensive new evidence to show that the observable morphological changes and differences in RNA quality between the POC and AOC cultured explants transcended beyond morphology to deeper changes in the biological and molecular pathways in the placenta; and that the changes impacted on interpretations of studies that have used explants cultured in AOC. I demonstrated further that explant culture in AOC not only affects individual gene expression but also induce relative changes to the enrichment of specific transcription factor targets, placental molecular functions, cellular components, biological processes and functional pathways.

6.1.1 Histamine has complex functions in the placenta: it regulates genetic pathways that have implications in PE placentae

I then undertook further work to extend our knowledge and understanding of gene expression in normal and PE placentae (Section 5.4). This work was an extension to a previous report on PE gene expression meta-analysis, which explored the relative relationship between NP and PE gene expression. In this work, I broadened the coverage to examine the roles of genes with consistent low or high level expressions in the placenta. One key finding was the identification for the first time, of subsets of genes that are consistently up or down-regulated in PE but not in NP placentae. This enabled the identification of specific biochemical pathways that characterises the contributory roles or causal effects of elevated histamine in the pathogenesis of PE. Through this work, I further showed that about 30% of genes currently published as being investigated for possible roles in the molecular pathology of PE were not consistently expressed in the PE placentae.

The work had interesting impact on further studies in PE. For, it not only provided a unique opportunity for researchers in the field to re-evaluate the relevance and focus of their investigations of PE, it offered further opportunities to refine the studies designed for molecular bio-markers for early detection of women at risk of preeclampsia. The work also developed on the framework for absolute gene expression analysis into modelling the baseline genomic blueprint for normal placental gene expression for comparison with expressions in complicated placentae.

Thus, this work was further extended to examine the effect of elevated histamine on placental gene expression in parallel with effect of PE on the placenta (Section 5.5).

In this extension work, new evidence was added to the knowledge and understanding of the roles of specific placental genes regulated by histamine and their implications in PE. The key finding was Histamine regulated a subset of placental genes that were significantly expressed in PE placentae but not in normal pregnancy placentae or cultured explants without histamine treatment. I provided evidence that the elevated histamine regulates specific genes in pre-eclampsia, and that the genes are involved in inflammation and immune system regulation; tissue proliferation and morphogenesis; regulation of junctional proteins; maintenance of placental contractile activity; regulation of growth; impairment of cell signalling; and regulation of placental gene expression. These suggested that histamine has significant mediating and or sustaining functions in the placenta, and that elevated levels do play key roles in the pathophysiology of pre-eclampsia.

6.1.2 Is histamine the missing link in PE pathophysiology?

In the introduction, it was stated that if histamine receptors are expressed in the placenta, then elevated histamine would have functional roles, and if the effects of elevated histamine were similar to the pathophysiological changes observed in PE placentae, then histamine could be the missing link that precipitates or decompensate PE pathophysiology. In the course of these discussions, I have provided evidence through the series of publication submitted here for consideration that: histamine receptor genes are expressed at the foeto-maternal interface; DAO genes are expressed in the placenta; HDC activity levels are increased in PE placentae; there is cross-talk between histamine, DAO and cytokines in the placenta; elevated histamine in dHDA regulated the expression of specific genes in the placenta and these genes are abnormally expressed in PE placentae; the functions

of these elevated histamine regulated genes also expressed in PE are involved in tissue morphology and possibly poor placentation, metabolic defects, endothelia dysfunction, inflammation, immunologic response, angiogenic and anti-angiogenic response in PE placentae. It is therefore suffice to suggest that elevated histamine fits the role of the missing link (Figure 6-1) in PE pathophysiology and that elevated histamine has causal effects on PE and is plausibly, the missing link that perpetuates the pathophysiological changes in the placental and maternal systems to culminate in pre-eclampsia complication. Therefore it is reasonable to conclude that the elevated histamine observed in PE would have pathophysiological roles in PE and early detection leading to effective control of maternal blood histamine levels could save lives and it's thus recommended.

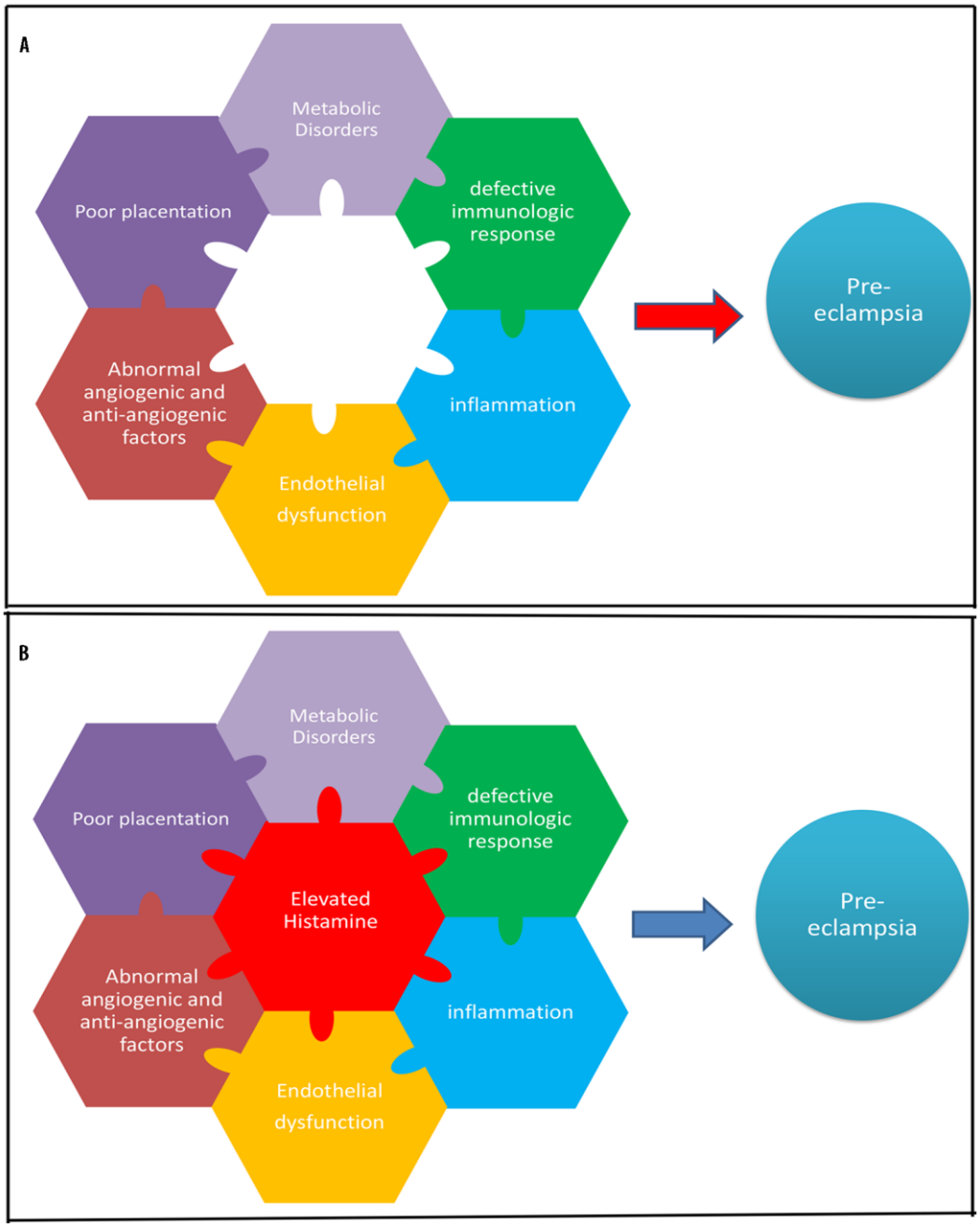


Figure 6-1: Histamine fits the missing link in Pre-eclampsia Pathogenesis

6.2 Reflection on my contributions and my professional development as a research practitioner

My contributions to the listed publications are described in Table 6-1 below according the CRediT Taxonomy of author contributions (Brand et al. 2015). My contributory role is described in column 1, followed by the role definition in column 2. The respective publications are numbered from 1 – 11 in line with the publication portfolio listing. The listings and completion (√) of this information with copies of the published author contribution accurately reflect my contributions to the works submitted here for the award.

Table 6-1: Summary of My Contributory

My Contributory Roles	Role Definition	1	2	3	4	5	6	7	8	9	10	11
Conceptualisation	Ideas; formulation or evolution of overarching research goals and aims.	√	√	√	√	√	√	√	√	√	√	√
Data Curation	Management activities to annotate (produce metadata), scrub data and maintain research data for initial use and later reuse.	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*
Formal Analysis	Application of statistical, mathematical, computational, or bioinformatics to analyse or synthesize study data.	√	√	√	√	√	√	√	√	√	√	√
Investigation**	Conducting research and investigation process, specifically performing the experiments	√	√	√*	√	√*	√	√	√	√	√	√
Methodology	Development or design of methodology; creation of models	√	√	√*	√*	√*	√*	√*	√*	√*	√*	√*
Project Administration	Management and coordination responsibility for the research activity planning and execution.	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*
Resources	Provision of study materials, reagents, materials, patients,	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*

	laboratory samples, animals, instrumentation, computing resources, or other analysis tools.											
Validation	Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.	√	√	√*	√	√*	√	√	√	√	√	√
Visualization	Preparation, creation and/or presentation of the published work, specifically visualization/data presentation.	√	√	√	√	√	√	√	√	√	√	√
Writing – Original Preparation	Creation and/or presentation of the published work, specifically writing the initial draft (including substantive interpretation).	√	√	√	√	√	√	√	√	√	√	√
Review & Editing	Presentation of the published work, specifically critical review, commentary or revision – including pre- or post-publication stages.	√*	√§	√§	√§	√§	√§	√§	√§	√§	√§	√§

√* = joint activity; √§ = I performed activity but consulted with named others; ** = list of specific experiments or investigations I performed (tissue dissection, tissue culture, protein and amine assays with ELISA, gene cloning, immuno-histochemistry; in situ hybridisation, RNA and DNA extraction, PCR, RT-PCR, real time RT-qPCR, systematic review, meta-analysis, all statistical analysis, all bioinformatics analysis)

Over the course of this work, it was a primary desire to improve on my skills for conceptualising research ideas. To achieve this, I used a 3Cs (Curiosity, Critical thinking, and Courage for idea generations) prong approach to improve on my conceptualisation skills. Development, harnessing and control of my curiosity for good research were particularly important for me. In the Old Man's Advice to Youth: "Never Lose a Holy Curiosity," LIFE magazine (2 May 1955, p. 64) Einstein is famously quoted as saying: “The important thing is not to stop questioning. Curiosity has its own reason for existence. One cannot help but be in awe when he contemplates the mysteries… of life, of the marvellous structure of reality. It is

enough if one tries merely to comprehend a little of this mystery each day. Never lose a holy curiosity". Thus, I desired to develop a higher sense of curiosity, a holy curiosity to continue to question, control my curiosity, amass courage to entertain and refine scientific questions including those others considered "bad ideas or red herrings" through critical thinking into clearly stated researchable ideas. The outcome for this is clearly outlined in Table 6-1, row 1. The ideas for all the studies underpinning the publications submitted here originated from my desire not to stop questioning.

Of course, my development was not limited to conceptualisation of good research ideas. I used the opportunities available to develop across all areas as an academic researcher. In Table 6-1, I also show that over the period of the studies, I developed quite considerable skills to position myself towards internationally and publicly renowned publications. I aligned my development with the UK workforce Researcher Development Framework (RDF) for researcher development. My primary goal for this initiative was to enhance my capacity to develop as a world-class researcher. I therefore took the initiatives to lead and manage the activities to annotate and produce metadata for initial use and later reuse. Some of the activities were more complex, for example, the curation, management and publication of raw data on public databases such as GEO required several standards to be met. Each stage of the material preparation was associated with critical learning that I found extremely helpful.

Similarly, I performed all statistical, mathematical, computational, and bioinformatics analyse or syntheses outlined in the studies underpinning the publications presented here. These processes involved determination and openness to extend my reach

and develop high throughput skills in computational biology and statistical analyses. Consequently, I over the period of investigations improved my knowledge, skills and capabilities in areas beyond biology, genetics and genomics to computer programming, data mining, complex statistics and bioinformatics. These culminated in the design and development of methodology; the creation of novel models, leading and conducting high throughput research and investigation, and in performing both wet and dry-laboratory experiments. Clearly, I received support and guidance and I made a very conscious effort to use every opportunity offered to develop as an all-round researcher in genomics and bioinformatics with core knowledge in human reproduction; and thus, actively contributed to all aspects of the research activities required for recognition as a publisher.

Also, I made conscious efforts during the studies to recognise, create and confidently act on opportunities with the potential to develop my career within both academia and industry. Therefore, I made it a career ambition to actively create and champion opportunities for others within my scope of practice. Hence, I developed a sense of responsiveness towards collaborative opportunities across research disciplines and with non-academic organisations. While I sought to develop substantial ability and confidence to set expectations, I also developed the courage and competence to advise peers and less experienced members of staff to engage in research and scholarly publications. In brief, the journey to develop as a research practitioner has been onerous but worthwhile. I still aspire to develop further to direct not just local policy, but enhance my contributions to shape wider policy and procedures within the HE sector and professional associations or bodies because human development is intrinsically linked to creation of new knowledge emanating from prudent questioning, and I believe I can still contribute.

6.3 Suggestions for future developments

The findings from the preceding studies have provided substantial insight into the roles of histamine in the human placenta and the ensuing effect on PE pathophysiology. While the studies have provided closure to some questions, they have also opened up novel areas for further investigations. In this section I outline new areas of investigations that have arisen from above studies.

6.3.1 Early Maternal Blood Pregnancy Histamine and links with Preeclampsia

In chapter 1, it was shown that PE is a major cause of perinatal mortality and it complicates up to 8% of all pregnancies in Western countries. In chapter 2, it was also shown that mothers who develop PE present with clinical symptoms akin to experimentally induced elevated histamine and the elevated histamine is harmful to the placenta. While it is widely accepted that the levels and activity of DAO in maternal blood rise a 100 and 1000 fold respectively during normal pregnancy, but diminishes after gestational week 10 in pregnancies that result in PE, the cause of the diminished DAO levels and activity in early pregnancy is unclear. It was further shown in chapter 4 that histamine production is increased in PE placentae, and increasing histamine levels diminishes DAO mRNA expression in placentae. Figure 2-4 further demonstrates that while maternal blood histamine levels in normal pregnancy and late PE pregnancy are well characterised, early maternal blood histamine levels in women who later develop PE remain unelucidated. Therefore, we are currently testing the hypothesis that maternal blood histamine in early pregnancy (first trimester) are elevated above normal pregnancy levels and the elevated histamine in early pregnancy is associated with diminished maternal serum DAO levels in mothers who later develop PE.

We are thus conducting a prospective association study to examine serial assay of maternal blood for histamine and serum for DAO levels of (1) women with no history of PE, (2) women with history of PE and (3) primigravid women with history of histamine intolerance. We anticipate that the findings from this investigation could provide evidence to show for the first time, differences in early pregnancy maternal blood histamine in mothers who later develop PE and those that do not. The findings are also expected to help determine the levels of maternal blood histamine that coincide with diminished maternal serum DAO. Thirdly, with predictive modelling the findings are also expected to suggest maternal histamine and DAO level cut-off points that could be used for early detection of PE.

6.3.2 Histamine Intolerance and Pregnancy Outcome

It was shown through the systematic reviews that maternal blood histamine is well controlled in normal pregnancy, and in such instances atopic women who usually have histamine intolerance symptoms fare well during normal pregnancy. In contrast, women whose blood histamine levels are poorly controlled during pregnancy or plausibly elevated from early pregnancy suffer from PE and experience most of the symptoms associated with histamine intolerance. The commentaries summarised the evidence that high blood and urine histamine occur in PE. It is established that placental HDC activity is increased while DAO activity is decreased in PE. It is also established that histamine intolerance can be triggered by certain foods such as beans and pulses, tomatoes, canned fish, cheese, cocoa and chocolate and other histamine releasing foods.

It is further established that maternal nutrient deficiency in copper and ascorbic acid increases maternal risk for PE. Copper and ascorbic acid are co-factors for histamine synthesis and metabolism. However, it is not known whether histamine releasing foods or deficiencies in copper and ascorbic acid have effects on the diminished DAO activity in maternal blood. It also uncertain which foods induce histamine elevation in pregnancy so that pregnant women can avoid such foods. We are therefore conducting further studies in this area to investigate the effect of environmental histamine releasers on pregnancy outcome. Specifically, the studies are focused to:

- prospectively survey the dietary type of a cohort of pregnant women and correlate with the outcome of pregnancy
- assay blood histamine, ascorbic acid and DAO levels and determine their relation with dietary intake and pregnancy outcome
- determine frequency of exposure to environmental histamine releasers such as ‘cigarette smoking’ alcohol, and lack of sleep that induce elevated blood histamine and how these correlate with symptoms of histamine intolerance during pregnancy.

These studies will have clear impact on maternal health and pregnancy outcomes. The information thus gained is expected to provide useful dietary and environmental advice to pregnant women who may be at a higher risk of developing trophoblastic diseases such as preeclampsia. It is also expected that the study will yield results to inform a predictive tool for early diagnosis of preeclampsia.

6.3.3 Genetic Variations in histamine pathways and Pregnancy Outcomes

It has been recognised that single nucleotide polymorphisms in key genes in histamine pathways including Methylenetetrahydrofolate reductase (MTHFR), DAO (also known as AOC1), Monoamine Oxidase (MAO), HNMT, and Phosphatidylethanolamine N-Methyltransferase (PEMT) are strongly linked with defective regulation of blood histamine with and histamine intolerance. Epigenetic regulation of histamine synthesis and metabolic genes has also been associated with defective histamine metabolism and elevated blood histamine. Although the placenta is the root cause of PE, previous studies examining variations in these genes in relation to PE have focused on the mother instead of the placenta. We have therefore adopted an integrated approach to examine the variations in these genes in the placenta in the context of clinical changes in maternal health. Currently we are focusing on characterising the genetic variations of these genes in placenta with high therapeutic potentials to develop and test a multi-sensory diagnostic intervention for early detection and management of PE.

6.3.4 Validation of HSPE genes and curation of histamine regulated pathways in PE placentae

A total of 270 significant genes were identified as consistently expressed, specifically in response to elevated histamine treatment in the placenta. These genes were observed to have functional effects that could have implications in the placental pathophysiology of PE. In order to establish the roles of elevated histamine in PE placental pathophysiology and curate the respective pathways, the expression of the HSPE genes require further validation assessment. It is therefore proposed to undertake further work using RT-qPCR to confirm the expression of the HSPE

genes. This validation study could confirm the effects of elevated histamine in the pathophysiology of PE and authenticate subsequent analysis to curate the pathways enriched by the elevated histamine in PE placentae.

6.3.5 Histamine effects on CD3ξ signal transduction in placental T and NK cells

Regulatory loops between histamine and cytokines in human placenta were confirmed in these studies. INF-gamma, IL-1beta and IL-10 transiently induced histamine production. The histamine thus produced was shown to sustain the production of pro-inflammatory and extra-thymus-like cytokines in placenta. Prolonged exposure of the placental tissues to higher histamine concentration up-regulates TH-1 like cytokine in human placenta explants. There is also emerging evidence that high histamine concentration up-regulates Th-1 response by reversing monocytes or macrophage down regulation of CD3ξ signal transduction on T and NK cells thereby allowing IL-2 and other Th-1 cytokines to potently sustain the Th-1 cytolytic response (Hellstrand et al. 2000, Johansson et al. 2000). I would like to conduct further studies on this subject to delineate the relationships between histamine receptors and the regulations of CD3ξ signal transduction in placental T and NK cells.

Reference

Aban, M., Cinel, L., Arslan, M., Dilek, U., Kaplanoglu, M., Arpaci, R. & Dilek, S. (2004) Expression of nuclear factor-kappa B and placental apoptosis in pregnancies complicated with intrauterine growth restriction and preeclampsia: an immunohistochemical study. *The Tohoku Journal of Experimental Medicine,* 204(0040-8727; 0040-8727; 3), p.195-202.

Achari, G., Achari, K. & Rao, K.K. (1971) Histaminase and histamine in normal and toxaemic pregnancy. *Jpn.J.Pharmacol.,* 21(0021-5198; 1), p.33-40.

Ahlmark, A. (1944) Studies on Histaminolytic Power of the Plasma with Special Reference to Pregnancy. *Acta Physiol Scand.,* 9.

Ahmed, A., Dunk, C., Kniss, D. & Wilkes, M. (1997) Role of VEGF receptor-1 (Flt-1) in mediating calcium-dependent nitric oxide release and limiting DNA synthesis in human trophoblast cells. *Lab Invest,* 76(0023-6837; 0023-6837; 6), p.779-791.

Ahmed, A., Li, X.F., Dunk, C., Whittle, M.J., Rushton, D.I. & Rollason, T. (1995) Colocalisation of vascular endothelial growth factor and its Flt-1 receptor in human placenta. *Growth Factors (Chur, Switzerland),* 12(0897-7194; 0897-7194; 3), p.235-243.

Ahmed, A. & Ramma, W. (2015) Unravelling the theories of pre-eclampsia: are the protective pathways the new paradigm? *British Journal of Pharmacology,* 172(6), p.1574-1586.

Akerman, S., Williamson, D.J., Kaube, H. & Goadsby, P.J. (2002) The role of histamine in dural vessel dilation. *Brain Res.,* 956(0006-8993; 1), p.96-102.

Andraweera, P.H., Dekker, G.A. & Roberts, C.T. (2012) The vascular endothelial growth factor family in adverse pregnancy outcomes. *Human Reproduction Update,* 18(4), p.436-457.

Arad, G., Nussinovich, R., Na'amad, M. & Kaempfer, R. (1996) Dual control of human interleukin-2 and interferon-gamma gene expression by histamine: activation and suppression. *Cellular Immunology,* 170(0008-8749; 0008-8749; 1), p.149-155.

Arngrimsson, R., Bjornsson, S., Geirsson, R.T., Bjornsson, H., Walker, J.J. & Snaedal, G. (1990) Genetic and familial predisposition to eclampsia and pre-eclampsia in a defined population. *Br.J.Obstet.Gynaecol.,* 97(0306-5456; 0306-5456; 9), p.762-769.

Asemissen, A.M., Scheibenbogen, C., Letsch, A., Hellstrand, K., Thoren, F., Gehlsen, K., Schmittel, A., Thiel, E. & Keilholz, U. (2005) Addition of histamine to interleukin 2 treatment augments type 1 T-cell responses in patients with melanoma in vivo: immunologic results from a randomized clinical trial of interleukin 2 with or without histamine (MP 104). *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research,* 11(1), p.290-297.

Baczyk, D., Dunk, C., Huppertz, B., Maxwell, C., Reister, F., Giannoulias, D. & Kingdom, J.C. (2006) Bi-potential behaviour of cytotrophoblasts in first trimester chorionic villi. *Placenta,* 27(0143-4004; 0143-4004; 4-5), p.367-374.

Bakker, R.A., Casarosa, P., Timmerman, H., Smit, M.J. & Leurs, R. (2004) Constitutively active Gq/11-coupled receptors enable signaling by co-expressed G(i/o)-coupled receptors. *Journal of Biological Chemistry,* 279(0021-9258; 0021-9258; 7), p.5152-5161.

Beaven, M.A. (1982) Factors Regulating Availability of Histamine at Tissue Receptors. In: C.R.Ganellin & M.E.Parsons (eds.) *The Pharmacology of Histamine Receptors.*: Butterworth Heinemann., p.103-145.

Beaven, M.A. (1978) Histamine: its role in physiological and pathological processes. *Monogr Allergy,* 13(0077-0760), p.1-113.

Beaven, M.A., Marshall, J.R., Baylin, S.B. & Sjoerdsma, A. (1975) Changes in plasma histaminase activity during normal early human pregnancy and pregnancy disorders. *Am.J.Obstet.Gynecol.,* 123(0002-9378; 6), p.605-609.

Beaven, M.A. & Shaff, R.E. (1975) Study of the relationship of histaminase and diamine oxidase activities in various rat tissues and plasma by sensitive isotopic assay procedures. *Biochem.Pharmacol.,* 24(0006-2952; 9), p.979-984.

Belo, L., Santos-Silva, A., Caslake, M., Cooney, J., Pereira-Leite, L., Quintanilha, A. & Rebelo, I. (2003) Neutrophil activation and C-reactive protein concentration in preeclampsia. *Hypertension in Pregnancy,* 22(2), p.129-141.

Best, C.H. (1929) The disappearance of histamine from autolysing lung tissue. *The Journal of Physiology,* 67(3), p.256-263.

Bjuro, T., Lindberg, S. & Westling, H. (1964) Further Observations on the Urinary Excretion of Histamine during and after Normal Pregnancy. *Acta Obstetricia Et Gynecologica Scandinavica,* 43(0001-6349), p.206-213.

Blalock, E. (2003) Experimental Design and Data Analysis. In: Blalock, E. (ed.) *A Beginner's Guide to Microarray.* London: Kluwer Academic Publishers., p.179-242.

Blanco, Y.S., Rozada, H., Remedio, M.R., Hendricks, C.H. & Alvarez, H. (1970) Human tubal motility in vivo. *American Journal of Obstetrics and Gynecology,* 106(0002-9378; 1), p.79-86.

Bode, C.J., Jin, H., Rytting, E., Silverstein, P.S., Young, A.M. & Audus, K.L. (2006) In vitro models for studying trophoblast transcellular transport. *Methods in Molecular Medicine,* 122, p.225-239.

Brand, A., Allen, L., Altman, M., Hlava, M. & Scott, J. (2015) Beyond authorship: attribution, contribution, collaboration, and credit. *Learned Publishing,* 28, p.151-155.

Breitling, R., Armengaud, P., Amtmann, A. & Herzyk, P. (2004) Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS Lett.,* 573(0014-5793; 0014-5793; 1-3), p.83-92.

Bronson, R.A. & Wallach, E.E. (1977) Lysis of periadnexal adhesions for correction of infertility. *Fertil.Steril.,* 28(0015-0282; 6), p.613-619.

Brunton, L. (1996) Agents Affecting Gastrointestinal Water Flux and Motility; Emesis and Antiemetics; Bile acids and Pancreatic Enzymes. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW & Gillman AG (eds.) *Goodman and Gillman's The Pharmacological Basis of Therapeutics.* New York: McGraw-Hill., p.917-937.

Buffoni, F. (1966) Histaminase and related amine oxidases. *Pharmacol.Rev.,* 18(0031-6997; 4), p.1163-1199.

Burton, G.J. & Caniggia, I. (2001) Hypoxia: implications for implantation to delivery-a workshop report. *Placenta,* 22 Suppl A(0143-4004), p.S63-S65.

Burton, G.J., Charnock-Jones, D.S. & Jauniaux, E. (2006) Working with oxygen and oxidative stress in vitro. *Methods in Molecular Medicine,* 122(1543-1894; 1543-1894), p.413-425.

Burton, G.J. & Jauniaux, E. (2004) Placental oxidative stress: from miscarriage to preeclampsia. *Journal of the Society for Gynecologic Investigation,* 11(1071-5576; 1071-5576; 6), p.342-352.

Buurma, A.J., Turner, R.J., Driessen, J.H., Mooyaart, A.L., Schoones, J.W., Bruijn, J.A., Bloemenkamp, K.W., Dekkers, O.M. & Baelde, H.J. (2013) Genetic variants in pre-eclampsia: a meta-analysis. *Human Reproduction Update,* 19(1460-2369; 1355-4786; 3), p.289-303.

Bytautiene, E., Romero, R., Vedernikov, Y.P., El Zeky, F., Saade, G.R. & Garfield, R.E. (2004a) Induction of premature labor and delivery by allergic reaction and prevention by histamine H1 receptor antagonist. *Am.J.Obstet.Gynecol.,* 191(0002-9378; 4), p.1356-1361.

Bytautiene, E., Vedernikov, Y.P., Saade, G.R., Romero, R. & Garfield, R.E. (2004b) Degranulation of uterine mast cell modifies contractility of isolated myometrium from pregnant women. *American Journal of Obstetrics and Gynecology,* 191(0002-9378; 5), p.1705-1710.

Bytautiene, E., Vedernikov, Y.P., Saade, G.R., Romero, R. & Garfield, R.E. (2003) Effect of histamine on phasic and tonic contractions of isolated uterine tissue from pregnant women. *Am.J.Obstet.Gynecol.,* 188(0002-9378; 3), p.774-778.

Caldwell, E.J., Carlson, S.E., Palmer, S.M. & Rhodes, P.G. (1988) Histamine and ascorbic acid: a survey of women in labor at term and significantly before term. *Int.J.Vitam.Nutr.Res.,* 58(0300-9831; 3), p.319-325.

Caniggia, I., Winter, J., Lye, S.J. & Post, M. (2000) Oxygen and placental development during the first trimester: implications for the pathophysiology of pre-eclampsia. *Placenta,* 21 Suppl A(0143-4004), p.S25-S30.

Castellucci, M. & Kaufmann, P. (1982) A three-dimensional study of the normal human placental villous core: II. Stromal architecture. *Placenta,* 3(0143-4004; 0143-4004; 3), p.269-285.

Castellucci, M., Scheper, M., Scheffen, I., Celona, A. & Kaufmann, P. (1990) The development of the human placental villous tree. *Anatomy and Embryology,* 181(2), p.117-128.

Chambers, J.C., Fusi, L., Malik, I.S., Haskard, D.O., De Swiet, M. & Kooner, J.S. (2001) Association of maternal endothelial dysfunction with preeclampsia. *Jama,* 285(0098-7484; 12), p.1607-1612.

Chatterjee, I.B., Gupta, S.D., Majumder, A.K., Nandi, B.K. & Subramanian, N. (1975) Effect of ascorbic acid on histamine metabolism in scorbutic guinea-pigs. *J.Physiol,* 251(2), p.271-279.

Chesley, L.C. & Cooper, D.W. (1986) Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women. *Br.J.Obstet.Gynaecol.,* 93(0306-5456; 0306-5456; 9), p.898-908.

Cincotta, R.B. & Brennecke, S.P. (1998) Family history of pre-eclampsia as a predictor for pre-eclampsia in primigravidas. *Int.J.Gynaecol.Obstet.,* 60(0020-7292; 0020-7292; 1), p.23-27.

Clemetson, C.A. (1980) Histamine and ascorbic acid in human blood. *J.Nutr.,* 110(0022-3166; 4), p.662-668.

Clemetson, C.A. & Cafaro, V. (1981) Abruptio placentae. *Int.J.Gynaecol.Obstet.,* 19(0020-7292; 6), p.453-460.

Cocchiara, R., Albeggiani, G., Di Trapani, G., Azzolina, A., Lampiasi, N., Rizzo, F., Diotallevi, L., Gianaroli, L. & Geraci, D. (1992) Oestradiol enhances in vitro the histamine release induced by embryonic histamine-releasing factor (EHRF) from uterine mast cells. *Hum.Reprod.,* 7(0268-1161; 8), p.1036-1041.

Cocchiara, R., Di Trapani, G., Azzolina, A., Albeggiani, G., Ciriminna, R., Cefalu, E., Cittadini, E. & Geraci, D. (1987) Isolation of a histamine releasing factor from human embryo culture medium after in-vitro fertilization. *Hum.Reprod.,* 2(0268-1161; 4), p.341-344.

Cocchiara, R., Lampiasi, N., Albeggiani, G., Azzolina, A., Bongiovanni, A., Gianaroli, L., Di Blasi, F. & Geraci, D. (1996) A factor secreted by human embryo stimulates cytokine release by uterine mast cell. *Mol.Hum.Reprod.,* 2(1360-9947; 10), p.781-791.

Connolly, M.R., Bitman, J., CECIL, H.C. & Wrenn, T.R. (1962) Water, electrolyte, glycogen, and histamine content of rat uterus during pregnancy. *Am.J.Physiol,* 203(0002-9513), p.717-719.

Cooper, J.A. & Schayer, R.W. (1956) Metabolism of C14 histamine in man. *J.Appl.Physiol,* 9(0021-8987; 3), p.481-483.

Cricco, G., Martin, G., Medina, V., Nunez, M., Mohamad, N., Croci, M., Crescenti, E., Bergoc, R. & Rivera, E. (2006) Histamine inhibits cell proliferation and modulates the expression of Bcl-2 family proteins via the H2 receptor in human pancreatic cancer cells. *Anticancer Research,* 26(0250-7005; 0250-7005; 6), p.4443-4450.

Dale, H.H. & Laidlaw, P.P. (1910) The physiological action of b-imidazolylethylamine. *The Journal of Physiology,* 41, p.318-344.

Damsky, C., Sutherland, A. & Fisher, S. (1993) Extracellular matrix 5: adhesive interactions in early mammalian embryogenesis, implantation, and placentation. *Faseb J.,* 7(0892-6638; 14), p.1320-1329.

Damsky, C., Schick, S.F., Klimanskaya, I., Stephens, L., Zhou, Y. & Fisher, S. (1997) Adhesive interactions in peri-implantation morphogenesis and placentation. *Reprod.Toxicol.,* 11(0890-6238; 0890-6238; 2-3), p.367-375.

Daoud, G., Barrak, J. & Abou-Kheir, W. (2016) Assessment Of Different Trophoblast Cell Lines As In Vitro Models For Placental Development. *The FASEB Journal,* 30(1 Supplement), p.1247.18-1247.18.

Darmochwal-Kolarz, D., Leszczynska-Gorzelak, B., Rolinski, J. & Oleszczuk, J. (1999) T helper 1- and T helper 2-type cytokine imbalance in pregnant women with pre-eclampsia. *European Journal of Obstetrics, Gynecology, and Reproductive Biology,* 86(0301-2115; 0301-2115; 2), p.165-170.

Davio, C., Mladovan, A., Lemos, B., Monczor, F., Shayo, C., Rivera, E. & Baldi, A. (2002) H1 and H2 histamine receptors mediate the production of inositol phosphates but not cAMP in human breast

epithelial cells. *Inflammation Research : Official Journal of the European Histamine Research Society ... [Et Al.],* 51(1023-3830; 1), p.1-7.

Dey, S.K. & Hubbard, C.J. (1981) Role of histamine and cyclic nucleotides in implantation in the rabbit. *Cell and Tissue Research,* 220(0302-766; 0302-766; 3), p.549-554.

Dey, S.K., Lim, H., Das, S.K., Reese, J., Paria, B.C., Daikoku, T. & Wang, H. (2004) Molecular cues to implantation. *Endocr.Rev.,* 25(0163-769; 3), p.341-373.

Dobbin, K. & Simon, R. (2005) Sample size determination in microarray experiments for class comparison and prognostic classification. *Biostatistics.,* 6(1465-4644; 1465-4644; 1), p.27-38.

Donahue, J.G., Lupton, J.B. & Golichowski, A.M. (1995) Cutaneous mastocytosis complicating pregnancy. *Obstet.Gynecol.,* 85(0029-7844; 5), p.813-815.

Dong, M., He, J., Wang, Z., Xie, X. & Wang, H. (2005) Placental imbalance of Th1- and Th2-type cytokines in preeclampsia. *Acta Obstetricia Et Gynecologica Scandinavica,* 84(8), p.788-793.

Dubois, A.M., Santais, M.C., Foussard, C., Dubois, F., Ruff, F., Taurelle, R. & Parrot, J.L. (1977) Blood histamine and plasma histaminase level during human pregnancy [proceedings]. *Agents Actions,* 7(0065-4299; 1), p.112.

Dudley, A.M., Aach, J., Steffen, M.A. & Church, G.M. (2002) Measuring absolute expression with microarrays with a calibrated reference sample and an extended signal intensity range. *Proc.Natl.Acad.Sci.U.S.A,* 99(0027-8424; 0027-8424; 11), p.7554-7559.

Duley, L. (2009) The global impact of pre-eclampsia and eclampsia. *Seminars in Perinatology,* 33(3), p.130-137.

Ebeigbe, A.B. & Talabi, O.O. (2014) Vascular Effects of Histamine. *Nigerian Journal of Physiological Sciences : Official Publication of the Physiological Society of Nigeria,* 29(1), p.7-10.

Efron, B. & Tibshirani, R. (2007) On testing the significance of sets of genes. *Ann.Appl.Stat.,* 1(1), p.107-129.

el-Gendi, M.A., Nassar, S.H. & el-Bassiouni, E.A. (1988) Histamine and histamine receptor antagonists in splanchnic compartments in schistosomal portal hypertension. *Clinical Pharmacology and Therapeutics,* 44(4), p.396-399.

Esplin, M.S., Fausett, M.B., Fraser, A., Kerber, R., Mineau, G., Carrillo, J. & Varner, M.W. (2001) Paternal and maternal components of the predisposition to preeclampsia. *N.Engl.J.Med.,* 344(0028-4793; 0028-4793; 12), p.867-872.

Fabregat, A., Sidiropoulos, K., Garapati, P., Gillespie, M., Hausmann, K., Haw, R., Jassal, B., Jupe, S., Korninger, F., McKay, S., Matthews, L., May, B., Milacic, M., Rothfels, K., Shamovsky, V., Webber, M., Weiser, J., Williams, M., Wu, G., Stein, L., Hermjakob, H. & D'Eustachio, P. (2016) The Reactome pathway Knowledgebase. *Nucleic Acids Research,* 44(D1), p.D481-7.

Fajardy, I., Moitrot, E., Vambergue, A., Vandersippe-Millot, M., Deruelle, P. & Rousseaux, J. (2009) Time course analysis of RNA stability in human placenta. *BMC Molecular Biology [Computer File],* 10(1471-2199; 1471-2199), p.21.

Falus, A. & Meretey, K. (1992) Histamine: an early messenger in inflammatory and immune reactions. *Immunology Today,* 13(0167-5699; 5), p.154-156.

Faye, A., Pornprasert, S., Dolcini, G., Ave, P., Taieb, J., Taupin, J.L., Derrien, M., Huerre, M., Barre-Sinoussi, F., Chaouat, G. & Menu, E. (2005) Evaluation of the placental environment with a new in vitro model of histocultures of early and term placentae: determination of cytokine and chemokine expression profiles. *Placenta,* 26(0143-4004; 0143-4004; 2-3), p.262-267.

Fisher, S.J. & Damsky, C.H. (1993) Human cytotrophoblast invasion. *Seminars in Cell Biology,* 4(1043-4682; 3), p.183-188.

Fukuda, M., Tanaka, S., Suzuki, S., Kusama, K., Kaneko, T. & Sakashita, H. (2007) Cimetidine induces apoptosis of human salivary gland tumor cells. *Oncology Reports,* 17(1021-335; 1021-335; 3), p.673-678.

Gammill, H.S. & Roberts, J.M. (2007) Emerging concepts in preeclampsia investigation. *Frontiers in Bioscience : A Journal and Virtual Library,* 12, p.2403-2411.

Genbacev, O., Joslin, R., Damsky, C.H., Polliotti, B.M. & Fisher, S.J. (1996) Hypoxia alters early gestation human cytotrophoblast differentiation/invasion in vitro and models the placental defects that occur in preeclampsia. *J.Clin.Invest,* 97(0021-9738; 2), p.540-550.

Genbacev, O. & Miller, R.K. (2000) Post-implantation differentiation and proliferation of cytotrophoblast cells: in vitro models--a review. *Placenta,* 21 Suppl A(0143-4004), p.S45-S49.

Ghosh, A.K., Hirasawa, N. & Ohuchi, K. (2001) Enhancement by histamine of vascular endothelial growth factor production in granulation tissue via H(2) receptors. *British Journal of Pharmacology,* 134(0007-1188; 0007-1188; 7), p.1419-1428.

Gierman, L.M., Stodle, G.S., Tangeras, L.H., Austdal, M., Olsen, G.D., Follestad, T., Skei, B., Rian, K., Gundersen, A.S., Austgulen, R. & Iversen, A.C. (2015) Toll-like receptor profiling of seven trophoblast cell lines warrants caution for translation to primary trophoblasts. *Placenta,* 36(11), p.1246-1253.

Greer, I.A., Dawes, J., Johnston, T.A. & Calder, A.A. (1991) Neutrophil activation is confined to the maternal circulation in pregnancy-induced hypertension. *Obstetrics and Gynecology,* 78(1), p.28-32.

Gunther, R.E. & Glick, D. (1967) Determination of histaminase activity in histologic samples and its quantitative distribution in intact human placenta and uterus. *J.Histochem.Cytochem.,* 15(0022-1554; 8), p.431-435.

Hamano, N., Terada, N., Maesako, K., Ikeda, T., Fukuda, S., Wakita, J., Yamashita, T. & Konno, A. (1998) Expression of histamine receptors in nasal epithelial cells and endothelial cells--the effects of sex hormones. *Int.Arch.Allergy Immunol.,* 115(1018-2438; 3), p.220-227.

Harris, L.K., Smith, S.D., Keogh, R.J., Jones, R.L., Baker, P.N., Knofler, M., Cartwright, J.E., Whitley, G.S. & Aplin, J.D. (2010) Trophoblast- and vascular smooth muscle cell-derived MMP-12 mediates elastolysis during uterine spiral artery remodeling. *American Journal of Pathology,* 177(1525-2191; 0002-9440; 4), p.2103-2115.

Hellstrand, K., Brune, M., Naredi, P., Mellqvist, U.H., Hansson, M., Gehlsen, K.R. & Hermodsson, S. (2000) Histamine: a novel approach to cancer immunotherapy. *Cancer Investigation,* 18(0735-7907; 4), p.347-355.

Hill, S.J. (1990) Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol.Rev.,* 42(0031-6997; 1), p.45-83.

Hine, R.J., Orsini, M.W. & Hegstrand, L.R. (1985) Changes in tissue histamine during the estrous cycle, pregnancy and pseudopregnancy in the golden hamster. *Proc.Soc.Exp.Biol.Med.,* 179(0037-9727; 3), p.271-278.

Hong, F. & Breitling, R. (2008) A comparison of meta-analysis methods for detecting differentially expressed genes in microarray experiments. *Bioinformatics,* 24(1367-4811; 1367-4803; 3), p.374-382.

Horakova, Z., Keiser, H.R. & Beaven, M.A. (1977) Blood and urine histamine levels in normal and pathological states as measured by a radiochemical assay. *Clinica Chimica Acta; International Journal of Clinical Chemistry,* 79(0009-8981; 2), p.447-456.

Huppertz, B., Kingdom, J., Caniggia, I., Desoye, G., Black, S., Korr, H. & Kaufmann, P. (2003) Hypoxia favours necrotic versus apoptotic shedding of placental syncytiotrophoblast into the maternal circulation. *Placenta,* 24(0143-4004; 2-3), p.181-190.

Ilekis, J.V., Tsilou, E., Fisher, S., Abrahams, V.M., Soares, M.J., Cross, J.C., Zamudio, S., Illsley, N.P., Myatt, L., Colvis, C., Costantine, M.M., Haas, D.M., Sadovsky, Y., Weiner, C., Rytting, E. & Bidwell, G. (2016) Placental origins of adverse pregnancy outcomes: potential molecular targets: an Executive Workshop Summary of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. *American Journal of Obstetrics and Gynecology,* .

Ind, P.W., Barnes, P.J., Brown, M.J., Causon, R. & Dollery, C.T. (1983) Measurement of plasma histamine in asthma. *Clin.Allergy,* 13(0009-9090; 1), p.61-67.

Ind, P.W., Brown, M.J., Lhoste, F.J., Macquin, I. & Dollery, C.T. (1982) Concentration effect relationships of infused histamine in normal volunteers. *Agents Actions,* 12(0065-4299; 1-2), p.12-16.

Irving, J.A., Lysiak, J.J., Graham, C.H., Hearn, S., Han, V.K. & Lala, P.K. (1995) Characteristics of trophoblast cells migrating from first trimester chorionic villus explants and propagated in culture. *Placenta,* 16(0143-4004; 0143-4004; 5), p.413-433.

James, J.L., Stone, P.R. & Chamley, L.W. (2006) The effects of oxygen concentration and gestational age on extravillous trophoblast outgrowth in a human first trimester villous explant model. *Hum.Reprod.,* 21(0268-1161; 0268-1161; 10), p.2699-2705.

James, J.L., Stone, P.R. & Chamley, L.W. (2005) Cytotrophoblast differentiation in the first trimester of pregnancy: evidence for separate progenitors of extravillous trophoblasts and syncytiotrophoblast. *Reproduction (Cambridge, England),* 130(1470-1626; 1470-1626; 1), p.95-103.

Jankovic, S.M., Varjacic, M. & Jankovic, S.V. (1998) Different roles of histamine receptor subtypes in ampullar & isthmic segments of human fallopian tube. *Indian J.Med.Res.,* 107(0971-5916), p.224-230.

Johansson, M., Henriksson, R., Bergenheim, A.T. & Koskinen, L.O. (2000) Interleukin-2 and histamine in combination inhibit tumour growth and angiogenesis in malignant glioma. *British Journal of Cancer,* 83(6), p.826-832.

Johnson, D.C. & Dey, S.K. (1980) Role of histamine in implantation: dexamethasone inhibits estradiol-induced implantation in the rat. *Biol.Reprod.,* 22(0006-3363; 5), p.1136-1141.

Juran, J.M. (1975) The non-Pareto principle; mea culpa. *Juran's Quality,* , p.1021.

Jutel, M., Watanabe, T., Klunker, S., Akdis, M., Thomet, O.A., Malolepszy, J., Zak-Nejmark, T., Koga, R., Kobayashi, T., Blaser, K. & Akdis, C.A. (2001) Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature,* 413(0028-0836; 0028-0836; 6854), p.420-425.

Kahlson, G., Nilsson, K., Rosengren, E. & Zederfeldt, B. (1960a) Wound healing as on rate of histamine formation. *Lancet,* 2, p.230-234.

Kahlson, G. & Rosengren, E. (1971) Biogenesis and physiology of histamine. *Monogr Physiol Soc.,* 21(0079-2020), p.1-318.

Kahlson, G., Rosengren, E. & WESTLING, H. (1958a) Increased formation of histamine in the pregnant rat. *J.Physiol,* 143(0022-3751; 1), p.91-103.

Kahlson, G., Rosengren, E., WESTLING, H. & WHITE, T. (1958b) The site of increased formation of histamine in the pregnant rat. *J.Physiol,* 144(0022-3751; 2), p.337-348.

Kahlson, G., Rosengren, E. & WHITE, T. (1960b) The formation of histamine in the rat foetus. *J.Physiol,* 151, p.131-138.

Kaliner, M., Shelhamer, J.H. & Ottesen, E.A. (1982) Effects of infused histamine: correlation of plasma histamine levels and symptoms. *J.Allergy Clin.Immunol.,* 69(0091-6749; 3), p.283-289.

Kambayashi, T. & Koretzky, G.A. (2007) Proximal signaling events in Fc epsilon RI-mediated mast cell activation. *The Journal of Allergy and Clinical Immunology,* 119(3), p.544-52; quiz 553-4.

Kapeller-Adler, R. (1965) Histamine Catabolism in vitro and in vivo. *Federation Proceedings,* 24(0014-9446), p.757-765.

Kapeller-Adler, R. (1952a) [Metabolism of histidine and histamine in normal and toxemic pregnancy]. *Brux.Med.,* 32(0068-3027; 31), p.1601-1617.

Kapeller-Adler, R. (1952b) Histamine metabolism in the human placenta and in the umbilical cord blood. *Biochem.J.,* 51(0264-6021; 5), p.610-613.

Kapeller-Adler, R. (1949) Histamine metabolism in pregnancy. *Lancet,* 2(0140-6736; 17), p.745-747.

Kapeller-Adler, R. (1944) Investigation on the activity of histaminase in normal and toxaemic pregnancy. *The Biochemical Journal,* 38, p.270-274.

Kapeller-Adler, R. (1941) Significance of Isolation of Histamine from the urine in Toxaemia of Pregnancy. *Journal of Obstetrics and Gynaecology (Tokyo, Japan),* 48, p.155-160.

Kauma, S.W., Walsh, S.W., Nestler, J.E. & Turner, T.T. (1992) Interleukin-1 is induced in the human placenta by endotoxin and isolation procedures for trophoblasts. *J.Clin.Endocrinol.Metab,* 75(0021-972; 3), p.951-955.

Kerr, M.K. & Churchill, G.A. (2001) Statistical design and the analysis of gene expression microarray data. *Genetical Research,* 77(0016-6723; 0016-6723; 2), p.123-128.

Khan, K.S., Wojdyla, D., Say, L., Gulmezoglu, A.M. & Van Look, P.F. (2006) WHO analysis of causes of maternal death: a systematic review. *Lancet (London, England),* 367(9516), p.1066-1074.

Khong, T.Y., Tee, J.H. & Kelly, A.J. (1997) Absence of innervation of the uteroplacental arteries in normal and abnormal human pregnancies. *Gynecologic and Obstetric Investigation,* 43(2), p.89-93.

King, A.E., Critchley, H.O. & Kelly, R.W. (2003) Innate immune defences in the human endometrium. *Reproductive Biology and Endocrinology : RB&E,* 1, p.116.

Kirkel', A.Z., Pekkel', V.A., Akhmetalieva, R.Z., Chernukha, E.A. & Gorkin, V.Z. (1983) [Decrease of placental amine oxidase activities in premature birth]. *Vopr.Med.Khim.,* 29(0042-8809; 4), p.83-87.

Kramer, M.S., Coates, A.L., Michoud, M.C., Dagenais, S., Moshonas, D., Davis, G.M., Hamilton, E.F., Nuwayhid, B., Joshi, A.K., Papageorgiou, A. & . (1995) Maternal asthma and idiopathic preterm labor. *Am.J.Epidemiol.,* 142(0002-9262; 10), p.1078-1088.

Kubo, Y. & Nakano, K. (1999) Regulation of histamine synthesis in mouse CD4+ and CD8+ T lymphocytes. *Inflammation Research : Official Journal of the European Histamine Research Society ... [Et Al.],* 48(1023-3830; 3), p.149-153.

Lassen, L.H., Christiansen, I., Iversen, H.K., Jansen-Olesen, I. & Olesen, J. (2003) The effect of nitric oxide synthase inhibition on histamine induced headache and arterial dilatation in migraineurs. *Cephalalgia : An International Journal of Headache,* 23(0333-1024; 9), p.877-886.

Leach, L., Eaton, B.M., Westcott, E.D. & Firth, J.A. (1995) Effect of histamine on endothelial permeability and structure and adhesion molecules of the paracellular junctions of perfused human placental microvessels. *Microvasc.Res.,* 50(3), p.323-337.

Leach, L. & Firth, J.A. (1997) Structure and permeability of human placental microvasculature. *Microsc.Res.Tech.,* 38(1059-910; 1-2), p.137-144.

Lee, M.L., Kuo, F.C., Whitmore, G.A. & Sklar, J. (2000) Importance of replication in microarray gene expression studies: statistical methods and evidence from repetitive cDNA hybridizations. *Proceedings of the National Academy of Sciences of the United States of America,* 97(0027-8424; 0027-8424; 18), p.9834-9839.

Legge, M. & Duff, G.B. (1981) Plasma diamine oxidase levels in pregnancy complicated by threatened abortion. *Journal of Clinical Pathology,* 34(0021-9746; 2), p.187-188.

Lewis, M.P., Clements, M., Takeda, S., Kirby, P.L., Seki, H., Lonsdale, L.B., Sullivan, M.H., Elder, M.G. & White, J.O. (1996) Partial characterization of an immortalized human trophoblast cell-line, TCL-1, which possesses a CSF-1 autocrine loop. *Placenta,* 17(0143-4004; 2-3), p.137-146.

Lim, K.J., Odukoya, O.A., Ajjan, R.A., Li, T.C., Weetman, A.P. & Cooke, I.D. (2000) The role of T-helper cytokines in human reproduction. *Fertil.Steril.,* 73(0015-0282; 0015-0282; 1), p.136-142.

Lindberg, S. (1963a) 14-C-histamine elimination from the blood of pregnant and non-pregnant women with special reference to the uterus. *Acta Obstetricia Et Gynecologica Scandinavica. Supplement,* 42(Suppl 1)(0300-8835), p.3-25.

Lindberg, S. (1963b) 14-C-histamine inactivation in vitro by human myometrial and placental tissues. *Acta Obstetricia Et Gynecologica Scandinavica. Supplement,* 42(Suppl 1)(0300-8835), p.26-34.

Lindberg, S., LINDELL, S.E. & WESTLING, H. (1963a) Formation and inactivation of histamine by human foetal tissues in vitro. *Acta Obstetricia Et Gynecologica Scandinavica. Supplement,* 42(Suppl 1)(0300-8835), p.49-58.

Lindberg, S., LINDELL, S.E. & WESTLING, H. (1963b) The metabolism of 14-C-labelled histamine injected into the umbilical artery. *Acta Obstetricia Et Gynecologica Scandinavica. Supplement,* 42(Suppl 1)(0300-8835), p.35-47.

Lindberg, S. & Tornqvist, A. (1966) The inhibitory effect of aminoguanidine on histamine catabolism in human pregnancy. *Acta Obstetricia Et Gynecologica Scandinavica,* 45(0001-6349; 2), p.131-139.

Linzke, N., Schumacher, A., Woidacki, K., Croy, B.A. & Zenclussen, A.C. (2014) Carbon monoxide promotes proliferation of uterine natural killer cells and remodeling of spiral arteries in pregnant hypertensive heme oxygenase-1 mutant mice. *Hypertension,* 63(3), p.580-588.

Liu, Z., Kilburn, B.A., Leach, R.E., Romero, R., Paria, B.C. & Armant, D.R. (2004) Histamine enhances cytotrophoblast invasion by inducing intracellular calcium transients through the histamine type-1 receptor. *Molecular Reproduction and Development,* 68(1040-452; 1040-452; 3), p.345-353.

Lonsdale, L.B., Elder, M.G. & Sullivan, M.H. (1996) A comparison of cytokine and hormone production by decidual cells and tissue explants. *J.Endocrinol.,* 151(0022-0795; 2), p.309-313.

Lorenz, M.O. (1905) Methods of Measuring the Concentration of Wealth. *American Statistical Association Publication,* 9(70), p.200-219.

Lorenz, W., Doenicke, A., Meyer, R., Reimann, H.J., Kusche, J., Barth, H., Geesing, H., Hutzel, M. & Weissenbacher, B. (1972) An improved method for the determination of histamine release in man: its application in studies with propanidid and thiopentone. *Eur.J.Pharmacol.,* 19(0014-2999; 2), p.180-190.

Lyall, F. (2006) Mechanisms regulating cytotrophoblast invasion in normal pregnancy and pre-eclampsia. *The Australian & New Zealand Journal of Obstetrics & Gynaecology,* 46(0004-8666; 0004-8666; 4), p.266-273.

Lyall, F. (2003) Development of the utero-placental circulation: the role of carbon monoxide and nitric oxide in trophoblast invasion and spiral artery transformation. *Microscopy Research and Technique,* 60(1059-910; 1059-910; 4), p.402-411.

Lyall, F. (2002) The human placental bed revisited. *Placenta,* 23(0143-4004; 0143-4004; 8-9), p.555-562.

Lynch, A.M., Murphy, J.R., Byers, T., Gibbs, R.S., Neville, M.C., Giclas, P.C., Salmon, J.E. & Holers, V.M. (2008) Alternative complement pathway activation fragment Bb in early pregnancy as a predictor of preeclampsia. *American Journal of Obstetrics and Gynecology,* 198(4), p.385.e1-385.e9.

Ma, R.Z., Gao, J., Meeker, N.D., Fillmore, P.D., Tung, K.S., Watanabe, T., Zachary, J.F., Offner, H., Blankenhorn, E.P. & Teuscher, C. (2002) Identification of Bphs, an autoimmune disease locus, as histamine receptor H1. *Science,* 297(1095-9203; 0036-8075; 5581), p.620-623.

Marcou, I., Athanasiu-Vergh, E., Chiriceanu, D., Cosma, G., Gingold, N. & Parhon, C.C. (1938) Sur le rôle physiologique de l'histamine. *Presse Med.,* 46, p.371.

Martin, C.B.,Jr (1965) Uterine Blood Flow and Placental Circulation. *Anesthesiology,* 26(0003-3022; 0003-3022), p.447-459.

Martinez-Varea, A., Pellicer, B., Perales-Marin, A. & Pellicer, A. (2014) Relationship between maternal immunological response during pregnancy and onset of preeclampsia. *Journal of Immunology Research,* 2014, p.210241.

Marzioni, D., Tamagnone, L., Capparuccia, L., Marchini, C., Amici, A., Todros, T., Bischof, P., Neidhart, S., Grenningloh, G. & Castellucci, M. (2004) Restricted innervation of uterus and placenta during pregnancy: evidence for a role of the repelling signal Semaphorin 3A. *Developmental Dynamics : An Official Publication of the American Association of Anatomists,* 231(4), p.839-848.

Matsubara, M., Ohmori, K. & Hasegawa, K. (2006) Histamine H1 receptor-stimulated interleukin 8 and granulocyte macrophage colony-stimulating factor production by bronchial epithelial cells requires extracellular signal-regulated kinase signaling via protein kinase C. *Int.Arch.Allergy Immunol.,* 139(1018-2438; 1018-2438; 4), p.279-293.

Matsuo, K., Kooshesh, S., Dinc, M., Sun, C.C., Kimura, T. & Baschat, A.A. (2007) Late postpartum eclampsia: report of two cases managed by uterine curettage and review of the literature. *Am.J.Perinatol.,* 24(0735-1631; 0735-1631; 4), p.257-266.

Matsuyama, K., Ichikawa, T., Nitta, Y., Ikoma, Y., Ishimura, K., Horio, S. & Fukui, H. (2006) Localized expression of histamine H1 receptors in syncytiotrophoblast cells of human placenta. *Journal of Pharmacological Sciences,* 102(3), p.331-337.

Matsuyama, K., Kawakami, N., Ichikawa, T., Nitta, Y., Ishimura, K., Horio, S. & Fukui, H. (2004) Expression of histamine H(1) receptor in placenta. *Inflammation Research : Official Journal of the European Histamine Research Society ...[Et Al.],* 53 Suppl 1, p.S85-6.

McMillan, J.C., Heskel, N.S. & Hanifin, J.M. (1985) Cyclic AMP-phosphodiesterase activity and histamine release in cord blood leukocyte preparations. *Acta Derm.Venereol.Suppl (Stockh),* 114(0365-8341), p.24-32.

Meekins, J.W., Pijnenborg, R., Hanssens, M., McFadyen, I.R. & van, A.A. (1994) A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *British Journal of Obstetrics and Gynaecology,* 101(0306-5456; 0306-5456; 8), p.669-674.

Menezo, Y.J., Nicollet, B., Dumont, M., Hazout, A. & Janny, L. (1993) Factors affecting human blastocyst formation in vitro and freezing at the blastocyst stage. *Acta Europaea Fertilitatis,* 24(0587-2421; 0587-2421; 5), p.207-213.

Miller, R.K., Genbacev, O., Turner, M.A., Aplin, J.D., Caniggia, I. & Huppertz, B. (2005) Human placental explants in culture: approaches and assessments. *Placenta,* 26(0143-4004; 6), p.439-448.

Minagawa, M., Narita, J., Tada, T., Maruyama, S., Shimizu, T., Bannai, M., Oya, H., Hatakeyama, K. & Abo, T. (1999) Mechanisms underlying immunologic states during pregnancy: possible association of the sympathetic nervous system. *Cell Immunol.,* 196(1), p.1-13.

Mohan, A.R., Sooranna, S.R., Lindstrom, T.M., Johnson, M.R. & Bennett, P.R. (2007) The effect of mechanical stretch on cyclooxygenase type 2 expression and activator protein-1 and nuclear factor-kappaB activity in human amnion cells. *Endocrinology,* 148(0013-7227; 0013-7227; 4), p.1850-1857.

Mol, B.W., Roberts, C.T., Thangaratinam, S., Magee, L.A., de Groot, C.J. & Hofmeyr, G.J. (2016) Pre-eclampsia. *Lancet (London, England),* 387(10022), p.999-1011.

Mondovi, B., Scioscia, S.A., Rotilio, G. & Costa, M.T. (1965) Studies on the Inhibition of Histaminase Activity by Histamine. *Enzymologia,* 28, p.228-234.

Morgan, D.M. & Hytten, F.E. (1984) Mid-trimester pregnancy--a time of tranquility or activity? *British Journal of Obstetrics and Gynaecology,* 91(0306-5456; 6), p.532-537.

Murakami, H., Sun-Wada, G.H., Matsumoto, M., Nishi, T., Wada, Y. & Futai, M. (1999) Human histamine H2 receptor gene: multiple transcription initiation and tissue-specific expression. *FEBS Letters,* 451(3), p.327-331.

Myatt, L. (2002) Role of placenta in preeclampsia. *Endocrine,* 19(1355-008; 1355-008; 1), p.103-111.

Naicker, T., Khedun, S.M., Moodley, J. & Pijnenborg, R. (2003) Quantitative analysis of trophoblast invasion in preeclampsia. *Acta Obstetricia Et Gynecologica Scandinavica,* 82(0001-6349; 0001-6349; 8), p.722-729.

Nandi, B.K., Subramanian, N., Majumder, A.K. & Chatterjee, I.B. (1974) Effect of ascorbic acid on detoxification of histamine under stress conditions. *Biochem.Pharmacol.,* 23(0006-2952; 3), p.643-647.

Nelson, D.M., Meister, R.K., Ortman-Nabi, J., Sparks, S. & Stevens, V.C. (1986) Differentiation and secretory activities of cultured human placental cytotrophoblast. *Placenta,* 7(0143-4004; 0143-4004; 1), p.1-16.

Neugebauer, E. & Lorenz, W. (1982) A modified Schayer procedure for the estimation of histidine decarboxylase activity: its application on tissue extracts from gastric mucosa of various mammals. *Agents Actions,* 12(0065-4299; 1-2), p.32-40.

NICE (2017) Pre-eclampsia. In: *Hypertension in pregnancy pathway.*: National Institute for Health and Care Excellence.

NICE (2011) *Hypertension in pregnancy: diagnosis and management. NICE clinical guideline 107.* :National Institute for Health and Clinical Excellence.

NICE (2008) *Antenatal care for uncomplicated pregnancies. Last updated: March 2016.* London :National Institute of Clinical Exellence.

Nilsson, E., Salonen, R.H., Cnattingius, S. & Lichtenstein, P. (2004) The importance of genetic and environmental effects for pre-eclampsia and gestational hypertension: a family study. *BJOG : An International Journal of Obstetrics and Gynaecology,* 111(1470-0328; 1470-0328; 3), p.200-206.

Nilsson, G., Lindell, S.E., Schayer, R.W. & Westling, H. (1959) Metabolism of 14C-labelled histamine in pregnant and non-pregnant women. *Clin.Sci.,* 18(0009-9287), p.313-319.

Norn, S. (1968) Antigenic histamine release from fractionated and unfractionated peritoneal cells from sensitized rats. *Acta Pharmacologica Et Toxicologica,* 26(4), p.373-383.

Norwitz, E.R., Schust, D.J. & Fisher, S.J. (2001) Implantation and the survival of early pregnancy. *The New England Journal of Medicine,* 345(0028-4793; 0028-4793; 19), p.1400-1408.

Orendi, K., Kivity, V., Sammar, M., Grimpel, Y., Gonen, R., Meiri, H., Lubzens, E. & Huppertz, B. (2011) Placental and trophoblastic in vitro models to study preventive and therapeutic agents for preeclampsia. *Placenta,* 32 Suppl(1532-3102; 0143-4004), p.S49-S54.

Packard, K.A. & Khan, M.M. (2003) Effects of histamine on Th1/Th2 cytokine balance. *International Immunopharmacology,* 3(1567-5769; 7), p.909-920.

Palmer, M.E., Watson, A.L. & Burton, G.J. (1997) Morphological analysis of degeneration and regeneration of syncytiotrophoblast in first trimester placental villi during organ culture. *Hum.Reprod.,* 12(0268-1161; 0268-1161; 2), p.379-382.

Panina-Bordignon, P., Mazzeo, D., Lucia, P.D., D'Ambrosio, D., Lang, R., Fabbri, L., Self, C. & Sinigaglia, F. (1997) Beta2-agonists prevent Th1 development by selective inhibition of interleukin 12. *The Journal of Clinical Investigation,* 100(6), p.1513-1519.

Pap, E., Falus, A., Mihalyi, D., Borck, H., Diel, F. & Pallinger, E. (2007) Histamine regulates placental cytokine expression--in vivo study on HDC knockout mice. *Placenta,* 28(0143-4004; 0143-4004; 2-3), p.239-244.

Peng, X. & Stromberg, A.J. (2003) Microarray Experiment Design and Statistical Analysis. In: *A Beginner's Guide to Microarray.* London: Kluwer Academic Publishers., p.243-275.

Pfeiffer, C.C., Iliev, V., Goldstein, L., Jenney, E.H. & Schultz, R. (1970) Blood histamine, polyamines and the schizophrenias. Computer correlations of the low and high blood histamine types. *Research Communications in Chemical Pathology and Pharmacology,* 1(2), p.247-265.

Pijnenborg, R. (1988) Establishment of uteroplacental circulation. *Reproduction, Nutrition, Development,* 28(0181-1916; 0181-1916; 6), p.1581-1586.

Pijnenborg, R., Ball, E., Bulmer, J.N., Hanssens, M., Robson, S.C. & Vercruysse, L. (2006a) In vivo analysis of trophoblast cell invasion in the human. *Methods in Molecular Medicine,* 122(1543-1894; 1543-1894), p.11-44.

Pijnenborg, R., Vercruysse, L. & Hanssens, M. (2006b) The uterine spiral arteries in human pregnancy: facts and controversies. *Placenta,* 27(0143-4004; 0143-4004; 9-10), p.939-958.

Pollock, I., Murdoch, R.D. & Lessof, M.H. (1991) Plasma histamine and clinical tolerance to infused histamine in normal, atopic and urticarial subjects. *Agents Actions,* 32(0065-4299; 3-4), p.359-365.

Prieto, J.A., Panyutich, A.V. & Heine, R.P. (1997) Neutrophil activation in preeclampsia. Are defensins and lactoferrin elevated in preeclamptic patients? *The Journal of Reproductive Medicine,* 42(1), p.29-32.

Pringle, K.G., Kind, K.L., Sferruzzi-Perri, A.N., Thompson, J.G. & Roberts, C.T. (2010) Beyond oxygen: complex regulation and activity of hypoxia inducible factors in pregnancy. *Human Reproduction Update,* 16(1460-2369; 1355-4786; 4), p.415-431.

Purcell, W.M. (1992) Human placental mast cells: a role in pre-eclampsia? *Med.Hypotheses,* 39(0306-9877; 3), p.281-283.

Purcell, W.M. & Hanahoe, T.H. (1991) A novel source of mast cells: the human placenta. *Agents Actions,* 33(0065-4299; 1-2), p.8-12.

Qin, S.Q., Kusuma, G.D., Al-Sowayan, B., Pace, R.A., Isenmann, S., Pertile, M.D., Gronthos, S., Abumaree, M.H., Brennecke, S.P. & Kalionis, B. (2016) Establishment and characterization of foetal and maternal mesenchymal stem/stromal cell lines from the human term placenta. *Placenta,* 39, p.134-146.

Raghupathy, R. (2001) Pregnancy: success and failure within the Th1/Th2/Th3 paradigm. *Seminars in Immunology,* 13(4), p.219-227.

Ramma, W., Buhimschi, I.A., Zhao, G., Dulay, A.T., Nayeri, U.A., Buhimschi, C.S. & Ahmed, A. (2012) The elevation in circulating anti-angiogenic factors is independent of markers of neutrophil activation in preeclampsia. *Angiogenesis,* 15(3), p.333-340.

Redman, C. (2014) Pre-eclampsia: A complex and variable disease. *Pregnancy Hypertension,* 4(3), p.241-242.

Redman, C.W. (1991) Current topic: pre-eclampsia and the placenta. *Placenta,* 12(4), p.301-308.

Redman, C.W., Sacks, G.P. & Sargent, I.L. (1999) Preeclampsia: an excessive maternal inflammatory response to pregnancy. *American Journal of Obstetrics and Gynecology,* 180(0002-9378; 2), p.499-506.

Regan, L. (1992) Recurrent early pregnancy failure. *Curr.Opin.Obstet Gynecol,* 4(2), p.220-228.

Rehn, D., Reimann, H.J., von der, O.M., Schmidt, U., Schmel, A. & Hennings, G. (1987) Biorhythmic changes of plasma histamine levels in healthy volunteers. *Agents Actions,* 22(0065-4299; 1-2), p.24-29.

Reilly, F.D. & Russell, P.T. (1977) Neurohistochemical evidence supporting an absence of adrenergic and cholinergic innervation in the human placenta and umbilical cord. *The Anatomical Record,* 188(3), p.277-286.

Reite, O.B. (1972) Comparative physiology of histamine. *Physiol Rev.,* 52(0031-9333; 3), p.778-819.

Reynolds, L.P., Borowicz, P.P., Caton, J.S., Vonnahme, K.A., Luther, J.S., Buchanan, D.S., Hafez, S.A., Grazul-Bilska, A.T. & Redmer, D.A. (2010) Uteroplacental vascular development and placental function: an update. *The International Journal of Developmental Biology,* 54(1696-3547; 0214-6282; 2-3), p.355-366.

Riedel, F., Achenbach, U. & Rieger, C.H. (1989) Prematurity and maternal bronchial hyperresponsiveness. *J.Perinat.Med.,* 17(0300-5577; 2), p.151-155.

Rivera, E.S., Cricco, G.P., Engel, N.I., Fitzsimons, C.P., Martin, G.A. & Bergoc, R.M. (2000) Histamine as an autocrine growth factor: an unusual role for a widespread mediator. *Seminars in Cancer Biology,* 10(1), p.15-23.

Roberts, J.M. & Hubel, C.A. (2009) The two stage model of preeclampsia: variations on the theme. *Placenta,* 30 Suppl A, p.S32-7.

Roberts, J.M., Taylor, R.N., Musci, T.J., Rodgers, G.M., Hubel, C.A. & McLaughlin, M.K. (1989) Preeclampsia: an endothelial cell disorder. *American Journal of Obstetrics and Gynecology,* 161(5), p.1200-1204.

Romero, R., Dey, S.K. & Fisher, S.J. (2014) Preterm labor: one syndrome, many causes. *Science (New York, N.Y.),* 345(6198), p.760-765.

Rothschild, Z. & Schayer, R.W. (1958) Synthesis and metabolism of a histamine metabolite, 1-methyl-4 (beta-aminoethyl)-imidazole. *Biochim.Biophys.Acta,* 30(0006-3002; 1), p.23-27.

RStudio, T. (2015) *RStudio: Integrated Development for R. RStudio, Inc., Boston, MA* [Online]. Available at: http://www.rstudio.com/.

Rukavina, D. & G, V. (2000) *Role of cytokines and immune cells at the foeto-maternal interface - a workshop report.*

Sacks, G., Sargent, I. & Redman, C. (1999) An innate view of human pregnancy. *Immunology Today,* 20(0167-5699; 3), p.114-118.

Sacks, G.P., Studena, K., Sargent, K. & Redman, C.W. (1998) Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *American Journal of Obstetrics and Gynecology,* 179(0002-9378; 1), p.80-86.

Saeed, A.I., Bhagabati, N.K., Braisted, J.C., Liang, W., Sharov, V., Howe, E.A., Li, J., Thiagarajan, M., White, J.A. & Quackenbush, J. (2006) TM4 microarray software suite. *Methods in Enzymology,* 411(0076-6879; 0076-6879), p.134-193.

Saeed, A.I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M., Currier, T., Thiagarajan, M., Sturn, A., Snuffin, M., Rezantsev, A., Popov, D., Ryltsov, A., Kostukovich, E.,

Borisovsky, I., Liu, Z., Vinsavich, A., Trush, V. & Quackenbush, J. (2003) TM4: a free, open-source system for microarray data management and analysis. *BioTechniques,* 34(0736-6205; 0736-6205; 2), p.374-378.

Saito, S. & Sakai, M. (2003) Th1/Th2 balance in preeclampsia. *Journal of Reproductive Immunology,* 59(2), p.161-173.

Saito, S., Umekage, H., Sakamoto, Y., Sakai, M., Tanebe, K., Sasaki, Y. & Morikawa, H. (1999) Increased T-helper-1-type immunity and decreased T-helper-2-type immunity in patients with preeclampsia. *American Journal of Reproductive Immunology (New York, N.Y. : 1989),* 41(1046-7408; 1046-7408; 5), p.297-306.

Salamonsen, L.A., Nie, G., Hannan, N.J. & Dimitriadis, E. (2009) Society for Reproductive Biology Founders' Lecture 2009. Preparing fertile soil: the importance of endometrial receptivity. *Reprod.Fertil.Dev.,* 21(1031-3613; 1031-3613; 7), p.923-934.

Sargent, I.L., Borzychowski, A.M. & Redman, C.W. (2006) Immunoregulation in normal pregnancy and pre-eclampsia: an overview. *Reproductive Biomedicine Online,* 13(5), p.680-686.

Sargent, I.L., Germain, S.J., Sacks, G.P., Kumar, S. & Redman, C.W. (2003) Trophoblast deportation and the maternal inflammatory response in pre-eclampsia. *Journal of Reproductive Immunology,* 59(0165-0378; 2), p.153-160.

Sasiak, K., Kierska, D., Boguslawski, M. & Maslinski, C. (1975) Phosphopyridoxal complexes with histamine and histidine. (3) The influence of presumed complex on activity of rat intestinal histaminase. *Agents Actions,* 5(0065-4299; 0065-4299; 1), p.25-30.

Sawin, S.W., Loret, d.M.,Jr., Monzon-Bordonaba, F., Wang, C.L. & Feinberg, R.F. (1997) Hydrosalpinx fluid enhances human trophoblast viability and function in vitro: implications for embryonic implantation in assisted reproduction. *Fertil.Steril.,* 68(0015-0282; 0015-0282; 1), p.65-71.

Say, L., Chou, D., Gemmill, A., Tuncalp, O., Moller, A.B., Daniels, J., Gulmezoglu, A.M., Temmerman, M. & Alkema, L. (2014) Global causes of maternal death: a WHO systematic analysis. *The Lancet.Global Health,* 2(6), p.e323-33.

Schayer, R.W. (1959) Catabolism of physiological quantities of histamine in vivo. *Physiol Rev.,* 39(0031-9333; 1), p.116-126.

Schayer, R.W. (1956) The metabolism of histamine in various species. *Br.J.Pharmacol.,* 11(0007-1188; 4), p.472-473.

Schayer, R.W. (1952a) Biogenesis of histamine. *J.Biol.Chem.,* 199(0021-9258; 1), p.245-250.

Schayer, R.W. (1952b) The metabolism of ring-labeled histamine. *J.Biol.Chem.,* 196(0021-9258; 2), p.469-475.

Schayer, R.W. & Karjala, S.A. (1956) Ring N methylation; a major route of histamine metabolism. *J.Biol.Chem.,* 221(0021-9258; 1), p.307-313.

Schayer, R.W. & Reilly, M.A. (1975) Methyl derivatives of histamine; interaction with histamine metabolism. *Agents Actions,* 5(0065-4299; 3), p.231-235.

Schena, M. (2003) *Microarray Analysis.* Hoboken, New Jersey: John Wiley & Sons Inc.

Schena, M., Shalon, D., Davis, R.W. & Brown, P.O. (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science,* 270(0036-8075; 0036-8075; 5235), p.467-470.

Schmetterer, L., Wolzt, M., Graselli, U., Findl, O., Strenn, K., Simak, S., Kastner, J., Eichler, H.G. & Singer, E.A. (1997) Nitric oxide synthase inhibition in the histamine headache model. *Cephalalgia : An International Journal of Headache,* 17(0333-1024; 3), p.175-182.

Seita, J., Sahoo, D., Rossi, D.J., Bhattacharya, D., Serwold, T., Inlay, M.A., Ehrlich, L.I., Fathman, J.W., Dill, D.L. & Weissman, I.L. (2012) Gene Expression Commons: an open platform for absolute gene expression profiling. *PloS One,* 7(7), p.e40321.

Sharma, S.C., Molloy, A., Walzman, M. & Bonnar, J. (1981) Levels of total ascorbic acid and histamine in the blood of women during the 3rd trimester of normal pregnancy. *Int.J.Vitam.Nutr.Res.,* 51(0300-9831; 3), p.266-273.

Sharma, S.C., Sabra, A., Molloy, A. & Bonnar, J. (1984) Comparison of blood levels of histamine and total ascorbic acid in pre-eclampsia with normal pregnancy. *Hum.Nutr.Clin.Nutr.,* 38(0263-8290; 1), p.3-9.

Sharma, S.C., Walzman, M., Bonnar, J. & Molloy, A. (1985) Blood ascorbic acid and histamine levels in patients with placental bleeding. *Hum.Nutr.Clin.Nutr.,* 39(0263-8290; 3), p.233-238.

Shrivastav, P., Gill, D.S., Jeremy, J.Y., Craft, I. & Dandona, P. (1988) Follicular fluid histamine concentrations in infertile women with pelvic adhesions. *Acta Obstetricia Et Gynecologica Scandinavica,* 67(0001-6349; 0001-6349; 8), p.727-729.

Shterental', I.S., Nikolaev, K.I., Merzhievskaia, V.M., Pikovskaia, N.B., Snegurova, V.G., Podkolodnaia, O.A., Skvortsova, I. & Pekker, M.S. (1991) Responses of the pressor and depressor regulatory systems to high sodium intake in borderline arterial hypertension. *Kardiologiia,* 31(10), p.47-50.

Siman, C.M., Sibley, C.P., Jones, C.J., Turner, M.A. & Greenwood, S.L. (2001) The functional regeneration of syncytiotrophoblast in cultured explants of term placenta. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology,* 280(0363-6119; 0363-6119; 4), p.R1116-R1122.

Simon, R., Lam, A., Li, M.C., Ngan, M., Menenzes, S. & Zhao, Y. (2007) Analysis of Gene Expression Data Using BRB-Array Tools. *Cancer Inform.,* 3(1176-9351), p.11-17.

Simon, R., Radmacher, M.D. & Dobbin, K. (2002) Design of studies using DNA microarrays. *Genetic Epidemiology,* 23(0741-0395; 1), p.21-36.

Simon, R., Radmacher, M.D., Dobbin, K. & McShane, L.M. (2003) Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification. *J.Natl.Cancer Inst.,* 95(0027-8874; 1), p.14-18.

Sitras, V., Fenton, C. & Acharya, G. (2015) Gene expression profile in cardiovascular disease and preeclampsia: a meta-analysis of the transcriptome based on raw data from human studies deposited in Gene Expression Omnibus. *Placenta,* 36(2), p.170-178.

Sooranna, S.R., Lee, Y., Kim, L.U., Mohan, A.R., Bennett, P.R. & Johnson, M.R. (2004) Mechanical stretch activates type 2 cyclooxygenase via activator protein-1 transcription factor in human myometrial cells. *Mol.Hum.Reprod.,* 10(1360-9947; 1360-9947; 2), p.109-113.

Sooranna, S.R., Oteng-Ntim, E., Meah, R., Ryder, T.A. & Bajoria, R. (1999) Characterization of human placental explants: morphological, biochemical and physiological studies using first and third trimester placenta. *Human Reproduction (Oxford, England),* 14(2), p.536-541.

Southren, A.L., Kobayashi, Y., Carmody, N.C. & Weingold, A.B. (1966a) Serial measurements of plasma diamine oxidase (DAO) during normal human pregnancy by an improved method. Evidence for the presence of a circulating DAO inhibitor. *American Journal of Obstetrics and Gynecology,* 95(0002-9378; 5), p.615-620.

Southren, A.L., Kobayashi, Y., Sherman, C.B., Levine, L., Gordon, G. & Weingold, A.B. (1963) Plasma Diamine Oxidase in Normal and Abnormal Human Pregnancy. *Surg.Forum,* 14(0071-8041), p.395-397.

Southren, A.L., Kobayashi, Y., Weingold, A.B. & Carmody, N.C. (1966b) Serial plasma diamine oxidase (DAO) assays in first and second trimester complications of pregnancy. *American Journal of Obstetrics and Gynecology,* 96(0002-9378; 4), p.502-510.

Steel, J.H., Malatos, S., Kennea, N., Edwards, A.D., Miles, L., Duggan, P., Reynolds, P.R., Feldman, R.G. & Sullivan, M.H. (2005) Bacteria and inflammatory cells in foetal membranes do not always cause preterm labor. *Pediatric Research,* 57(3), p.404-411.

Subramanian, A., Kuehn, H., Gould, J., Tamayo, P. & Mesirov, J.P. (2007) GSEA-P: a desktop application for Gene Set Enrichment Analysis. *Bioinformatics (Oxford, England),* 23(1460-2059; 23), p.3251-3253.

Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S. & Mesirov, J.P. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc.Natl.Acad.Sci.U.S.A,* 102(0027-8424; 43), p.15545-15550.

Sutherland, A., Cooper, D.W., Howie, P.W., Liston, W.A. & MacGillivray, I. (1981) The indicence of severe pre-eclampsia amongst mothers and mothers-in-law of pre-eclamptics and controls. *Br.J.Obstet.Gynaecol.,* 88(0306-5456; 0306-5456; 8), p.785-791.

Szelag, A., Merwid-Lad, A. & Trocha, M. (2002a) [Histamine receptors in the female reproductive system. Part II. The role of histamine in the placenta, histamine receptors and the uterus contractility]. *Ginekol.Pol.,* 73(0017-0011; 7), p.636-644.

Szelag, A., Merwid-Lad, A. & Trocha, M. (2002b) Histamine receptors in the female reproductive system. Part I. Role of the mast cells and histamine in female reproductive system. *Ginekologia Polska,* 73(7), p.627-635.

Szewczyk, G., Szewczyk, A., Pyzlak, M., Klimkiewicz, J., Smiertka, W. & Szukiewicz, D. (2005) [Mast cells, histamine and development of the placental vascular network in pregnancies complicated by preeclampsia and intrauterine growth retardation]. *Ginekol.Pol.,* 76(0017-0011; 0017-0011; 9), p.727-734.

Szukiewicz, D., Maslinska, D., Stelmachow, J. & Wojtecka-Lukasik, E. (1995) Biogenetic amines in placental tissue. Relation to the contractile activity of the human uterus. Preliminary communication. *Clin.Exp.Obstet.Gynecol.,* 22(0390-6663; 1), p.66-70.

Szukiewicz, D., Szukiewicz, A., Maslinska, D., Gujski, M., Poppe, P. & Mazurek-Kantor, J. (1999) Mast cell number, histamine concentration and placental vascular response to histamine in preeclampsia. *Inflammation Research : Official Journal of the European Histamine Research Society ... [Et Al.],* 48 Suppl 1(1023-3830), p.S39-S40.

Tamura, H., Horiike, K., Fukuda, H. & Watanabe, T. (1989) Kinetic studies on the inhibition mechanism of diamine oxidase from porcine kidney by aminoguanidine. *Journal of Biochemistry,* 105(2), p.299-306.

Tao, T.W. & Hertig, A.T. (1965) Viability and Differentiation of Human Trophoblast in Organ Culture. *The American Journal of Anatomy,* 116(0002-9106; 0002-9106), p.315-327.

Tasaka, K. (1991) Histamine and Blood. In: Uvnas (ed.) *Histamine and Histamine Antagonists.* Berlin: Springer-Verlag., p.473-510.

Taylor, P.V. & Hancock, K.W. (1973) Viability of human trophoblast in vitro. *The Journal of Obstetrics and Gynaecology of the British Commonwealth,* 80(0022-3204; 0022-3204; 9), p.834-838.

Taylor, R.N., de Groot, C.J., Cho, Y.K. & Lim, K.H. (1998) Circulating factors as markers and mediators of endothelial cell dysfunction in preeclampsia. *Seminars in Reproductive Endocrinology,* 16(1), p.17-31.

Tetlow, L.C. & Woolley, D.E. (2003) Histamine stimulates the proliferation of human articular chondrocytes in vitro and is expressed by chondrocytes in osteoarthritic cartilage. *Annals of the Rheumatic Diseases,* 62(0003-4967; 10), p.991-994.

Textor, J., Hardt, J. & Knuppel, S. (2011) DAGitty: a graphical tool for analyzing causal diagrams. *Epidemiology (Cambridge, Mass.),* 22(5), p.745.

Thomas, A.G., Curtis, N. & Walker-Smith, A. (1991) Cyclical vomiting and hypertension with dermatographism and histamine release. *Journal of the Royal Society of Medicine,* 84(0141-0768; 10), p.629.

Thompson, K.L., Pine, P.S., Rosenzweig, B.A., Turpaz, Y. & Retief, J. (2007) Characterization of the effect of sample quality on high density oligonucleotide microarray data using progressively degraded rat liver RNA. *BMC Biotechnology [Computer File],* 7(1472-6750; 1472-6750), p.57.

Thomson, N.F., Thornton, S. & Clark, J.F. (2000) The effects of placental extracts from normotensive and preeclamptic women on vasoconstriction and oxidative metabolism. *Am.J.Obstet.Gynecol.,* 183(0002-9378; 1), p.206-210.

Tornqvist, A. (1969) The effect of histaminase inhibition on the concentration and distribution of 14C histamine in blood during pregnancy. *Acta Obstetricia Et Gynecologica Scandinavica,* 48(0001-6349; 2), p.272-284.

Tranquilli, A.L., Dekker, G., Magee, L., Roberts, J., Sibai, B.M., Steyn, W., Zeeman, G.G. & Brown, M.A. (2014) The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertension,* 4(2), p.97-104.

Tryding, N. & Willert, B. (1968) Determination of plasma diamine oxidase (histaminase) in clinical practice. A comparison between a biological method and a radiochemical micromethod. *Scand.J.Clin.Lab Invest,* 22(0036-5513; 1), p.29-32.

Tufvesson, G. (1978) Purification and properties of human amniotic fluid diamine oxidase. *Scand.J.Clin.Lab Invest,* 38(0036-5513; 5), p.463-472.

Tuimala, J., Korpelainen, E., Gentile, M., Kallio, A. & Hupponen, T. (2008) *Chipster Manual* [Online]. Available at: https://extras.csc.fi/biosciences/chipster-manual/.

van der Pouw Kraan, T.C., Snijders, A., Boeije, L.C., de Groot, E.R., Alewijnse, A.E., Leurs, R. & Aarden, L.A. (1998) Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. *J.Clin.Invest,* 102(0021-9738; 10), p.1866-1873.

Vanina, L.V., Strongina, T.N. & Meshcheriakova, S.A. (1971) [The state of the blood histamine--histaminase system in late toxemia of pregnancy]. *Akush.Ginekol.(Mosk),* 47(0002-3906; 4), p.25-28.

Walker, D.W. & McLean, J.R. (1971) Absence of adrenergic nerves in the human placenta. *Nature,* 229(5283), p.344-345.

Wang, J., Duncan, D., Shi, Z. & Zhang, B. (2013) WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): update 2013. *Nucleic Acids Research,* 41(1362-4962; 0305-1048), p.W77-W83.

Wantke, F., Proud, D., Siekierski, E. & Kagey-Sobotka, A. (1998) Daily variations of serum diamine oxidase and the influence of H1 and H2 blockers: a critical approach to routine diamine oxidase assessment. *Inflammation Research : Official Journal of the European Histamine Research Society ... [Et Al.],* 47(10), p.396-400.

Watson, A.L., Palmer, M.E. & Burton, G. (1995) Human chorionic gonadotrophin release and tissue viability in placental organ culture. *Hum.Reprod.,* 10(0268-1161; 0268-1161; 8), p.2159-2164.

Weetman, A.P. (1999) The immunology of pregnancy. *Thyroid : Official Journal of the American Thyroid Association,* 9(1050-7256; 1050-7256; 7), p.643-646.

Wegmann, T.G., Lin, H., Guilbert, L. & Mosmann, T.R. (1993) Bidirectional cytokine interactions in the maternal-foetal relationship: is successful pregnancy a TH2 phenomenon? *Immunology Today,* 14(0167-5699; 7), p.353-356.

Weingold, A.B. & Southren, A.L. (1968) Diamine oxidase as an index of the foetoplacental unit. Clinical applications. *Obstet Gynecol,* 32(0029-7844; 5), p.593-606.

Wen, S.W., Demissie, K. & Liu, S. (2001) Adverse outcomes in pregnancies of asthmatic women: results from a Canadian population. *Annals of Epidemiology,* 11(1047-2797; 1), p.7-12.

WHO (2014) *World Health Statistics.* Geneva: World Health Organisation.

Wicksell, F. (1949) Observations on Histamine and Histaminolysis in Pregnancy. *Acta Physiologica Scandinavica,* 17, p.395-414.

Wilczynski, J.R. (2006) Immunological analogy between allograft rejection, recurrent abortion and pre-eclampsia - the same basic mechanism? *Human Immunology,* 67(7), p.492-511.

Wit, E. & McClure, J. (2004) *Statistics for Microarray: Design, Analysis and Inference.*: John Wiley and Sons, Ltd.

Wright, G.W. & Simon, R.M. (2003) A random variance model for detection of differential gene expression in small microarray experiments. *Bioinformatics (Oxford, England),* 19(1367-4803; 18), p.2448-2455.

Xia, J., Fjell, C.D., Mayer, M.L., Pena, O.M., Wishart, D.S. & Hancock, R.E. (2013) INMEX--a web-based tool for integrative meta-analysis of expression data. *Nucleic Acids Research,* 41(1362-4962; 0305-1048), p.W63-W70.

Xia, J., Gill, E.E. & Hancock, R.E. (2015) NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. *Nature Protocols,* 10(6), p.823-844.

Yang, Y.H., Dudoit, S., Luu, P., Lin, D.M., Peng, V., Ngai, J. & Speed, T.P. (2002) Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Research,* 30(1362-4962; 4), p.e15.

Yang, Y.H. & Speed, T. (2002) Design issues for cDNA microarray experiments. *Nature Reviews. Genetics,* 3(1471-0056; 1471-0056; 8), p.579-588.

Zeller, E.A. (1938) Über den enzymatischen Abbau von Histamin und Diaminen. 2. Mitteilung. *Helvetica Chimica Acta,* 21(1), p.880-890.

Zhang, B., Schmoyer, D., Kirov, S. & Snoddy, J. (2004) GOTree Machine (GOTM): a web-based platform for interpreting sets of interesting genes using Gene Ontology hierarchies. *BMC Bioinformatics,* 5, p.16.

Zhao, X., Ma, W., Das, S.K., Dey, S.K. & Paria, B.C. (2000) Blastocyst H(2) receptor is the target for uterine histamine in implantation in the mouse. *Development,* 127(0950-1991; 12), p.2643-2651.

Zhou, Y., Fisher, S.J., Janatpour, M., Genbacev, O., Dejana, E., Wheelock, M. & Damsky, C.H. (1997) Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? *The Journal of Clinical Investigation,* 99(0021-9738; 0021-9738; 9), p.2139-2151.

Appendix

Appendix 1: Statistical Analysis results of maternal blood and urine histamine

Table A-1: Maternal Blood Histamine Levels

Condition	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NP Trimester 1	2	64.0000	8.48528	6.00000	-12.2372	140.2372	58.00	70.00
NP Trimester 2	3	43.3333	11.54701	6.66667	14.6490	72.0177	30.00	50.00
NP Trimester 3	38	52.9108	8.80811	1.42886	50.0156	55.8059	35.00	74.30
PE	31	63.5613	10.49342	1.88467	59.7123	67.4103	49.10	94.30
Eclampsia	14	69.6714	10.03145	2.68102	63.8794	75.4634	54.20	87.30
Total	88	59.2547	11.86594	1.26491	56.7405	61.7688	30.00	94.30

Table A-2: Maternal Blood Histamine Trimester Multiple Comparisons

Dependent Variable: BloodHA

Bonferroni

(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NP Trimester 1	NP Trimester 2	20.66667	8.86125	.221	-4.8893	46.2226
	NP Trimester 3	11.08921	7.04221	1.000	-9.2206	31.3990
	PE	.43871	7.08185	1.000	-19.9854	20.8628
	Eclampsia	-5.67143	7.33781	1.000	-26.8338	15.4909
NP Trimester 2	NP Trimester 1	-20.66667	8.86125	.221	-46.2226	4.8893
	NP Trimester 3	-9.57746	5.82137	1.000	-26.3663	7.2114
	PE	-20.22796*	5.86926	.009	-37.1550	-3.3009
	Eclampsia	-26.33810*	6.17569	.001	-44.1488	-8.5273
NP Trimester 3	NP Trimester 1	-11.08921	7.04221	1.000	-31.3990	9.2206
	NP Trimester 2	9.57746	5.82137	1.000	-7.2114	26.3663
	PE	-10.65050*	2.34930	.000	-17.4259	-3.8751
	Eclampsia	-16.76064*	3.03481	.000	-25.5131	-8.0082
PE	NP Trimester 1	-.43871	7.08185	1.000	-20.8628	19.9854
	NP Trimester 2	20.22796*	5.86926	.009	3.3009	37.1550
	NP Trimester 3	10.65050*	2.34930	.000	3.8751	17.4259
	Eclampsia	-6.11014	3.12570	.540	-15.1247	2.9044
Eclampsia	NP Trimester 1	5.67143	7.33781	1.000	-15.4909	26.8338
	NP Trimester 2	26.33810*	6.17569	.001	8.5273	44.1488
	NP Trimester 3	16.76064*	3.03481	.000	8.0082	25.5131

	PE	6.11014	3.12570	.540	-2.9044	15.1247

*. The mean difference is significant at the 0.05 level.

Table A-3: Maternal Urine Histamine after Aminoguanidine Treatment

Gestational Age		Mean HA before AG	Mean HA after AG	Significance
Non-Pregnant	Mean (µg/24h)	6.6667	7.8333	p>0.05
	N	3	3	
	Std. Deviation	5.13160	8.80814	
	Std. Error of Mean	2.96273	5.08538	
	Minimum (µg/24h)	2.33	2.50	
	Maximum (µg/24h)	12.33	18.00	
	Range (µg/24h)	10.00	15.50	
Trimester 1	Mean (µg/24h)	44.1333	52.4000	p=0.137
	N	5	5	
	Std. Deviation	18.31605	17.67201	
	Std. Error of Mean	8.19119	7.90316	
	Minimum (µg/24h)	17.67	21.00	
	Maximum (µg/24h)	61.00	62.00	
	Range (µg/24h)	43.33	41.00	
	Percentage (%) rise in urine histamine			

An analysis of maternal urine histamine (HA) levels after inhibition of Diamine oxidase (DAO) with aminoguanidine (AG) based on Bjuro et al 1964. Percentage (%) rise in urine histamine in pregnancy from non-pregnant = 562.00%. Percentage rise in maternal urine histamine after inhibition of DAO with aminoguanidine = 18.73%.

Appendix 2: Causal effect identification-Testable Implications

A.	**Adjustment for Total effect:** No adjustment is necessary to estimate the total effect of Elevated Histamine on Pre-eclampsia. **Adjustments for Direct Effect:** Minimal sufficient adjustment sets for estimating the direct effect of Elevated Histamine on Pre-eclampsia: • Cell Signalling, Gene Regulation, Inflammation and Immune regulation, Junctional proteins regulation, Oxygen Sensing, Placental like Innervation, Placental like contractile activity, Placental growth, Tissue proliferation and morphogenesis **Instrumental and conditional Covariates:** • Defective_DAO_Activity • Increased_HDC_Activity
B.	**Testable implications-** The model implies the following conditional independences:
1.	Cell_Signaling ⊥ Defective_DAO_Activity | Elevated Histamine
2.	Cell_Signaling ⊥ Gene_Regulation | Elevated Histamine
3.	Cell_Signaling ⊥ Increased_HDC_Activity | Elevated Histamine
4.	Cell_Signaling ⊥ Inflammation_and_Immune_regulation | Elevated Histamine
5.	Cell_Signaling ⊥ Junctional_proteins_regulation | Elevated Histamine
6.	Cell_Signaling ⊥ Oxygen_Sensing | Elevated Histamine
7.	Cell_Signaling ⊥ Placental_Innervation | Elevated Histamine
8.	Cell_Signaling ⊥ Placental_contractile_activity | Elevated Histamine
9.	Cell_Signaling ⊥ Placental_growth | Elevated Histamine
10.	Cell_Signaling ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine
11.	Defective_DAO_Activity ⊥ Gene_Regulation | Elevated Histamine
12.	Defective_DAO_Activity ⊥ Increased_HDC_Activity
13.	Defective_DAO_Activity ⊥ Inflammation_and_Immune_regulation | Elevated Histamine
14.	Defective_DAO_Activity ⊥ Junctional_proteins_regulation | Elevated Histamine
15.	Defective_DAO_Activity ⊥ Oxygen_Sensing | Elevated Histamine
16.	Defective_DAO_Activity ⊥ Placental_Innervation | Elevated Histamine
17.	Defective_DAO_Activity ⊥ Placental_contractile_activity | Elevated Histamine

18.	Defective_DAO_Activity ⊥ Placental_growth | Elevated Histamine
19.	Defective_DAO_Activity ⊥ Pre-eclampsia | Elevated Histamine
20.	Defective_DAO_Activity ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine
21.	Gene_Regulation ⊥ Increased_HDC_Activity | Elevated Histamine
22.	Gene_Regulation ⊥ Inflammation_and_Immune_regulation | Elevated Histamine
23.	Gene_Regulation ⊥ Junctional_proteins_regulation | Elevated Histamine
24.	Gene_Regulation ⊥ Oxygen_Sensing | Elevated Histamine
25.	Gene_Regulation ⊥ Placental_Innervation | Elevated Histamine
26.	Gene_Regulation ⊥ Placental_contractile_activity | Elevated Histamine
27.	Gene_Regulation ⊥ Placental_growth | Elevated Histamine
28.	Gene_Regulation ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine
29.	Increased_HDC_Activity ⊥ Inflammation_and_Immune_regulation | Elevated Histamine
30.	Increased_HDC_Activity ⊥ Junctional_proteins_regulation | Elevated Histamine
31.	Increased_HDC_Activity ⊥ Oxygen_Sensing | Elevated Histamine
32.	Increased_HDC_Activity ⊥ Placental_Innervation | Elevated Histamine
33.	Increased_HDC_Activity ⊥ Placental_contractile_activity | Elevated Histamine
34.	Increased_HDC_Activity ⊥ Placental_growth | Elevated Histamine
35.	Increased_HDC_Activity ⊥ Pre-eclampsia | Elevated Histamine
36.	Increased_HDC_Activity ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine
37.	Inflammation_and_Immune_regulation ⊥ Junctional_proteins_regulation | Elevated Histamine
38.	Inflammation_and_Immune_regulation ⊥ Oxygen_Sensing | Elevated Histamine
39.	Inflammation_and_Immune_regulation ⊥ Placental_Innervation | Elevated Histamine
40.	Inflammation_and_Immune_regulation ⊥ Placental_contractile_activity | Elevated Histamine
41.	Inflammation_and_Immune_regulation ⊥ Placental_growth | Elevated Histamine
42.	Inflammation_and_Immune_regulation ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine
43.	Junctional_proteins_regulation ⊥ Oxygen_Sensing | Elevated Histamine

44.	Junctional_proteins_regulation ⊥ Placental_Innervation | Elevated Histamine
45.	Junctional_proteins_regulation ⊥ Placental_contractile_activity | Elevated Histamine
46.	Junctional_proteins_regulation ⊥ Placental_growth | Elevated Histamine
47.	Junctional_proteins_regulation ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine
48.	Oxygen_Sensing ⊥ Placental_Innervation | Elevated Histamine
49.	Oxygen_Sensing ⊥ Placental_contractile_activity | Elevated Histamine
50.	Oxygen_Sensing ⊥ Placental_growth | Elevated Histamine
51.	Oxygen_Sensing ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine
52.	Placental_Innervation ⊥ Placental_contractile_activity | Elevated Histamine
53.	Placental_Innervation ⊥ Placental_growth | Elevated Histamine
54.	Placental_Innervation ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine
55.	Placental_contractile_activity ⊥ Placental_growth | Elevated Histamine
56.	Placental_contractile_activity ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine
57.	Placental_growth ⊥ Tissue_proliferation_and_morphogenesis | Elevated Histamine

Appendix 3: Scatterplot Up-regulated Genes

ProbeSet	Accession	UGCluster	Symbol
1552509_a_at	NM_145273	Hs.657365	CD300LG
1552575_a_at	NM_153344	Hs.485528	C6orf141
1553151_at	AY079172	Hs.436360	ATP6V0D2
1553482_at	NM_153040	Hs.367879	C15orf32
1553574_at	NM_176891	Hs.682604	IFNE
1553789_a_at	NM_058180	Hs.236572	C21orf58
1553973_a_at	BC032003	Hs.334274	SPINK6
1554020_at	BC010091	Hs.505202	BICD1
1554097_a_at	BC021861	Hs.458096	LOC554202
1554640_at	BC039306	Hs.591908	PALM2
1554663_a_at	BC043499	Hs.325978	NUMA1
1554906_a_at	BC029395	Hs.344400	MPHOSPH6
1554997_a_at	AY151286	Hs.196384	PTGS2
1555758_a_at	AF213040	Hs.84113	CDKN3
1556364_at	AK057923	Hs.660881	LOC730057
1556409_a_at	AF086184	Hs.103068	LOC100129932
1556423_at	BE220445	Hs.525479	VASH1
1556695_a_at	AK095719	Hs.457407	FLJ42709
1556698_a_at	AI819722	Hs.605082	GPRIN3
1558075_at	BM989131	Hs.619009	LOC339047
1558834_s_at	AL832216	Hs.716732	C1orf62
1558930_at	BC009533	Hs.559194	LOC728192
1559277_at	AK093019	Hs.78061	FLJ35700
1560558_at	BC013097	Hs.658575	C9orf80
1561171_a_at	AK093450	Hs.569472	LOC727832
1561720_at	BC042989	Hs.632229	RECQL5
1562102_at	BC014579	Hs.460260	AKR1C1
1562337_at	AK095468	Hs.531755	OR7D2
1562591_a_at	AF548116	Hs.532138	OFCC1
1564231_at	AK025109	Hs.478095	IFT80
1564315_at	AK055534	Hs.545529	C8orf49
1564474_at	AK055144	Hs.161338	LOC728723
1565716_at	BE930017	Hs.513522	FUS
1566785_x_at	AK025172	Hs.646586	LOC728806
1568638_a_at	BQ024836	Hs.676257	IDO2
1570515_a_at	BC029425	Hs.696158	FILIP1
201430_s_at	W72516	Hs.519659	DPYSL3
201508_at	NM_001552	Hs.462998	IGFBP4
201820_at	NM_000424	Hs.433845	KRT5
201860_s_at	NM_000930	Hs.491582	PLAT
202273_at	NM_002609	Hs.509067	PDGFRB
202291_s_at	NM_000900	Hs.365706	MGP
202363_at	AF231124	Hs.643338	SPOCK1
202465_at	NM_002593	Hs.202097	PCOLCE
202709_at	NM_002023	Hs.519168	FMOD
202834_at	NM_000029	Hs.19383	AGT

202936_s_at	NM_000346	Hs.647409	SOX9
203083_at	NM_003247	Hs.371147	THBS2
203085_s_at	BC000125	Hs.645227	TGFB1
203151_at	AW296788	Hs.194301	MAP1A
203153_at	NM_001548	Hs.20315	IFIT1
203263_s_at	AI625739	Hs.54697	ARHGEF9
203325_s_at	AI130969	Hs.210283	COL5A1
203649_s_at	NM_000300	Hs.466804	PLA2G2A
203886_s_at	NM_001998	Hs.198862	FBLN2
204114_at	NM_007361	Hs.369840	NID2
204301_at	NM_014867	Hs.5333	KBTBD11
204337_at	AL514445	Hs.386726	RGS4
204411_at	NM_017596	Hs.169182	KIF21B
204533_at	NM_001565	Hs.632586	CXCL10
204596_s_at	U46768	Hs.25590	STC1
204719_at	NM_007168	Hs.58351	ABCA8
204733_at	NM_002774	Hs.79361	KLK6
204749_at	NM_004538	Hs.21365	NAP1L3
204818_at	NM_002153	Hs.162795	HSD17B2
204932_at	BF433902	Hs.81791	TNFRSF11B
204945_at	NM_002846	Hs.89655	PTPRN
204951_at	NM_004310	Hs.654594	RHOH
205048_s_at	NM_003832	Hs.512656	PSPH
205051_s_at	NM_000222	Hs.479754	KIT
205112_at	NM_016341	Hs.655033	PLCE1
205236_x_at	NM_003102	Hs.2420	SOD3
205267_at	NM_006235	Hs.654525	POU2AF1
205290_s_at	NM_001200	Hs.73853	BMP2
205347_s_at	NM_021992	Hs.56145	TMSB15A
205364_at	NM_003500	Hs.444959	ACOX2
205404_at	NM_005525	Hs.195040	HSD11B1
205447_s_at	BE222201	Hs.713539	MAP3K12
205475_at	NM_007281	Hs.7122	SCRG1
205481_at	NM_000674	Hs.77867	ADORA1
205499_at	NM_014467	Hs.306339	SRPX2
205500_at	NM_001735	Hs.494997	C5
205624_at	NM_001870	Hs.646	CPA3
205660_at	NM_003733	Hs.118633	OASL
205680_at	NM_002425	Hs.2258	MMP10
205695_at	NM_006843	Hs.439023	SDS
205767_at	NM_001432	Hs.115263	EREG
205782_at	NM_002009	Hs.567268	FGF7
205801_s_at	NM_015376	Hs.143674	RASGRP3
205870_at	NM_000623	Hs.654542	BDKRB2
205890_s_at	NM_006398	Hs.44532	UBD
206224_at	NM_001898	Hs.123114	CST1
206227_at	NM_003613	Hs.442180	CILP
206304_at	NM_004997	Hs.927	MYBPH

206481_s_at	NM_001290	Hs.714330	LDB2
206510_at	AF332197	Hs.101937	SIX2
206574_s_at	NM_007079	Hs.43666	PTP4A3
206618_at	NM_003855	Hs.469521	IL18R1
206637_at	NM_014879	Hs.2465	P2RY14
206806_at	NM_004717	Hs.242947	DGKI
206924_at	NM_000641	Hs.467304	IL11
206994_at	NM_001899	Hs.654549	CST4
207016_s_at	AB015228	Hs.643455	ALDH1A2
207191_s_at	NM_005545	Hs.699822	ISLR
207276_at	NM_004065	Hs.446675	CDR1
207387_s_at	NM_000167	Hs.1466	GK
207510_at	NM_000710	Hs.525572	BDKRB1
207889_at	NM_007101	Hs.198003	SARDH
208307_at	NM_005058	Hs.380450	RBMY1A1
208851_s_at	AL161958	Hs.644697	THY1
209047_at	AL518391	Hs.660192	AQP1
209198_s_at	BC004291	Hs.32984	SYT11
209335_at	AI281593	Hs.718429	DCN
209396_s_at	M80927	Hs.382202	CHI3L1
209496_at	BC000069	Hs.647064	RARRES2
209560_s_at	U15979	Hs.533717	DLK1
209596_at	AF245505	Hs.369422	MXRA5
209747_at	J03241	Hs.592317	TGFB3
209914_s_at	AW149405	Hs.637685	NRXN1
209955_s_at	U76833	Hs.654370	FAP
210029_at	M34455	Hs.840	IDO1
210119_at	U73191	Hs.411299	KCNJ15
210140_at	AF031824	Hs.143212	CST7
210145_at	M68874	Hs.497200	PLA2G4A
210401_at	U45448	Hs.41735	P2RX1
210605_s_at	BC003610	Hs.3745	MFGE8
210619_s_at	AF173154	Hs.75619	HYAL1
210632_s_at	L35853	Hs.463412	SGCA
210687_at	BC000185	Hs.503043	CPT1A
211617_at	M21191	Hs.652473	ALDOAP2
211743_s_at	BC005929	Hs.512633	PRG2
211958_at	R73554	Hs.607212	IGFBP5
212134_at	AB014538	Hs.504062	PHLDB1
212354_at	BE500977	Hs.409602	SULF1
212358_at	AL117468	Hs.466539	CLIP3
212624_s_at	BF339445	Hs.654534	CHN1
212670_at	AA479278	Hs.647061	ELN
212713_at	R72286	Hs.296049	MFAP4
212736_at	BE299456	Hs.401798	C16orf45
212865_s_at	BF449063	Hs.409662	COL14A1
212942_s_at	AB033025	Hs.459088	KIAA1199
213001_at	AF007150	Hs.653262	ANGPTL2

213075_at	AL050002	Hs.357004	OLFML2A
213249_at	AU145127	Hs.433057	FBXL7
213265_at	AI570199	Hs.601055	PGA3
213280_at	AK000478	Hs.499659	GARNL4
213422_s_at	AW888223	Hs.558570	MXRA8
213800_at	X04697	Hs.363396	CFH
213820_s_at	T54159	Hs.513075	STARD5
213832_at	AA530995	Hs.666367	KCND3
213974_at	AB033059	Hs.459162	ADAMTSL3
214022_s_at	AA749101	Hs.458414	IFITM1
214079_at	AK000345	Hs.272499	DHRS2
214236_at	AA166684	Hs.463295	CDC27
214761_at	AW149417	Hs.530930	ZNF423
214951_at	AL050358	Hs.159481	SLC26A10
214978_s_at	AK023365	Hs.153648	PPFIA4
215002_at	BE000837	Hs.552700	LOC100132247
215184_at	AK026801	Hs.237886	DAPK2
215192_at	D38500	Hs.712714	PMS2L4
215388_s_at	X56210	Hs.575869	CFHR1
215543_s_at	AB011181	Hs.474667	LARGE
215646_s_at	R94644	Hs.643801	VCAN
215856_at	AK025833	Hs.287692	SIGLEC15
216176_at	AK025343	Hs.675399	HCRP1
217525_at	AW305097	Hs.503500	OLFML1
217590_s_at	AA502609	Hs.716816	TRPA1
217897_at	NM_022003	Hs.714294	FXYD6
218285_s_at	NM_020139	Hs.124696	BDH2
218332_at	NM_018476	Hs.334370	BEX1
218484_at	NM_020142	Hs.718455	NDUFA4L2
218638_s_at	NM_012445	Hs.302963	SPON2
218729_at	NM_020169	Hs.478067	LXN
218820_at	NM_020215	Hs.6434	C14orf132
218950_at	NM_022481	Hs.25277	ARAP3
219025_at	NM_020404	Hs.195727	CD248
219054_at	NM_024563	Hs.13528	C5orf23
219059_s_at	AL574194	Hs.655332	LYVE1
219087_at	NM_017680	Hs.435655	ASPN
219230_at	NM_018286	Hs.173233	TMEM100
219315_s_at	NM_024600	Hs.459652	TMEM204
219359_at	NM_025092	Hs.353181	ATHL1
219407_s_at	NM_006059	Hs.201805	LAMC3
219478_at	NM_021197	Hs.36688	WFDC1
219500_at	NM_013246	Hs.502977	CLCF1
219554_at	NM_016321	Hs.459284	RHCG
219602_s_at	NM_022068	Hs.585839	FAM38B
219629_at	NM_017911	Hs.265018	FAM118A
219689_at	NM_020163	Hs.59729	SEMA3G
219700_at	NM_020405	Hs.125036	PLXDC1

219743_at	NM_012259	Hs.144287	HEY2
219837_s_at	NM_018659	Hs.13872	CYTL1
219855_at	NM_018159	Hs.200016	NUDT11
220092_s_at	NM_018153	Hs.165859	ANTXR1
220116_at	NM_021614	Hs.98280	KCNN2
220191_at	NM_019617	Hs.69319	GKN1
220301_at	NM_024781	Hs.280781	CCDC102B
220480_at	NM_021973	Hs.388245	HAND2
220872_at	NM_018547	Hs.621377	PRO2964
221305_s_at	NM_019076	Hs.554822	UGT1A8
221572_s_at	AF288410	Hs.631925	SLC26A6
221730_at	NM_000393	Hs.445827	COL5A2
221914_at	H19843	Hs.225936	SYN1
221992_at	AI925734	Hs.448833	NPIPL2
222253_s_at	AL117484	Hs.534980	POM121L9P
222379_at	AI002715	Hs.348522	KCNE4
222450_at	AL035541	Hs.517155	PMEPA1
223316_at	AL136562	Hs.498720	CCDC3
223467_at	AF069506	Hs.25829	RASD1
223499_at	AF329841	Hs.632102	C1QTNF5
223618_at	AF225426	Hs.24889	FMN2
223672_at	AL136561	Hs.132121	SGIP1
223816_at	AF242557	Hs.512668	SLC46A2
223987_at	AF332891	Hs.432379	CHRDL2
224212_s_at	AF169689	Hs.199343	PCDHA10
224339_s_at	AB056476	Hs.591474	ANGPTL1
224403_at	AF343661	Hs.120260	FCRL4
224976_at	R37335	Hs.191911	NFIA
226069_at	AA404269	Hs.524348	PRICKLE1
226304_at	AA563621	Hs.534538	HSPB6
226408_at	AA905942	Hs.515534	TEAD2
226474_at	AA005023	Hs.528836	NLRC5
226571_s_at	N38920	Hs.712625	PTPRS
226614_s_at	BE856336	Hs.124299	FAM167A
226658_at	AW590196	Hs.468675	PDPN
226677_at	AF141339	Hs.116935	ZNF521
226766_at	AB046788	Hs.13305	ROBO2
226814_at	AI431730	Hs.656071	ADAMTS9
226828_s_at	AL040198	Hs.472566	HEYL
226847_at	BF438173	Hs.9914	FST
226971_at	AI678057	Hs.521178	CCDC136
226997_at	W74476	Hs.12680	ADAMTS12
227006_at	AA156998	Hs.631569	PPP1R14A
227058_at	AW084730	Hs.646647	C13orf33
227059_at	AI651255	Hs.444329	GPC6
227099_s_at	AW276078	Hs.714890	LOC387763
227154_at	AL566367	Hs.212511	IGSF21
227289_at	AU119437	Hs.106511	PCDH17

227300_at	AL521682	Hs.449718	TMEM119
227475_at	AI676059	Hs.591352	FOXQ1
227557_at	AI127800	Hs.474251	SCARF2
227566_at	AW085558	Hs.504352	NTM
227654_at	AI056877	Hs.372578	FAM65C
227688_at	AK022128	Hs.65366	LRCH2
227850_x_at	AW084544	Hs.415791	CDC42EP5
227923_at	BF439330	Hs.149035	SHANK3
227946_at	AI955239	Hs.463320	OSBPL7
227984_at	BE464483	Hs.371980	LOC650392
228384_s_at	AI690274	Hs.238303	C10orf33
228537_at	AI797248	Hs.111867	GLI2
228554_at	AL137566	Hs.32405	PGR
228580_at	AI828007	Hs.479119	HTRA3
228593_at	AI271425	Hs.471067	LOC339483
228608_at	N49852	Hs.525146	NALCN
228617_at	AA142842	Hs.441975	XAF1
228875_at	AI540210	Hs.126712	FAM162B
229019_at	AI694320	Hs.655005	ZNF385B
229088_at	BF591996	Hs.527295	ENPP1
229151_at	BE673587	Hs.101307	SLC14A1
229158_at	AW082836	Hs.105448	WNK4
229177_at	AI823572	Hs.11782	C16orf89
229288_at	BF439579	Hs.73962	EPHA7
229529_at	AI827830	Hs.78061	TCF21
229678_at	AA418402	Hs.380738	LOC728431
229902_at	AW083785	Hs.646917	FLT4
230015_at	AV729651	Hs.634380	PRCD
230109_at	AI638433	Hs.594417	PDE7B
230204_at	AU144114	Hs.2799	HAPLN1
230453_s_at	AW188009	Hs.513870	ATP2A3
231358_at	BE465760	Hs.30495	MRO
231406_at	AW205664	Hs.363308	ORAI2
232001_at	AW193600	Hs.590987	LOC439949
232224_at	AI274095	Hs.89983	MASP1
232313_at	AL122107	Hs.49599	TMEM132C
232570_s_at	AL356755	Hs.173716	ADAM33
232602_at	AL050348	Hs.419126	WFDC3
232645_at	AW665885	Hs.259625	LOC153684
232686_at	AI801574	Hs.132045	SIGLECP3
233092_s_at	AL133561	Hs.648234	LOC100271840
233947_s_at	U47671	Hs.567915	LOC255480
234994_at	AA088177	Hs.591341	TMEM200A
235126_at	N51468	Hs.552649	LQK1
235334_at	AW963951	Hs.337040	ST6GALNAC3
235521_at	AW137982	Hs.659337	HOXA3
235548_at	BG326592	Hs.119286	APCDD1L
235588_at	AA740849	Hs.99480	ESCO2

235874_at	AL574912	Hs.98381	PRSS35
235944_at	BF446673	Hs.58877	HMCN1
236044_at	BF130943	Hs.40479	PPAPDC1A
236141_at	AA156933	Hs.61435	NBLA00301
236738_at	AW057589	Hs.710781	LOC401097
236918_s_at	AW975772	Hs.591289	LRRC34
237721_s_at	BE220587	Hs.666357	ASB4
238370_x_at	AI252081	Hs.515329	RPL22
238542_at	AA831769	Hs.656778	ULBP2
238577_s_at	AA628481	Hs.271605	TSHZ2
238654_at	W79425	Hs.293236	LOC147645
239077_at	W81648	Hs.657569	CSGALNACT2
239286_at	AI038737	Hs.116471	CDH11
239580_at	BF724601	Hs.24258	GUCY1A3
239627_at	BG034114	Hs.279929	TMED9
239739_at	AW452218	Hs.483200	SNX24
240068_at	H08345	Hs.106234	C21orf130
241221_at	BE644691	Hs.505601	SEC14L3
241394_at	AA213799	Hs.464224	LOC284120
241926_s_at	AA296657	Hs.473819	ERG
242100_at	AI076484	Hs.213137	CHSY3
242625_at	AW189843	Hs.17518	RSAD2
243526_at	AI968904	Hs.647083	WDR86
244413_at	AW237307	Hs.560087	CLECL1
244723_at	BF510430	Hs.656497	LOC100129488
336_at	D38081	Hs.442530	TBXA2R
37408_at	AB014609	Hs.7835	MRC2
40687_at	M96789	Hs.296310	GJA4
61734_at	AI797684	Hs.567550	RCN3

Appendix 4: Scatterplot Down-regulated Genes

ProbeSet	Accession	UGCluster	Symbol
1553243_at	NM_032817	Hs.498586	ITIH5
1553333_at	NM_152367	Hs.376194	C1orf161
1553352_x_at	AF513360		ERVWE1
1553405_a_at	NM_033225	Hs.571466	CSMD1
1553698_a_at	NM_145257	Hs.715507	C1orf96
1553986_at	BC023566	Hs.657750	RASEF
1554036_at	BC036731	Hs.409876	ZBTB24
1554099_a_at	BC032490	Hs.522672	SPIN3
1554122_a_at	BC012536	Hs.132513	HSD17B12
1554195_a_at	BC021680	Hs.660038	C5orf46
1554737_at	AF193046	Hs.519294	FBN2
1554875_at	BC029359	Hs.468349	C2orf34
1554916_a_at	BC043351	Hs.535903	JRK
1555038_at	BC031042	Hs.584954	EPB41L4A
1556200_a_at	BC021140	Hs.587663	C10orf90
1557047_at	BC032368	Hs.632575	YEATS2
1557161_at	BC039503	Hs.62646	LOC100132735
1557321_a_at	AA743820	Hs.468059	CAPN14
1557636_a_at	BC031107	Hs.258357	C7orf57
1558212_at	BC004474	Hs.416043	FLJ35024
1558451_at	AK094945	Hs.435077	LOC285500
1558523_at	AJ420563	Hs.443789	FAM184A
1559254_at	BI826147	Hs.534828	NCRNA00162
1560932_at	AK055918	Hs.562970	FLJ31356
1561514_at	BC034583	Hs.579378	LOC400655
1562054_at	BF377197	Hs.350673	SMEK3P
1562966_at	BC017424	Hs.445885	KIAA1217
1563900_at	AK055204	Hs.657974	FAM83B
1563933_a_at	AK091691	Hs.672452	PLD5
1564166_s_at	AK098276	Hs.406395	PRKRIP1
1568849_at	BC009635	Hs.382029	C21orf135
1568882_at	BC035223	Hs.317243	LRTOMT
1569112_at	AW020413	Hs.654821	SLC44A5
1569464_at	BC001560	Hs.172445	PPFIBP1
1569555_at	BC012859	Hs.494163	GDA
1569723_a_at	BC011119	Hs.461786	SPIRE2
1570394_at	BC039314	Hs.435103	XRN1
201909_at	NM_001008	Hs.282376	RPS4Y1
202203_s_at	NM_001144	Hs.295137	AMFR
202966_at	NM_014289	Hs.496593	CAPN6
203589_s_at	NM_006286	Hs.379018	TFDP2
203591_s_at	NM_000760	Hs.524517	CSF3R
203815_at	NM_000853	Hs.268573	GSTT1
203824_at	NM_004616	Hs.170563	TSPAN8
203949_at	NM_000250	Hs.458272	MPO
204154_at	NM_001801	Hs.442378	CDO1

204199_at	NM_014636	Hs.648175	RALGPS1
204359_at	NM_013231	Hs.533710	FLRT2
204409_s_at	BC005248	Hs.461178	EIF1AY
204529_s_at	AI961231	Hs.491805	TOX
204548_at	NM_000349	Hs.521535	STAR
204614_at	NM_002575	Hs.594481	SERPINB2
204624_at	NM_000053	Hs.492280	ATP7B
204750_s_at	BF196457	Hs.95612	DSC2
204844_at	L12468	Hs.435765	ENPEP
205000_at	NM_004660	Hs.99120	DDX3Y
205073_at	NM_000775	Hs.152096	CYP2J2
205190_at	NM_002670	Hs.203637	PLS1
205287_s_at	NM_003222	Hs.473152	TFAP2C
205363_at	NM_003986	Hs.591996	BBOX1
205543_at	NM_014278	Hs.135554	HSPA4L
205916_at	NM_002963	Hs.112408	S100A7
205922_at	NM_004665	Hs.293130	VNN2
206239_s_at	NM_003122	Hs.407856	SPINK1
206424_at	NM_000783	Hs.150595	CYP26A1
206605_at	NM_006025	Hs.997	P11
206698_at	NM_021083	Hs.78919	XK
206700_s_at	NM_004653	Hs.80358	JARID1D
206801_at	NM_002521	Hs.219140	NPPB
206882_at	NM_005071	Hs.515217	SLC1A6
207111_at	NM_001974	Hs.2375	EMR1
207220_at	NM_021071	Hs.591158	ART4
207291_at	NM_024081	Hs.471695	PRRG4
207423_s_at	AF029899	Hs.177984	ADAM20
207790_at	NM_025168	Hs.646997	LRRC1
208607_s_at	NM_030754	Hs.1955	SAA2
209521_s_at	AF286598	Hs.528051	AMOT
209686_at	BC001766	Hs.422181	S100B
209757_s_at	BC002712	Hs.25960	MYCN
210141_s_at	M13981	Hs.407506	INHA
210172_at	D26121	Hs.502829	SF1
210437_at	BC002351	Hs.512582	MAGEA9
210548_at	U58913	Hs.169191	CCL23
210984_x_at	U95089	Hs.488293	EGFR
211149_at	AF000994	Hs.115277	UTY
211170_s_at	AF127480	Hs.348762	PDE10A
211469_s_at	U73531	Hs.34526	CXCR6
213307_at	AF131790	Hs.268726	SHANK2
213816_s_at	AA005141	Hs.132966	MET
213849_s_at	AA974416	Hs.655213	PPP2R2B
214051_at	BF677486	Hs.675540	TMSB15B
214357_at	AL035295	Hs.517991	C1orf105
214397_at	AI827820	Hs.25674	MBD2
214920_at	R33964	Hs.120855	THSD7A

214983_at	AL080135	Hs.433656	TTTY15
214998_at	AF090100	Hs.468878	AAK1
215047_at	AL080170	Hs.323858	TRIM58
215153_at	AF037070	Hs.655000	NOS1AP
215491_at	AI273812	Hs.437922	MYCL1
216213_at	AF155113	Hs.481181	NEK1
217477_at	U78581	Hs.534371	PIP5K1B
217589_at	AW300309	Hs.27453	RAB40A
218182_s_at	NM_021101	Hs.439060	CLDN1
218701_at	NM_016027	Hs.118554	LACTB2
219263_at	NM_024539	Hs.496542	RNF128
219304_s_at	NM_025208	Hs.352298	PDGFD
219424_at	NM_005755	Hs.501452	EBI3
219756_s_at	NM_024921	Hs.267038	POF1B
219768_at	NM_024626	Hs.546434	VTCN1
219814_at	NM_018388	Hs.105134	MBNL3
220100_at	NM_018484	Hs.220844	SLC22A11
220196_at	NM_024690	Hs.432676	MUC16
220410_s_at	NM_018627	Hs.522493	CAMSAP1
220528_at	NM_018399	Hs.183656	VNN3
222456_s_at	BF197289	Hs.525419	LIMA1
224168_at	AL136742	Hs.98712	TXNDC2
224482_s_at	BC006240	Hs.406788	RAB11FIP4
226665_at	AI986239	Hs.655602	AHSA2
227128_s_at	AI345950	Hs.23582	TACSTD2
227174_at	Z98443	Hs.122125	WDR72
227241_at	R79759	Hs.407152	MUC15
228212_at	AL574699	Hs.29742	ISM2
228236_at	AA903862	Hs.283865	C20orf54
228492_at	AV681765	Hs.598540	USP9Y
229215_at	AI393930	Hs.152475	ASCL2
229403_at	AI572046	Hs.272011	B4GALT1
229546_at	AI378035	Hs.657296	LOC653602
229599_at	AA675917	Hs.390599	LOC440335
230760_at	BF592062	Hs.522845	ZFY
230763_at	AA905508	Hs.171130	SPATA17
230792_at	BE671210	Hs.496205	FAAH2
230863_at	R73030	Hs.657729	LRP2
230882_at	AA129217	Hs.34969	FLJ34048
230910_s_at	AI828018	Hs.69517	LY6K
231186_at	AI184196	Hs.445241	FLJ43390
231240_at	AI038059	Hs.202354	DIO2
231569_at	N58489	Hs.98843	TMEM31
232056_at	AW470178	Hs.534699	SCEL
232124_at	AL117530	Hs.146346	LOC729085
232170_at	AJ243672	Hs.442337	S100A7A
232388_at	AB051550	Hs.461389	CNTNAP4
232452_at	AI808477	Hs.23491	OR2C3

232771_at	Z83850	Hs.209527	NRK
232849_at	AI761436	Hs.204945	LOC100128988
233520_s_at	AL359338	Hs.482625	CMYA5
233737_s_at	AK023548	Hs.504540	LOC284561
233830_at	AK023635	Hs.635164	LOC90246
234766_at	AF162668	Hs.504212	OR8D2
235382_at	AI246369	Hs.98288	LVRN
235942_at	AI272059	Hs.568127	LOC401629
236255_at	BG026457	Hs.535800	PLEKHG4B
236518_at	BE208843	Hs.370555	C9orf86
236707_at	AA521016	Hs.436271	DAPP1
237229_at	AI268287	Hs.145717	JMJD5
237265_at	BF062257	Hs.662737	C16orf73
237449_at	BF447038	Hs.195922	SP8
237577_at	BE465316	Hs.545311	PCNP
238263_at	AW590543	Hs.642649	LOC285965
238763_at	AI539118	Hs.715766	RBM20
238778_at	AI244661	Hs.499159	MPP7
238790_at	BE738988	Hs.659158	LOC374443
239186_at	AI347139	Hs.8162	MGC39372
239243_at	AA279654	Hs.434401	ZNF638
239398_at	AI743156	Hs.131064	KLHL31
239430_at	AA195677	Hs.546554	IGFL1
239921_at	AA995791	Hs.491104	COL28A1
240091_at	AI001156	Hs.464813	PSMA8
240189_at	BF064226	Hs.253320	ACOXL
241111_at	AI032819	Hs.364941	HSD3B1
241782_at	AI932350	Hs.5025	NEBL
241881_at	N54813	Hs.269151	OR2W3
241931_at	AI168338	Hs.179675	XG
243541_at	AI123586	Hs.55378	IL31RA
243683_at	H43976	Hs.326387	MORF4L2
243709_at	BG054799	Hs.649685	SLC38A9
244198_at	AA885461	Hs.410810	RANBP17
244374_at	N39767	Hs.515575	PLAC2
244385_at	AA766126	Hs.709425	JMJD2C
244802_at	AA909218	Hs.500409	GLUD1

Appendix 5: Differentially expressed genes among EHM and Control classes

	ProbeSet	Symbol	P-value	Fold-change
1	220579_at	FLJ14100	0.0003589	1.44
2	221267_s_at	FAM108A1	0.0011862	1.24
3	235409_at	MGA	0.001332	0.7
4	201407_s_at	PPP1CB	0.0014617	0.91
5	217358_at	DNAJC16	0.0017172	0.82
6	202076_at	BIRC2	0.0023647	0.93
7	224151_s_at	AK3	0.0026289	0.82
8	205987_at	CD1C	0.002642	0.75
9	34689_at	TREX1	0.0027493	1.15
10	220410_s_at	CAMSAP1	0.002781	0.66
11	238790_at	LOC374443	0.0028797	0.63
12	214540_at	HIST1H2BO	0.0028801	0.77
13	1563933_a_at	PLD5	0.0029399	0.56
14	207423_s_at	ADAM20	0.0030885	0.65
15	212649_at	DHX29	0.0030945	0.86
16	236329_at	FLJ33996	0.0031796	1.31
17	244565_at	HMX2	0.0032257	1.24
18	225210_s_at	FAM103A1	0.0032481	0.82
19	1564240_at	LOC100130856	0.003337	1.45
20	237229_at	JMJD5	0.0034946	0.62
21	216203_at	SPTLC2	0.0035257	0.75
22	215248_at	GRB10	0.0035407	0.67
23	243406_at	LOC440268	0.0036221	1.21
24	207959_s_at	DNAH9	0.0036241	1.21
25	230015_at	PRCD	0.0037443	1.7
26	243238_at	PYGB	0.0037485	1.26
27	212420_at	ELF1	0.0039983	0.84
28	229941_at	FAM166B	0.0042202	0.9
29	216213_at	NEK1	0.004344	0.59
30	217486_s_at	ZDHHC17	0.0044159	0.67
31	237205_at	C14orf53	0.0044451	1.15
32	238600_at	JAKMIP1	0.0044965	1.31
33	240827_at	FLJ45983	0.0046887	0.78
34	232318_s_at	LOC121838	0.0047704	0.73
35	1553756_at	C9orf70	0.0049188	1.28
36	231667_at	SLC39A5	0.0049354	1.18
37	223120_at	FUCA2	0.0050835	0.93
38	220947_s_at	TBC1D10B	0.0052046	0.89
39	222438_at	MED4	0.0052299	0.82
40	232001_at	LOC439949	0.005269	1.93
41	229542_at	C20orf85	0.0054122	1.24
42	217803_at	GOLPH3	0.0054213	0.88
43	224827_at	UBTD2	0.0055323	0.89
44	212185_x_at	MT2A	0.0056176	1.1
45	228621_at	HFE2	0.0058128	0.87

46	205500_at	C5	0.0062228	1.59
47	204638_at	ACP5	0.0062257	1.34
48	203250_at	RBM16	0.0062952	0.93
49	224069_x_at	P2RX2	0.0063464	1.3
50	202371_at	TCEAL4	0.0064324	0.81
51	218171_at	VPS4B	0.0065387	0.85
52	236918_s_at	LRRC34	0.0065704	1.6
53	205553_s_at	CSRP3	0.0065788	1.14
54	1566363_at	DNTT	0.0067486	0.78
55	229860_x_at	C4orf48	0.0069419	1.26
56	203167_at	TIMP2	0.0071573	1.26
57	218529_at	CD320	0.0071899	1.29
58	204617_s_at	ACD	0.0071951	1.21
59	244119_at	LOC283483	0.0073746	0.85
60	223579_s_at	APOB	0.0073762	1.12
61	228154_at	C19orf44	0.0074435	0.8
62	1561232_at	LOC100270680	0.007454	1.11
63	230012_at	C17orf44	0.007469	1.41
64	209300_s_at	NECAP1	0.0076702	0.87
65	46142_at	LMF1	0.007714	1.42
66	1558795_at	LOC728052	0.0079144	0.87
67	222742_s_at	RABL5	0.0081637	1.22
68	238644_at	MYSM1	0.0082074	0.74
69	239585_at	KAT2B	0.0084524	0.84
70	223430_at	SIK2	0.0084569	1.16
71	208308_s_at	GPI	0.008541	1.13
72	225603_s_at	C8orf83	0.0086517	0.83
73	1555343_at	MEGF10	0.0088899	1.29
74	209523_at	TAF2	0.0089522	0.86
75	204548_at	STAR	0.0089635	0.66
76	215452_x_at	SUMO4	0.0090376	0.89
77	240784_at	C7orf52	0.0090543	0.86
78	1554801_at	C5orf40	0.0094134	0.88
79	207403_at	IRS4	0.0095524	1.24
80	203593_at	CD2AP	0.0095917	0.87
81	236283_x_at	LOC646214	0.0096327	0.87
82	206875_s_at	SLK	0.0098347	0.87
83	1559682_at	TRIM16L	0.0099596	1.27
84	221336_at	ATOH1	0.0100868	0.9
85	221220_s_at	SCYL2	0.010151	0.68
86	233681_at	KRTAP3-3	0.0106159	0.87
87	203614_at	UTP14C	0.0112371	0.94
88	206355_at	GNAL	0.0113893	1.39
89	221005_s_at	PTDSS2	0.0115387	1.18
90	210033_s_at	SPAG6	0.0115842	0.7
91	1570394_at	XRN1	0.0115887	0.56
92	203686_at	MPG	0.0116844	1.17
93	220022_at	ZNF334	0.0119879	0.78

94	204992_s_at	PFN2	0.012005	0.91
95	201348_at	GPX3	0.0122545	0.78
96	220971_at	IL25	0.0122726	0.85
97	216551_x_at	PLCG1	0.0122809	1.26
98	235261_at	UNC45B	0.0123232	1.25
99	230993_s_at	C6orf118	0.0123761	0.91
100	234617_at	OR52D1	0.0126402	0.85
101	210406_s_at	RAB6C	0.0127851	0.95
102	1564974_at	KRTAP8-1	0.0129876	1.21
103	206842_at	KCND1	0.0130475	0.86
104	200661_at	CTSA	0.0130818	1.19
105	236033_at	ASB12	0.0130968	0.83
106	234226_at	OPN4	0.0131863	0.9
107	237056_at	INSC	0.0132595	1.4
108	223816_at	SLC46A2	0.0133179	1.8
109	1563685_at	LOC285422	0.0133496	0.8
110	222705_s_at	SLC25A15	0.0134727	0.72
111	227120_at	FOXP4	0.0134956	1.33
112	216380_x_at	RPS28P6	0.0135994	1.13
113	233353_at	FER1L5	0.013662	1.28
114	243683_at	MORF4L2	0.0137011	0.46
115	1557296_at	FLJ12825	0.0139896	1.27
116	244132_x_at	ZNF518A	0.0140383	0.8
117	1558622_a_at	ZNF548	0.0140483	1.23
118	203982_s_at	ABCD4	0.0141782	1.19
119	238978_at	LMBRD2	0.0143239	0.7
120	225193_at	KIAA1967	0.0143674	0.75
121	210057_at	SMG1	0.0144536	0.71
122	238833_at	LOC729088	0.0144885	1.36
123	231791_at	ASAH2B	0.0146016	0.68
124	202600_s_at	NRIP1	0.0149217	0.71
125	212682_s_at	LMF2	0.015119	1.19
126	1554882_at	ERCC8	0.0152081	0.71
127	241736_at	FBXW2	0.0152111	0.74
128	206427_s_at	MLANA	0.0152622	1.16
129	1553106_at	C5orf24	0.0153748	0.75
130	227946_at	OSBPL7	0.0153901	1.76
131	1564660_at	LOC100131864	0.0154189	1.09
132	213303_x_at	ZBTB7A	0.0154385	1.13
133	215244_at	DGCR5	0.0157839	1.28
134	209112_at	CDKN1B	0.0158879	0.88
135	209752_at	REG1A	0.0159607	1.22
136	229458_s_at	GALK1	0.0161022	1.24
137	208551_at	HIST1H4G	0.0161741	0.85
138	227617_at	TMEM201	0.0161898	1.14
139	232810_at	AIG1	0.016286	0.73
140	216088_s_at	PSMA7	0.0163512	1.11
141	206505_at	UGT2B4	0.0163536	0.9

142	222259_s_at	SPO11	0.0164153	1.16
143	225024_at	RPRD1B	0.0167583	0.88
144	216921_s_at	KRT35	0.0168356	1.15
145	224252_s_at	FXYD5	0.016973	1.19
146	205775_at	FAM50B	0.0170546	1.43
147	214432_at	ATP1A3	0.0171428	1.19
148	214903_at	SYT2	0.0171937	0.9
149	1567060_at	OR8G1	0.0173169	0.75
150	238577_s_at	TSHZ2	0.0173521	2.59
151	237619_at	C6orf146	0.01744	1.12
152	209705_at	MTF2	0.0175313	0.87
153	216699_s_at	KLK1	0.0177079	1.2
154	228026_at	RP5-1000E10.4	0.0177405	0.84
155	32209_at	FAM89B	0.0177597	1.2
156	240521_at	NCRNA00153	0.0178521	1.4
157	232486_at	LRFN1	0.0180814	1.14
158	215959_at	PPFIBP2	0.0182769	0.78
159	241256_at	LOC100131283	0.0183674	1.21
160	206424_at	CYP26A1	0.0184207	0.6
161	1561384_a_at	LOC284661	0.0185284	1.17
162	223273_at	C14orf142	0.0186446	0.8
163	205140_at	FPGT	0.0189011	0.84
164	233344_x_at	KIAA1875	0.0191119	0.86
165	1564435_a_at	KRT72	0.0191456	1.18
166	223780_s_at	MED13	0.0192048	0.68
167	214161_at	OSGIN2	0.0192178	0.76
168	241599_at	LSM11	0.0192427	1.5
169	1562209_at	WDR21B	0.0192807	0.75
170	226617_at	ARL5A	0.0196498	0.87
171	227637_at	TFCP2	0.0196867	0.76
172	219695_at	SMPD3	0.0200129	0.88
173	208745_at	ATP5L	0.0200659	0.88
174	231783_at	CHRM1	0.0202329	0.8
175	225028_at	LOC550643	0.0203	0.82
176	214995_s_at	APOBEC3F	0.0203374	1.25
177	204382_at	NAT9	0.0204292	1.11
178	227005_at	RPP14	0.0206063	0.87
179	226390_at	STARD4	0.0206713	0.9
180	1557146_a_at	LOC146336	0.0207754	0.87
181	200943_at	HMGN1	0.0208001	0.9
182	219273_at	CCNK	0.0208133	0.73
183	1553246_a_at	SGK196	0.0210413	0.77
184	220217_x_at	SPANXC	0.0211315	1.13
185	219443_at	TASP1	0.0213244	0.8
186	242685_at	GTPBP8	0.0214123	0.77
187	209361_s_at	PCBP4	0.0215662	1.39
188	217269_s_at	PRSS7	0.0215683	1.34
189	230418_s_at	GALNTL1	0.021664	1.21

190	206080_at	PLCH2	0.021834	1.15
191	206358_at	PRM1	0.0219524	1.19
192	206872_at	SLC17A1	0.0220032	1.08
193	223754_at	MGC13057	0.0220641	0.72
194	215790_at	AJAP1	0.0220801	1.31
195	1552751_a_at	CIB3	0.0221359	0.82
196	1560932_at	FLJ31356	0.0222339	0.66
197	232039_at	KIAA1383	0.0222371	0.85
198	212440_at	SNRNP27	0.0224666	0.92
199	223752_at	CFC1	0.02253	1.17
200	220223_at	ATAD5	0.0227945	1.27
201	223506_at	ZC3H8	0.022919	0.87
202	238163_at	LOC100133229	0.0231235	1.18
203	1566524_a_at	hCG_1986447	0.0231327	0.68
204	206605_at	P11	0.023154	0.64
205	208093_s_at	NDEL1	0.0237543	0.95
206	206967_at	CCNT1	0.0239101	0.78
207	205948_at	PTPRT	0.0239316	1.26
208	1552602_at	CACNG5	0.0240234	0.78
209	209323_at	PRKRIR	0.0240316	0.92
210	232291_at	MIRHG1	0.0240519	0.85
211	205765_at	CYP3A5	0.0241927	0.9
212	244672_at	WDR1	0.0242403	0.85
213	203413_at	NELL2	0.0242406	0.83
214	1555330_at	GCLC	0.0243718	0.76
215	1564166_s_at	PRKRIP1	0.024386	0.65
216	223330_s_at	SUGT1	0.0245407	0.82
217	206151_x_at	ELA3B	0.024579	1.14
218	205350_at	CRABP1	0.0246988	1.42
219	226268_at	RAB21	0.0247677	0.87
220	213838_at	NOL7	0.024782	0.83
221	233229_at	SCFD1	0.0251655	0.7
222	237449_at	SP8	0.0251984	0.65
223	228139_at	RIPK3	0.0252366	1.32
224	212844_at	RRP1B	0.025475	0.87
225	238586_at	LOC731489	0.025525	1.11
226	210454_s_at	KCNJ6	0.0255429	1.37
227	208548_at	IFNA6	0.0255546	1.17
228	220627_at	CST8	0.0258015	1.22
229	236514_at	ACOT8	0.0258242	0.8
230	238893_at	LOC338758	0.0258614	1.49
231	209593_s_at	TOR1B	0.0258656	1.07
232	243362_s_at	LOC641518	0.0258671	1.41
233	219171_s_at	ZNF236	0.025871	0.78
234	221641_s_at	ACOT9	0.0261025	1.07
235	238135_at	AGTRAP	0.0261472	0.8
236	226167_at	SYT7	0.0261505	1.36
237	205475_at	SCRG1	0.0263258	2

238	34225_at	WHSC2	0.026431	0.84
239	1563581_at	LOC285456	0.0264972	1.32
240	1553314_a_at	KIF19	0.0265424	0.85
241	220224_at	HAO1	0.0265452	0.86
242	226800_at	EFCAB7	0.0265954	0.87
243	1555238_at	PTH2	0.0266333	1.13
244	229566_at	LOC645638	0.0266682	0.73
245	216607_s_at	CYP51A1	0.0267076	1.33
246	209937_at	TM4SF4	0.0267185	1.23
247	241779_at	MTX3	0.0268559	0.77
248	238016_s_at	LOC100129105	0.0268726	1.1
249	201574_at	ETF1	0.0270324	0.91
250	232907_at	UBR4	0.0270394	0.69
251	1562966_at	KIAA1217	0.027046	0.6
252	229094_at	LOC401431	0.0270462	1.22
253	226962_at	ZBTB41	0.0270543	0.82
254	227365_at	ATCAY	0.0273438	0.93
255	230763_at	SPATA17	0.0273701	0.61
256	231731_at	OTX2	0.0274355	0.88
257	218760_at	COQ6	0.0276837	1.11
258	205364_at	ACOX2	0.0277763	1.61
259	225235_at	TSPAN17	0.0277944	1.17
260	1554339_a_at	COG3	0.0279185	0.72
261	225580_at	MRPL50	0.0279466	0.85
262	208440_at	C3orf27	0.0280131	1.15
263	208578_at	SCN10A	0.0280748	1.2
264	218174_s_at	C10orf57	0.0281064	0.84
265	227358_at	ZBTB46	0.0281615	1.24
266	235310_at	GCET2	0.028259	0.79
267	1558027_s_at	PRKAB2	0.0282756	0.88
268	237721_s_at	ASB4	0.0283536	1.58
269	205911_at	PTH1R	0.0283936	1.28
270	234979_at	BCDIN3D	0.0284326	0.81
271	233532_x_at	IFT52	0.0285195	1.19
272	208040_s_at	MYBPC3	0.0287075	0.89
273	220235_s_at	C1orf103	0.0287579	0.77
274	239492_at	SEC14L4	0.0287998	1.4
275	230839_at	PRMT8	0.0289504	1.16
276	209668_x_at	CES2	0.0292073	1.13
277	204951_at	RHOH	0.0292183	1.62
278	211646_at	LOC100126583	0.0295025	0.88
279	203924_at	GSTA2	0.0295472	1.3
280	218297_at	C10orf97	0.0295589	0.84
281	208953_at	LARP5	0.0296576	0.84
282	214411_x_at	CTRB2	0.0297295	0.79
283	207844_at	IL13	0.0297325	0.86
284	1553193_at	ZNF441	0.0303803	0.73
285	210753_s_at	EPHB1	0.0303997	1.47

286	229422_at	NRD1	0.0304688	0.71
287	200627_at	PTGES3	0.030551	0.93
288	231765_at	ZFYVE20	0.030568	1.25
289	242284_at	LOC199899	0.0305789	1.21
290	203965_at	USP20	0.0305844	1.14
291	219916_s_at	RNF39	0.0306563	1.32
292	212339_at	EPB41L1	0.0307202	1.18
293	206578_at	NKX2-5	0.0307973	1.21
294	216460_at	FLJ00049	0.030816	1.23
295	210873_x_at	APOBEC3A	0.0308197	1.11
296	1559561_at	FBXO18	0.0308873	1.18
297	206411_s_at	ABL2	0.0309285	1.36
298	1564000_at	ANKRD31	0.031043	0.84
299	238061_at	LGI3	0.0310584	0.83
300	235588_at	ESCO2	0.0314097	1.7
301	210556_at	NFATC3	0.0317492	1.39
302	242441_at	LOC646548	0.0318012	0.88
303	221966_at	GPR137	0.0318496	0.86
304	227720_at	ANKRD13B	0.032017	1.33
305	244175_at	RP3-439I14.1	0.0320294	1.11
306	205271_s_at	CCRK	0.0320322	0.84
307	244198_at	RANBP17	0.0320327	0.6
308	1556266_a_at	LOC400831	0.0320601	1.34
309	243916_x_at	UBLCP1	0.0321357	0.89
310	227601_at	METTL14	0.0323088	0.88
311	230141_at	ARID4A	0.0323968	0.82
312	232570_s_at	ADAM33	0.0324026	1.7
313	1553191_at	DST	0.0326154	0.71
314	208259_x_at	IFNA7	0.0326415	0.87
315	215112_x_at	MCF2L2	0.0326861	1.11
316	218299_at	C11orf24	0.0327883	1.13
317	220077_at	CCDC134	0.0328582	1.14
318	234025_at	LOC100129890	0.0329924	1.28
319	223928_s_at	GUCA1C	0.0331593	0.85
320	235866_at	C9orf85	0.033266	0.72
321	228782_at	SCGB3A2	0.0333221	0.88
322	215324_at	SEMA3D	0.0333565	0.84
323	228001_at	TMEM50B	0.0334288	1.2
324	201822_at	TIMM17A	0.0334318	0.74
325	243268_at	PATE2	0.0337116	0.85
326	222792_s_at	CCDC59	0.0337128	1.16
327	230136_at	LOC400099	0.0337718	0.8
328	219833_s_at	EFHC1	0.0338373	1.24
329	242883_at	OTOS	0.0338653	0.91
330	217620_s_at	PIK3CB	0.0338695	0.7
331	1556133_s_at	LOC100169752	0.0338836	1.12
332	204501_at	NOV	0.0339183	1.18
333	237120_at	KRT77	0.0339548	1.05

334	233171_at	GRIN3A	0.0340149	0.84
335	224846_at	SHKBP1	0.0340186	1.16
336	213322_at	C6orf130	0.0340868	0.89
337	224110_at	LOC100133319	0.0342204	1.49
338	242210_at	ZNF24	0.034362	0.78
339	236421_at	ANKRD45	0.0343994	0.86
340	239198_at	EZH1	0.0345462	0.75
341	237513_at	TRYX3	0.0346088	0.9
342	205044_at	GABRP	0.0346971	0.81
343	1567238_at	OR2L2	0.0349928	0.77
344	1553822_at	RTP1	0.035195	1.21
345	217515_s_at	CACNA1S	0.0352024	0.87
346	212160_at	XPOT	0.0352788	0.89
347	1569436_at	LOC400128	0.0353156	1.38
348	204030_s_at	SCHIP1	0.0355554	0.91
349	233913_at	WFDC10A	0.035772	0.78
350	205029_s_at	FABP7	0.0359774	0.8
351	235707_at	LOC221710	0.0360783	0.74
352	1552990_at	LOC100133695	0.0363598	0.81
353	239143_x_at	RNF138	0.0363651	0.69
354	218861_at	RNF25	0.0367013	1.15
355	215491_at	MYCL1	0.0368251	0.62
356	214554_at	HIST1H2AL	0.0368598	1.22
357	216937_s_at	RS1	0.0369858	0.76
358	219101_x_at	ABHD8	0.0370082	0.9
359	206612_at	CACNG1	0.0370709	0.85
360	200025_s_at	RPL27	0.0370973	0.96
361	243927_x_at	KIAA1429	0.0371189	0.86
362	1558584_at	UBL4B	0.0371305	0.85
363	1563696_at	HSD17B4	0.0375333	0.83
364	224436_s_at	NIPSNAP3A	0.0376867	0.81
365	1556472_s_at	SCML4	0.0377806	1.13
366	222949_at	NXF3	0.0378359	0.86
367	220186_s_at	PCDH24	0.0379349	1.14
368	222825_at	OTUD6B	0.0379432	0.84
369	231403_at	TRIO	0.0380465	0.81
370	1570006_at	LOC400958	0.0380896	1.33
371	204936_at	MAP4K2	0.0381501	1.19
372	237493_at	IL22RA2	0.0382551	1.16
373	231719_at	IFRG15	0.0383874	0.67
374	224311_s_at	CAB39	0.0384198	0.86
375	205984_at	CRHBP	0.0385009	1.31
376	219632_s_at	TRPV1	0.0385879	1.25
377	224242_at	GALP	0.0386116	1.12
378	223030_at	TRAF7	0.0386461	0.84
379	218164_at	SPATA20	0.0387422	1.31
380	217378_x_at	LOC100130100	0.0389593	1.11
381	226654_at	MUC12	0.038983	1.34

382	225949_at	NRBP2	0.0389924	1.31
383	217731_s_at	ITM2B	0.0390139	0.91
384	207694_at	POU3F4	0.0392589	1.14
385	211339_s_at	ITK	0.0392954	0.87
386	223740_at	C6orf59	0.0393114	1.15
387	1562388_at	LOC285819	0.0393585	1.11
388	219345_at	BOLA1	0.0393812	1.23
389	224678_at	KIAA1219	0.0394098	0.88
390	220116_at	KCNN2	0.0394641	1.56
391	202941_at	NDUFV2	0.0395118	1.07
392	1560147_at	LOC100131176	0.0395294	1.19
393	203749_s_at	RARA	0.0395419	1.3
394	235469_at	FAM133B	0.0395577	0.76
395	1557571_at	VPS13D	0.0397487	1.18
396	230341_x_at	ADAMTS10	0.0397793	1.4
397	1559627_at	LOC285941	0.0398172	1.12
398	1556200_a_at	C10orf90	0.0398429	0.59
399	203253_s_at	HISPPD1	0.0398832	0.87
400	213589_s_at	B3GNTL1	0.0399597	1.44
401	205716_at	SLC25A40	0.0399974	0.81
402	205839_s_at	BZRAP1	0.0400035	1.42
403	210720_s_at	NECAB3	0.0400495	1.28
404	211147_s_at	P2RX6	0.0403632	0.77
405	215503_at	SPINT3	0.0403652	0.88
406	1560806_at	LOC150527	0.0405062	1.29
407	207849_at	IL2	0.0405218	1.11
408	220972_s_at	KRTAP9-9	0.0406294	0.92
409	210175_at	C2orf3	0.0406319	0.69
410	204611_s_at	PPP2R5B	0.0406393	1.29
411	232373_at	NOXA1	0.0406835	1.08
412	239243_at	ZNF638	0.0407022	0.64
413	211116_at	SLC9A2	0.040738	1.17
414	231068_at	SLC47A2	0.0407413	1.09
415	208479_at	KCNA1	0.0408819	1.24
416	237288_at	TGM7	0.0409264	0.87
417	223398_at	C9orf89	0.0409987	1.16
418	209497_s_at	RBM4B	0.0410305	0.93
419	1570128_at	DDX19A	0.0411041	0.84
420	244694_at	IGLON5	0.0411146	1.45
421	222040_at	LOC728844	0.0411901	0.84
422	225607_at	CCDC43	0.0412916	0.93
423	219359_at	ATHL1	0.0412942	1.6
424	1558292_s_at	PIGW	0.0413222	0.83
425	232143_at	DNM1P41	0.0413762	1.26
426	213526_s_at	LIN37	0.0413856	1.11
427	225844_at	POLE4	0.0413904	1.15
428	225455_at	TADA1L	0.0414091	0.85
429	238149_at	ZNF818P	0.0414375	0.73

430	207557_s_at	RYR2	0.0415176	1.48
431	221240_s_at	B3GNT4	0.0415369	1.23
432	1564386_at	TXNDC8	0.041683	0.85
433	241955_at	HECTD1	0.0418456	0.77
434	219369_s_at	OTUB2	0.0420378	0.75
435	225277_at	SLC39A13	0.042045	1.16
436	212589_at	RRAS2	0.0420794	0.86
437	220573_at	KLK14	0.0422841	0.86
438	239802_at	SAP30L	0.0423883	0.75
439	221392_at	TAS2R4	0.0423904	0.76
440	1555240_s_at	GNG12	0.042565	0.78
441	218103_at	FTSJ3	0.0425963	1.09
442	232366_at	KIAA0232	0.0426389	0.78
443	205720_at	POMC	0.042663	0.85
444	207021_at	ZPBP	0.0428135	1.1
445	224553_s_at	TNFRSF18	0.0429597	0.81
446	209151_x_at	TCF3	0.0429873	1.24
447	219095_at	JMJD7-PLA2G4B	0.0431288	1.17
448	243311_at	DEFB132	0.0431385	0.9
449	219730_at	MED18	0.0432211	1.2
450	1563137_at	MGAT5B	0.0432279	0.87
451	1554875_at	C2orf34	0.0432354	0.6
452	214226_at	POL3S	0.0434023	1.22
453	216176_at	HCRP1	0.0434029	1.77
454	1554916_a_at	JRK	0.043474	0.67
455	211152_s_at	HTRA2	0.0437694	1.09
456	225076_s_at	ZNFX1	0.0437904	1.21
457	226325_at	ADSSL1	0.0438138	1.25
458	241963_at	ZNF704	0.0439061	1.33
459	229690_at	FAM109A	0.0439114	1.15
460	205447_s_at	MAP3K12	0.0439787	1.91
461	218458_at	GMCL1	0.0440709	0.81
462	207830_s_at	PPP1R8	0.0441136	0.95
463	222759_at	SUV420H1	0.0443464	0.82
464	203765_at	GCA	0.0444953	0.85
465	237021_at	LOC144486	0.0445005	1.15
466	207972_at	GLRA1	0.044605	1.17
467	226493_at	KCTD18	0.0447031	0.71
468	235619_at	LOC285986	0.0447732	1.33
469	202957_at	HCLS1	0.0448217	0.9
470	231197_at	PPP1R3F	0.0449499	0.8
471	228384_s_at	C10orf33	0.0451921	1.53
472	215567_at	FCF1	0.0453041	0.88
473	208244_at	BMP3	0.0454621	1.15
474	222817_at	HSD3B7	0.0454939	1.15
475	201467_s_at	NQO1	0.0455492	1.4
476	230076_at	PITPNM3	0.0455623	1.23

477	208843_s_at	GORASP2	0.0455965	0.83
478	239894_at	LOC100128511	0.0456483	0.82
479	1561169_at	LOC727818	0.0456823	1.2
480	1555947_at	FAM120A	0.0457091	0.71
481	1553267_a_at	CNOT6L	0.0457881	0.68
482	234903_at	OR2B3	0.0459378	0.89
483	213866_at	SAMD14	0.0459837	1.43
484	219011_at	PLEKHA4	0.0460468	1.41
485	1562591_a_at	OFCC1	0.0461233	1.62
486	1565728_at	LOC284630	0.0461407	1.18
487	206915_at	NKX2-2	0.0462666	1.18
488	228880_at	LOC339984	0.0465128	0.8
489	220007_at	METTL8	0.0465492	0.76
490	223823_at	KCNMB2	0.0465625	0.76
491	218513_at	C4orf43	0.0466076	0.84
492	1553525_at	NLRP13	0.0466533	1.4
493	212713_at	MFAP4	0.0467887	2.8
494	1557864_x_at	LOC100129266	0.0467995	1.31
495	229451_at	GALNT9	0.0468526	1.11
496	1564785_at	LOC196913	0.0468907	1.11
497	211170_s_at	PDE10A	0.0469142	0.55
498	242200_at	ADAMTSL5	0.0470495	1.27
499	218745_x_at	TMEM161A	0.047207	1.21
500	1569974_x_at	SEP13	0.0472981	1.14
501	212391_x_at	LOC100130107	0.0473749	0.97
502	202412_s_at	USP1	0.0474036	0.78
503	229399_at	C10orf118	0.0475045	0.81
504	236055_at	DQX1	0.0475116	0.67
505	210380_s_at	CACNA1G	0.0475193	1.29
506	220347_at	SMG6	0.0475337	1.2
507	203983_at	TSNAX	0.047564	0.94
508	1553558_at	TAS2R41	0.047615	1.23
509	219751_at	SETD6	0.0476513	0.79
510	1554309_at	EIF4G3	0.0478381	0.74
511	242427_at	WAC	0.0478621	0.88
512	236675_at	RPA1	0.0478876	0.71
513	222220_s_at	TSNAXIP1	0.0478912	1.25
514	213200_at	SYP	0.0479142	1.4
515	1569468_at	ZNF732	0.0479338	0.84
516	231070_at	IYD	0.0479356	1.13
517	206020_at	SOCS6	0.0479646	0.88
518	1559252_a_at	C20orf29	0.0479771	1.31
519	227325_at	LOC255783	0.0480529	1.23
520	225754_at	AP1G1	0.0481797	0.88
521	230759_at	SNX14	0.0482163	0.91
522	218677_at	S100A14	0.0483573	0.72
523	218411_s_at	MBIP	0.0483993	0.89
524	235129_at	PPP1R1A	0.0484194	1.31

525	221456_at	TAS2R3	0.0488843	1.12
526	210119_at	KCNJ15	0.0489331	1.95
527	209064_x_at	PAIP1	0.0489473	0.83
528	213611_at	AQP5	0.0489659	0.86
529	1555757_at	C7orf34	0.0489967	0.9
530	1552594_at	TMEM190	0.0490474	1.23
531	205014_at	FGFBP1	0.04912	0.8
532	204873_at	PEX1	0.049123	0.8
533	225883_at	ATG16L2	0.0492266	1.33
534	1554794_a_at	UBE3C	0.0492977	1.27
535	237002_at	NCDN	0.0493018	1.16
536	214237_x_at	LOC100133105	0.0493116	1.21
537	1568690_a_at	LOC728853	0.0495655	0.88
538	239594_at	LOC145837	0.0496205	1.21
539	202393_s_at	KLF10	0.0498015	0.91
540	226346_at	MEX3A	0.0499647	1.42

Appendix 6: Up-regulated HSPE Genes

Gene Symbol	Gene Name	EntrezGene	Ensembl
ACTR3	ARP3 actin-related protein 3 homolog (yeast)	10096	ENSG00000115091
ADAR	adenosine deaminase, RNA-specific	103	ENSG00000160710
AFF1	AF4/FMR2 family, member 1	4299	ENSG00000172493
AIDA	axin interactor, dorsalization associated	64853	ENSG00000186063
ANXA3	annexin A3	306	ENSG00000138772
ANXA8L2	annexin A8-like 2	244	ENSG00000186807
APP	amyloid beta (A4) precursor protein	351	ENSG00000142192
ARF1	ADP-ribosylation factor 1	375	ENSG00000143761
ARPC4	actin related protein 2/3 complex, subunit 4, 20kDa	10093	ENSG00000241553
ATP1B3	ATPase, Na+/K+ transporting, beta 3 polypeptide	483	ENSG00000069849
ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	488	ENSG00000174437
ATP6V1D	ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D	51382	ENSG00000100554
BZW1	basic leucine zipper and W2 domains 1	9689	ENSG00000082153
C15orf48	chromosome 15 open reading frame 48	84419	ENSG00000166920
CALD1	caldesmon 1	800	ENSG00000122786
CCDC6	coiled-coil domain containing 6	8030	ENSG00000108091
CCND2	cyclin D2	894	ENSG00000118971
CCT3	chaperonin containing TCP1, subunit 3 (gamma)	7203	ENSG00000163468
CD164	CD164 molecule, sialomucin	8763	ENSG00000135535
CDC42	cell division cycle 42 (GTP binding protein, 25kDa)	998	ENSG00000070831
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1026	ENSG00000124762
CKAP4	cytoskeleton-associated protein 4	10970	ENSG00000136026
CLDND1	claudin domain containing 1	56650	ENSG00000080822
CMTM6	CKLF-like MARVEL transmembrane domain containing 6	54918	ENSG00000091317
COX7B	cytochrome c oxidase subunit VIIb	1349	ENSG00000131174
CYR61	cysteine-rich, angiogenic inducer, 61	3491	ENSG00000142871
DEK	DEK oncogene	7913	ENSG00000124795
DLG5	discs, large homolog 5 (Drosophila)	9231	ENSG00000151208
DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6	10049	ENSG00000105993
DYNLRB1	dynein, light chain, roadblock-type 1	83658	ENSG00000125971
EGR1	early growth response 1	1958	ENSG00000120738
ERGIC1	endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1	57222	ENSG00000113719
ERP29	endoplasmic reticulum protein 29	10961	ENSG00000089248
ETV5	ets variant 5	2119	ENSG00000244405
FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	2207	ENSG00000158869
FDFT1	farnesyl-diphosphate farnesyltransferase 1	2222	ENSG00000079459
FLNB	filamin B, beta	2317	ENSG00000136068
GABARAP	GABA(A) receptor-associated protein	11337	ENSG00000170296
GJA1	gap junction protein, alpha 1, 43kDa	2697	ENSG00000152661
H2AFY	H2A histone family, member Y	9555	ENSG00000113648
H3F3B	H3 histone, family 3B (H3.3B)	3021	ENSG00000132475
HDGF	hepatoma-derived growth factor	3068	ENSG00000143321
HMGB3	high mobility group box 3	3149	ENSG00000029993

HN1	hematological and neurological expressed 1	51155	ENSG00000189159
HNRNPA3	heterogeneous nuclear ribonucleoprotein A3	220988	ENSG00000170144
HNRNPH3	heterogeneous nuclear ribonucleoprotein H3 (2H9)	3189	ENSG00000096746
HNRNPM	heterogeneous nuclear ribonucleoprotein M	4670	ENSG00000099783
HNRPDL	heterogeneous nuclear ribonucleoprotein D-like	9987	ENSG00000152795
HSPA1A	heat shock 70kDa protein 1A	3303	ENSG00000204389
IER2	immediate early response 2	9592	ENSG00000160888
IL24	interleukin 24	11009	ENSG00000162892
INHBC	inhibin, beta C	3626	ENSG00000175189
INSR	insulin receptor	3643	ENSG00000171105
IRS2	insulin receptor substrate 2	8660	ENSG00000185950
ITGA6	integrin, alpha 6	3655	ENSG00000091409
ITPRIPL2	inositol 1,4,5-trisphosphate receptor interacting protein-like 2	162073	ENSG00000205730
KARS	lysyl-tRNA synthetase	3735	ENSG00000065427
KHDRBS1	KH domain containing, RNA binding, signal transduction associated 1	10657	ENSG00000121774
KIF5B	kinesin family member 5B	3799	ENSG00000170759
LARP1	La ribonucleoprotein domain family, member 1	23367	ENSG00000155506
LDHB	lactate dehydrogenase B	3945	ENSG00000111716
LGALS3	lectin, galactoside-binding, soluble, 3	3958	ENSG00000131981
LHFPL2	lipoma HMGIC fusion partner-like 2	10184	ENSG00000145685
LSM4	LSM4 homolog, U6 small nuclear RNA associated (S. cerevisiae)	25804	ENSG00000130520
MAP1LC3B	microtubule-associated protein 1 light chain 3 beta	81631	ENSG00000140941
MATR3	matrin 3	9782	ENSG00000015479
MCL1	myeloid cell leukemia sequence 1 (BCL2-related)	4170	ENSG00000143384
MGEA5	meningioma expressed antigen 5 (hyaluronidase)	10724	ENSG00000198408
MLLT4	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4	4301	ENSG00000130396
MPRIP	myosin phosphatase Rho interacting protein	23164	ENSG00000133030
MRC1	mannose receptor, C type 1	4360	ENSG00000120586
MRFAP1L1	Morf4 family associated protein 1-like 1	114932	ENSG00000178988
MTPN	myotrophin	136319	ENSG00000105887
MYADM	myeloid-associated differentiation marker	91663	ENSG00000179820
NDUFB4	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa	4710	ENSG00000065518
NHP2L1	NHP2 non-histone chromosome protein 2-like 1 (S. cerevisiae)	4809	ENSG00000100138
NPTN	neuroplastin	27020	ENSG00000156642
NUB1	negative regulator of ubiquitin-like proteins 1	51667	ENSG00000013374
NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1	64710	ENSG00000069275
OXSR1	oxidative-stress responsive 1	9943	ENSG00000172939
PAFAH1B1	platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (45kDa)	5048	ENSG00000007168
PALLD	palladin, cytoskeletal associated protein	23022	ENSG00000129116
PDCD4	programmed cell death 4 (neoplastic transformation inhibitor)	27250	ENSG00000150593

PDGFA	platelet-derived growth factor alpha polypeptide	5154	ENSG00000197461
PDIA3	protein disulfide isomerase family A, member 3	2923	ENSG00000167004
PDIA4	protein disulfide isomerase family A, member 4	9601	ENSG00000155660
PFN2	profilin 2	5217	ENSG00000070087
PGAM1	phosphoglycerate mutase 1 (brain)	5223	ENSG00000171314
PHLDB2	pleckstrin homology-like domain, family B, member 2	90102	ENSG00000144824
PICALM	phosphatidylinositol binding clathrin assembly protein	8301	ENSG00000073921
PIP4K2C	phosphatidylinositol-5-phosphate 4-kinase, type II, gamma	79837	ENSG00000166908
POLR2B	polymerase (RNA) II (DNA directed) polypeptide B, 140kDa	5431	ENSG00000047315
PPIC	peptidylprolyl isomerase C (cyclophilin C)	5480	ENSG00000168938
PSMA2	proteasome (prosome, macropain) subunit, alpha type, 2	5683	ENSG00000106588,ENSG
PSMD14	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14	10213	ENSG00000115233
PSMD4	proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	5710	ENSG00000159352
PSME4	proteasome (prosome, macropain) activator subunit 4	23198	ENSG00000068878
PTGES	prostaglandin E synthase	9536	ENSG00000148344
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	5743	ENSG00000073756
PURB	purine-rich element binding protein B	5814	ENSG00000146676
RAB1A	RAB1A, member RAS oncogene family	5861	ENSG00000138069
RALA	v-ral simian leukemia viral oncogene homolog A (ras related)	5898	ENSG00000006451
RAN	RAN, member RAS oncogene family	5901	ENSG00000132341
RAP2A	RAP2A, member of RAS oncogene family	5911	ENSG00000125249
RBM39	RNA binding motif protein 39	9584	ENSG00000131051
RBMS1	RNA binding motif, single stranded interacting protein 1	5937	ENSG00000153250
RCAN1	regulator of calcineurin 1	1827	ENSG00000159200
RDH10	retinol dehydrogenase 10 (all-trans)	157506	ENSG00000121039
RPS21	ribosomal protein S21	6227	ENSG00000171858
RREB1	ras responsive element binding protein 1	6239	ENSG00000124782
RSL24D1	ribosomal L24 domain containing 1	51187	ENSG00000137876
SAR1A	SAR1 homolog A (S. cerevisiae)	56681	ENSG00000079332
SEC13	SEC13 homolog (S. cerevisiae)	6396	ENSG00000157020
SEPT2	septin 2	4735	ENSG00000168385
SF3B14	splicing factor 3B, 14 kDa subunit	51639	ENSG00000115128
SFPQ	splicing factor proline/glutamine-rich	6421	ENSG00000116560
SKP1	S-phase kinase-associated protein 1	6500	ENSG00000113558
SKP2	S-phase kinase-associated protein 2, E3 ubiquitin protein ligase	6502	ENSG00000145604
SNRPE	small nuclear ribonucleoprotein polypeptide E	6635	ENSG00000182004
SNX3	sorting nexin 3	8724	ENSG00000112335
SSB	Sjogren syndrome antigen B (autoantigen La)	6741	ENSG00000138385
SSR1	signal sequence receptor, alpha	6745	ENSG00000124783
SSR2	signal sequence receptor, beta (translocon-associated protein beta)	6746	ENSG00000163479

STAT2	signal transducer and activator of transcription 2, 113kDa	6773	ENSG00000170581
STC1	stanniocalcin 1	6781	ENSG00000159167
SYNCRIP	synaptotagmin binding, cytoplasmic RNA interacting protein	10492	ENSG00000135316
TALDO1	transaldolase 1	6888	ENSG00000177156
TAPBP	TAP binding protein (tapasin)	6892	ENSG00000231925
TARS	threonyl-tRNA synthetase	6897	ENSG00000113407
TAX1BP3	Tax1 (human T-cell leukemia virus type I) binding protein 3	30851	ENSG00000213977
TFAP2A	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	7020	ENSG00000137203
TJP1	tight junction protein 1	7082	ENSG00000104067
TM2D3	TM2 domain containing 3	80213	ENSG00000184277
TPBG	trophoblast glycoprotein	7162	ENSG00000146242
TPM2	tropomyosin 2 (beta)	7169	ENSG00000198467
TPM4	tropomyosin 4	7171	ENSG00000167460
TRIM8	tripartite motif containing 8	81603	ENSG00000171206
TSPAN13	tetraspanin 13	27075	ENSG00000106537
TUBB6	tubulin, beta 6 class V	84617	ENSG00000176014
TXNL1	thioredoxin-like 1	9352	ENSG00000091164
U2AF1	U2 small nuclear RNA auxiliary factor 1	7307	ENSG00000160201
UBE2D3	ubiquitin-conjugating enzyme E2D 3	7323	ENSG00000109332
VCAN	versican	1462	ENSG00000038427
YTHDC1	YTH domain containing 1	91746	ENSG00000083896
YTHDF2	YTH domain family, member 2	51441	ENSG00000198492
ZBTB4	zinc finger and BTB domain containing 4	57659	ENSG00000174282
ZFAND6	zinc finger, AN1-type domain 6	54469	ENSG00000086666
ZFP36L1	zinc finger protein 36, C3H type-like 1	677	ENSG00000185650

HSPE = Histamine Specific genes in PE (elevated histamine regulated significant genes also expressed consistently in PE placentae); PE = Pre-eclampsia

Appendix 7: Down-Regulated HSPE genes

Gene Symbol	Gene Name	EntrezGene	Ensembl
ABCB5	ATP-binding cassette, sub-family B (MDR/TAP), member 5	340273	ENSG00000004846
ABRA	actin-binding Rho activating protein	137735	ENSG00000174429
ACSM2B	acyl-CoA synthetase medium-chain family member 2B	348158	ENSG00000066813
ADCYAP1R1	adenylate cyclase activating polypeptide 1 (pituitary) receptor type I	117	ENSG00000078549
APOF	apolipoprotein F	319	ENSG00000175336
ARG1	arginase, liver	383	ENSG00000118520
ASB12	ankyrin repeat and SOCS box containing 12	142689	ENSG00000198881
ASB5	ankyrin repeat and SOCS box containing 5	140458	ENSG00000164122
B3GALT2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	8707	ENSG00000162630
BCL2L14	BCL2-like 14 (apoptosis facilitator)	79370	ENSG00000121380
C12orf40	chromosome 12 open reading frame 40	283461	ENSG00000180116
C14orf39	chromosome 14 open reading frame 39	317761	ENSG00000179008
C15orf43	chromosome 15 open reading frame 43	145645	ENSG00000167014
C1orf110	chromosome 1 open reading frame 110	339512	ENSG00000185860
C3orf15	chromosome 3 open reading frame 15	89876	ENSG00000183833
C6orf10	chromosome 6 open reading frame 10	10665	ENSG00000204296
C8orf34	chromosome 8 open reading frame 34	116328	ENSG00000165084
CA6	carbonic anhydrase VI	765	ENSG00000131686
CADPS	Ca++-dependent secretion activator	8618	ENSG00000163618
CCDC67	coiled-coil domain containing 67	159989	ENSG00000165325
CFTR	cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7)	1080	ENSG00000001626
CNBD1	cyclic nucleotide binding domain containing 1	168975	ENSG00000176571
CNTNAP4	contactin associated protein-like 4	85445	ENSG00000152910
CNTNAP5	contactin associated protein-like 5	129684	ENSG00000155052
CPEB2	cytoplasmic polyadenylation element binding protein 2	132864	ENSG00000137449
CRB1	crumbs homolog 1 (Drosophila)	23418	ENSG00000134376
CRYGB	crystallin, gamma B	1419	ENSG00000182187
CSN2	casein beta	1447	ENSG00000135222
DEFB126	defensin, beta 126	81623	NA
EFCAB6	EF-hand calcium binding domain 6	64800	ENSG00000186976
EMCN	endomucin	51705	ENSG00000164035
ENAM	enamelin	10117	ENSG00000132464
ESRRB	estrogen-related receptor beta	2103	ENSG00000119715
FABP6	fatty acid binding protein 6, ileal	2172	ENSG00000170231
FCRL5	Fc receptor-like 5	83416	ENSG00000143297
FLG	filaggrin	2312	ENSG00000143631
FOXA1	forkhead box A1	3169	ENSG00000129514
FSTL5	follistatin-like 5	56884	ENSG00000168843
GC	group-specific component (vitamin D binding protein)	2638	ENSG00000145321
GCM2	glial cells missing homolog 2 (Drosophila)	9247	ENSG00000124827
GLRA3	glycine receptor, alpha 3	8001	ENSG00000145451
GLYAT	glycine-N-acyltransferase	10249	ENSG00000149124
GPM6A	glycoprotein M6A	2823	ENSG00000150625

GPR17	G protein-coupled receptor 17	2840	ENSG00000144230
GPR22	G protein-coupled receptor 22	2845	ENSG00000172209
GRIN2B	glutamate receptor, ionotropic, N-methyl D-aspartate 2B	2904	ENSG00000150086
HAL	histidine ammonia-lyase	3034	ENSG00000084110
HAO1	hydroxyacid oxidase (glycolate oxidase) 1	54363	ENSG00000101323
HEPACAM2	HEPACAM family member 2	253012	ENSG00000188175
HHLA2	HERV-H LTR-associating 2	11148	ENSG00000114455
IGFBP1	insulin-like growth factor binding protein 1	3484	ENSG00000146678
INTS4	integrator complex subunit 4	92105	ENSG00000149262
IQGAP2	IQ motif containing GTPase activating protein 2	10788	ENSG00000145703
KCNK10	potassium channel, subfamily K, member 10	54207	ENSG00000100433
KLF12	Kruppel-like factor 12	11278	ENSG00000118922
KRT38	keratin 38	8687	ENSG00000171360
LAMA2	laminin, alpha 2	3908	ENSG00000196569
LHX9	LIM homeobox 9	56956	ENSG00000143355
LMO3	LIM domain only 3 (rhombotin-like 2)	55885	ENSG00000048540
LOC400891	chromosome 14 open reading frame 166B pseudogene	400891	NA
MAGEC2	melanoma antigen family C, 2	51438	ENSG00000046774
MAGEE2	melanoma antigen family E, 2	139599	ENSG00000186675
MC2R	melanocortin 2 receptor (adrenocorticotropic hormone)	4158	ENSG00000185231
MIPOL1	mirror-image polydactyly 1	145282	ENSG00000151338
MPL	myeloproliferative leukemia virus oncogene	4352	ENSG00000117400
MTMR8	myotubularin related protein 8	55613	ENSG00000102043
MYO3B	myosin IIIB	140469	ENSG00000071909
MYT1L	myelin transcription factor 1-like	23040	ENSG00000186487
NCAM1	neural cell adhesion molecule 1	4684	ENSG00000149294
NCR2	natural cytotoxicity triggering receptor 2	9436	ENSG00000096264
NDST4	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 4	64579	ENSG00000138653
NOVA1	neuro-oncological ventral antigen 1	4857	ENSG00000139910
NPBWR2	neuropeptides B/W receptor 2	2832	ENSG00000125522
OLFM3	olfactomedin 3	118427	ENSG00000118733
OPRM1	opioid receptor, mu 1	4988	ENSG00000112038
OR2J2	olfactory receptor, family 2, subfamily J, member 2	26707	ENSG00000204700
OR5V1	olfactory receptor, family 5, subfamily V, member 1	81696	ENSG00000243729
P2RY10	purinergic receptor P2Y, G-protein coupled, 10	27334	ENSG00000078589
PCDH20	protocadherin 20	64881	ENSG00000197991
PDE11A	phosphodiesterase 11A	50940	ENSG00000128655
PER3	period homolog 3 (Drosophila)	8863	ENSG00000049246
PMS1	PMS1 postmeiotic segregation increased 1 (S. cerevisiae)	5378	ENSG00000064933
POF1B	premature ovarian failure, 1B	79983	ENSG00000124429
POU1F1	POU class 1 homeobox 1	5449	ENSG00000064835
PPP1R3A	protein phosphatase 1, regulatory subunit 3A	5506	ENSG00000154415
PPP3R2	protein phosphatase 3, regulatory subunit B, beta	5535	ENSG00000188386
PREX2	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2	80243	ENSG00000046889
PTCHD1	patched domain containing 1	139411	ENSG00000165186
PTH	parathyroid hormone	5741	ENSG00000152266

PTN	pleiotrophin	5764	ENSG00000105894
RALYL	RALY RNA binding protein-like	138046	ENSG00000184672
RNF165	ring finger protein 165	494470	ENSG00000141622
SCEL	sciellin	8796	ENSG00000136155
SCML4	sex comb on midleg-like 4 (Drosophila)	256380	ENSG00000146285
SCN2B	sodium channel, voltage-gated, type II, beta subunit	6327	ENSG00000149575
SDR16C5	short chain dehydrogenase/reductase family 16C, member 5	195814	ENSG00000170786
SERPINB4	serpin peptidase inhibitor, clade B (ovalbumin), member 4	6318	ENSG00000206073
SFRP4	secreted frizzled-related protein 4	6424	ENSG00000106483
SIM1	single-minded homolog 1 (Drosophila)	6492	ENSG00000112246
SLC13A1	solute carrier family 13 (sodium/sulfate symporters), member 1	6561	ENSG00000081800
SLC17A1	solute carrier family 17 (sodium phosphate), member 1	6568	ENSG00000124568
SLC17A8	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 8	246213	ENSG00000179520
SLC26A7	solute carrier family 26, member 7	115111	ENSG00000147606
SLC5A7	solute carrier family 5 (choline transporter), member 7	60482	ENSG00000115665
SOX6	SRY (sex determining region Y)-box 6	55553	ENSG00000110693
SRP72	signal recognition particle 72kDa	6731	ENSG00000174780
TAS2R16	taste receptor, type 2, member 16	50833	ENSG00000128519
TCTE3	t-complex-associated-testis-expressed 3	6991	ENSG00000184786
TMED5	transmembrane emp24 protein transport domain containing 5	50999	ENSG00000117500
TMEM67	transmembrane protein 67	91147	ENSG00000164953
TPPP2	tubulin polymerization-promoting protein family member 2	122664	ENSG00000179636
TRDN	triadin	10345	ENSG00000186439
TRPC5	transient receptor potential cation channel, subfamily C, member 5	7224	ENSG00000072315
TRPM1	transient receptor potential cation channel, subfamily M, member 1	4308	ENSG00000134160
TSHR	thyroid stimulating hormone receptor	7253	ENSG00000165409
UGT2A3	UDP glucuronosyltransferase 2 family, polypeptide A3	79799	ENSG00000135220
UNC5C	unc-5 homolog C (C. elegans)	8633	ENSG00000182168
UPP2	uridine phosphorylase 2	151531	ENSG00000007001
VASH2	vasohibin 2	79805	ENSG00000143494
ZDHHC15	zinc finger, DHHC-type containing 15	158866	ENSG00000102383
ZNF407	zinc finger protein 407	55628	ENSG00000215421
ZNF780B	zinc finger protein 780B	163131	ENSG00000128000

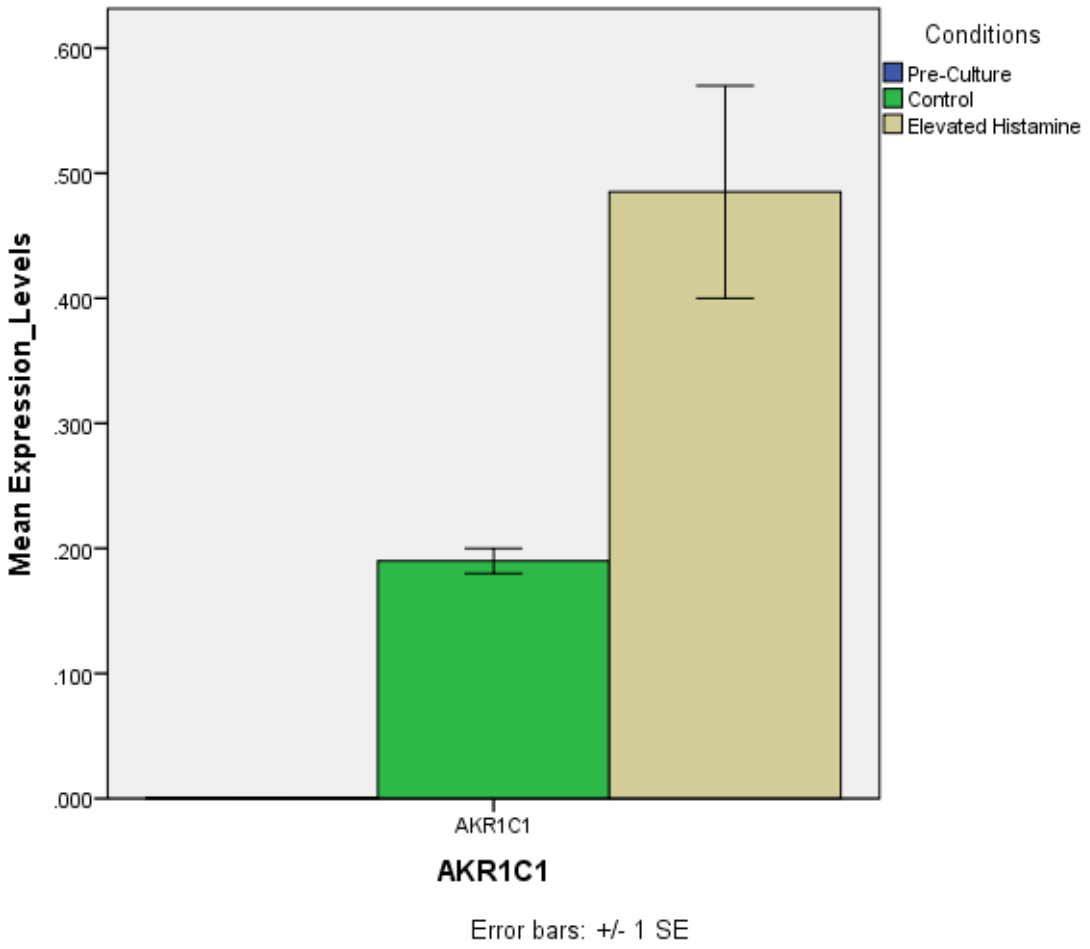
HSPE = Histamine Specific genes in PE (elevated histamine regulated significant genes also expressed consistently in PE placentae); PE = Pre-eclampsia

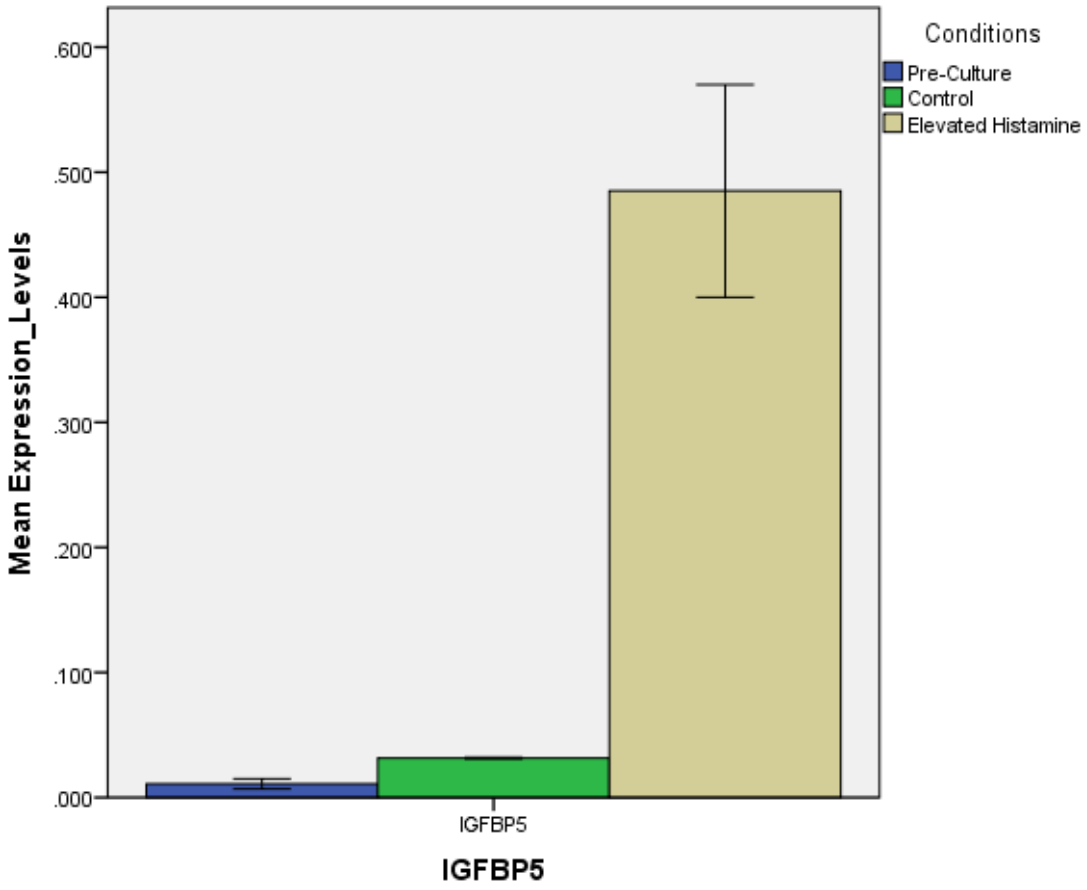
Appendix 8: Validation Genes Primer sequences for RT-qPCR

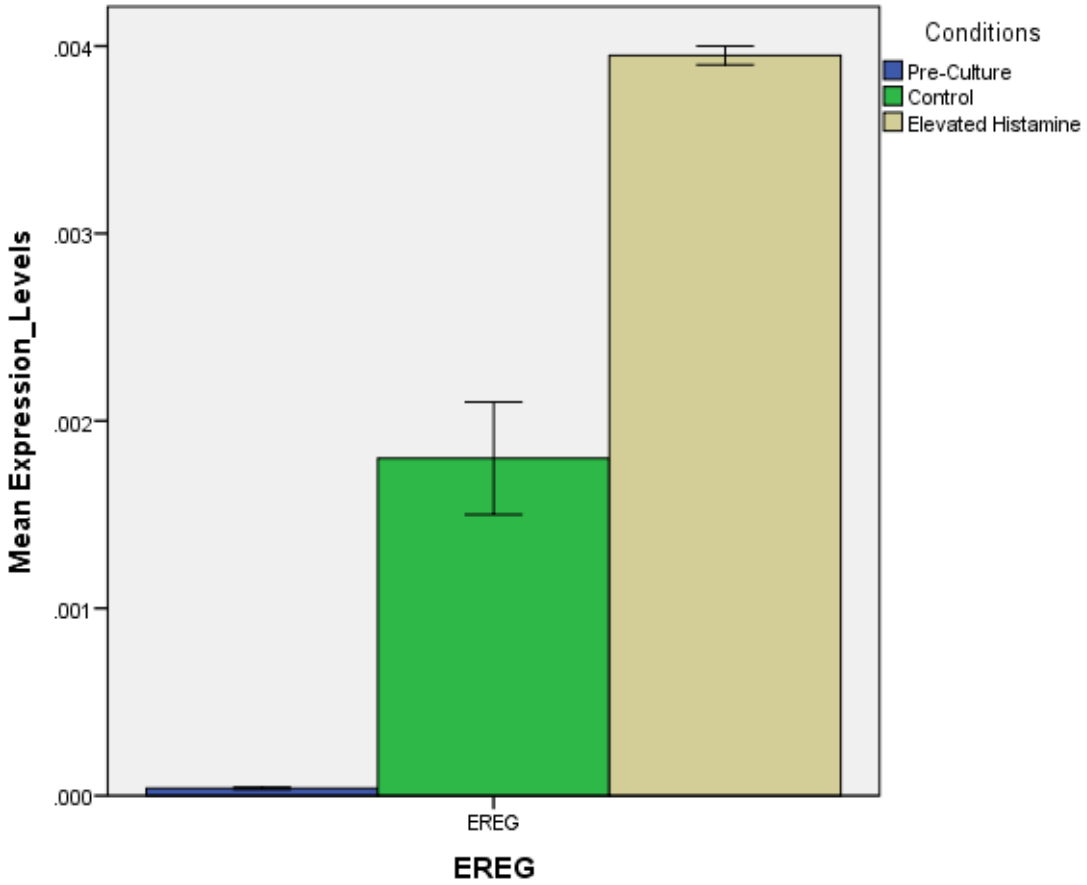
Target	Primer sequence (5' to 3')	Product length (bp)	GenBank ® accession number
L19	F: GCGGAAGGGTACAGCCAAT R: GCAGCCGGCGCAAA	140	NM_000981.3
AKR1C1	F: ATTTGCCAGCCAGGCTAGTG R: ACTTTTAGGACCTCTGCAGGC	122	NM_001353.5
EREG	F: CTGCCTGGGTTTCCATCTTCT R: GCCACACGTGGATTGTCTTC	123	NM_001412.2
IGFBP5	F: AAAGCAGTGCAAACCTTCCC R: CACTCAACGTTGCTGCTGTC	138	NM_001353.5

AKR1C1, aldo-ketose reductase family 1 member C1; EREG, epiregulin; IGFBP5, insulin-like growth factor binding protein 5; RT-qPCR, real-time quantitative polymerase chain reaction;

Appendix 9: RT-qPCR Validation results relative to L19




Conditions
Pre-Culture
Control
Elevated Histamine
Mean Expression_Levels
.600
.500
.400
.300
.200
.100
.000
IGFBP5
IGFBP5
Error bars: +/- 1 SE


Conditions
Pre-Culture
Control
Elevated Histamine
Mean Expression_Levels
.004
.003
.002
.001
.000
EREG
EREG
Error bars: +/- 1 SE