# The Effect of Maternal Asthma During Pregnancy on Placental Function and Fetal Development

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## Declaration

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution

Signed .....

Vanessa E Murphy

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Philippians 4:13

I can do all things through Christ who strengthens me

# Publication List

The work presented in this thesis has directly resulted in the following publications:

1. **Murphy VE**, Zakar T, Smith R, Giles WB, Gibson PG, Clifton VL. Reduced 11β-hydroxysteroid dehydrogenase type 2 activity is associated with decreased birth weight centile in pregnancies complicated by asthma. Journal of Clinical Endocrinology and Metabolism 2002; 87(4), 1660-1668.
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9. **Murphy VE**, Gibson PG, Giles WB, Zakar T, Smith R, Kessell CG and Clifton VL. Maternal asthma affects female fetal growth and is associated with reduced placental 11β-HSD2 activity and altered sensitivity to cortisol. 11th NSW Scientific Meeting of the Australian Society for Medical Research, Sydney, Australia, 3 June 2002 (won Best Poster Presentation by a Student).

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# Abbreviations

| | |
|---|---|
| 11β-HSD | 11 beta-hydroxysteroid dehydrogenase |
| 2D-PAGE | Two dimensional-polyacrylamide gel electrophoresis |
| $^3H$ | Tritium (Tritiated) |
| ACTH | Adrenocorticotropic hormone |
| AME | Apparent mineralocorticoid excess |
| AMS | Asthma Management Service |
| ANOVA | Analysis of variance |
| ATP | Adenosine triphosphate |
| $\beta_2$-agonist | Beta two adrenergic receptor agonist |
| BDP | Beclomethasone dipropionate |
| BMI | Body Mass Index |
| BPD | Biparietal diameter |
| BSA | Bovine serum albumin |
| BWC | Birth weight centile |
| C section | Caesarean section |
| cAMP | Cyclic 3',5'-adenosine monophosphate |
| cGMP | Cyclic 3',5'-guanosine monophosphate |
| CHAPS | 3-[(3-chloamidopropyl)-dimethylammonio]-1-propanesulfonate |
| CHCA | α-cyano-4-hydroxycinnamic acid |
| cpm | Counts per minute |
| CRH | Corticotropin releasing hormone |
| $C_T$ | Threshold cycle |
| Da | Daltons |
| dATP | 2'-deoxyadenosine 5'-triphosphate |
| dCTP | 2'-deoxycytidine 5'-triphosphate |
| dGTP | 2'-deoxyguanosine 5'-triphosphate |
| DHEA-S | Dehydroepiandrosterone sulfate |
| dNTPs | Deoxynucleotide triphosphates |
| dpm | Disintegrations per minute |
| DTT | Dithiothreitol |
| dTTP | 2'-deoxythymidine 5'-triphosphate |
| dUTP | 2'-deoxyuridine 5'-triphosphate |
| EAM | Energy absorbing molecule |
| EDTA | Ethylene-diamine-tetra-acetic acid |
| EGTA | Ethylene-glycol-bis(β-aminoethyl ether)-tetra-acetic acid |
| ELISA | Enzyme linked immunosorbent assay |
| $FEV_1$ | Forced expiratory volume at one second |
| FRI | Ferring Research Institute |
| FSH | Follicle stimulating hormone |
| FVC | Forced vital capacity |
| fwd | Forward (primer) |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase |
| GH | Growth hormone |
| GM-CSF | Granulocyte macrophage-colony stimulating factor |
| GR | Glucocorticoid receptor |
| HC:AC | Head circumference to abdominal circumference ratio |

| | |
|---|---|
| hCG | Human chorionic gonadotropin |
| HPA | Hypothalamic-pituitary-adrenal |
| HPLC | High performance liquid chromatography |
| IFN-γ | Interferon gamma |
| IgE | Immunoglobulin E |
| IGF | Insulin-like growth factor |
| IGFBP | Insulin-like growth factor binding protein |
| IL | Interleukin |
| IMAC | Immobilised metal affinity capture |
| IQ | Intelligence quotient |
| IU | International units |
| IUGR | Intrauterine growth restriction |
| KCl | Potassium chloride |
| $K_m$ | Michaelis constant |
| LPS | Lipopolysaccharide |
| $LTB_4$ | Leukotriene $B_4$ |
| MALDI | Matrix assisted laser desorption/ionisation |
| MR | Mineralocorticoid receptor |
| mRNA | Messenger RNA |
| MS | Mass spectrometry |
| MW | Molecular weight |
| m/z | Mass to charge ratio |
| n | Number of subjects |
| NaCl | Sodium chloride |
| NAD | Nicotinamide adenine dinucleotide |
| NADH | Nicotinamide adenine dinucleotide reduced form |
| NADP | Nicotinamide adenine dinucleotide phosphate |
| NADPH | Nicotinamide adenine dinucleotide phosphate reduced form |
| NBT/BCIP | Nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate |
| Ni-NTA | Nickel nitrilotriacetic acid |
| No. | Number |
| NO | Nitric oxide |
| NTC | No template control |
| PBS | Phosphate buffered saline |
| PCA | Principal component analysis |
| PCR | Polymerase chain reaction |
| $PCO_2$ | Partial pressure of carbon dioxide |
| PEF | Peak expiratory flow |
| PEFR | Peak expiratory flow rate |
| $PGE_2$ | Prostaglandin $E_2$ |
| $PGF_{2\alpha}$ | Prostaglandin $F_{2\alpha}$ |
| pI | Isoelectric point |
| PIH | Pregnancy induced hypertension |
| $PO_2$ | Partial pressure of oxygen |
| PPROM | Preterm premature rupture of membranes |
| PVDF | Polyvinylidene fluoride |
| RDS | Respiratory distress syndrome |
| rev | Reverse (primer) |
| RIA | Radioimmunoassay |
| rRNA | Ribosomal RNA |

| | |
|---|---|
| RT-PCR | Reverse transcriptase-polymerase chain reaction |
| SAX | Strong anion exchange |
| SD | Systolic/diastolic ratio |
| SDS | Sodium dodecyl sulfate |
| SDS-PAGE | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| SELDI-TOF | Surface enhanced laser desorption/ionisation-time of flight |
| SEM | Standard error of the mean |
| SGA | Small for gestational age |
| SOD | Superoxide dismutase |
| SPA | Sinapinic acid |
| TBE | Tris borate EDTA |
| TBS | Tris buffered saline |
| TFA | Trifluoroacetic acid |
| TGF-β | Transforming growth factor-beta |
| Th1/2 | T helper type 1/2 |
| TLC | Thin layer chromatography |
| $T_m$ | Melting temperature |
| TNF-α | Tumour necrosis factor-alpha |
| TOF | Time of flight |
| TTTS | Twin to twin transfusion syndrome |
| TX-100 | Triton X-100 |
| UV | Ultraviolet |
| VC | Vital capacity |
| WCX | Weak cation exchange |

# Abstract

Maternal asthma is associated with low birth weight, a risk factor for disease in adult life. To determine the mechanisms involved, the relationships between mother, placenta and fetus were examined in asthmatic and non-asthmatic pregnancies.

Maternal asthma and its treatment (no glucocorticoid or glucocorticoid) was monitored throughout pregnancy. Fetal growth was examined during gestation, and at birth, neonatal size and sex were determined. Placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme activity and umbilical vein plasma cortisol and estriol concentrations were measured. Placental cytokine, growth factor and glucocorticoid receptor (GR) mRNA were determined using quantitative RT-PCR.

Birth weight of female neonates in the no glucocorticoid asthmatic group only, was significantly reduced compared to females of the non-asthmatic group. Male neonates were unaffected by asthma or its treatment. Asthmatic women pregnant with a female fetus showed a significant increase in circulating monocytes and glucocorticoid treatment as pregnancy progressed, while those pregnant with a male fetus did not, suggesting that maternal asthma worsens in the presence of a female fetus. 11β-HSD2 activity was significantly reduced in placentae from female neonates of the no glucocorticoid group compared to other female neonates and was associated with a trend towards higher plasma cortisol, reduced fetal adrenal activity demonstrated by lower cord blood estriol, reduced placental GR expression, no alteration in placental or fetal insulin-like growth factors or their binding proteins and a significantly increased Th2:Th1 cytokine mRNA ratio, which was inversely correlated with 11β-HSD2 activity in all females. Reduced placental 11β-HSD2 activity may be an important component leading to decreased female fetal growth in pregnancies complicated by asthma.

This study provides strong evidence for a fetal sex-specific effect on the maternal immune system which can have adverse effects on the female fetus. The female fetus alters maternal inflammatory pathways, which when not controlled by the use of inhaled glucocorticoids results in reduced placental 11β-HSD2 activity, contributing to suppressed fetal adrenal function and a late gestation decrease in female fetal growth.