CORTICOSTEROID BINDING GLOBULIN:

HIGH RESOLUTION SEPARATION OF PLASMA GLYCAN ISOFORMS FROM PREGNANT AND COMBINED ORAL CONTRACEPTIVE PILL TAKING WOMEN

This thesis is submitted for award of the degree

of Master of Medical Science by

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CONTENTS

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

DECLARATION

I, Elizabeth Mitchell declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university and that to my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the text of the thesis.

In addition, I give consent to this copy of my thesis, when deposited in the University Library, being available for photocopying and loan if accepted for the award of Masters of Medical Science.

13th day of February the year 2007

Miss Elizabeth Mitchell

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ABSTRACT

Corticosteroid binding globulin (CBG) is the main carrier of cortisol in the circulation. Generally, the glycoprotein bears a mixture of bi- and tri-antennary N-linked glycosylation. During pregnancy, the concentration of CBG increases and the glycosylation converts to more tri-antennary with appearance of a sub-fraction, known as pregnancy-specific CBG, lacking any bi-antennary glycosylation. Previous methods including one dimension SDS-PAGE and crossed immunoelectrophoresis, have not been able to elucidate the full repertoire of CBG glycoforms during pregnancy due to low resolution. Therefore, a high-resolution analytical method, two-dimensional electrophoresis (2D-E), capable of broader examination of CBG glycosylation changes during pregnancy was employed.

It is postulated that glycoforms specific to pregnancy appearing in the first trimester may vary with gestational age. Furthermore, it is postulated that CBG glycosylation may be altered by exogenous oestrogen from the oral contraceptive pill (OCP).

Plasma samples were analysed from five pregnant women at various gestational ages, ten samples from women at 3-16 weeks and forty consecutive samples from ten women at 4 time points between 16-36 weeks. In addition, ten plasma samples were analysed from women taking the OCP, as well as five control samples, from three non-pregnant non-OCP women and two healthy men.

A method developed combining 2D-E and Western blotting detected 9-15 CBG glycoforms, an improvement on the afore mentioned lower resolution, methods. During pregnancy, CBG glycoforms were found to become generally more acidic, presumably as a result of incorporation of increased sialic acid residues.

Experiments using Concanavalin A, which binds bi-antennary but not tri-antennary glycosylated proteins, demonstrated the appearance of a portion, 10-15%, of CBG glycoforms exhibiting solely tri-antennary glycosylation during mid to late pregnancy. This form of CBG, previously termed pregnancy-specific CBG, had a heterogeneous profile on 2D-E that substantially overlapped the CBG profiles from non-con A treated samples.

The OCP 2D-E sample profiles demonstrated overlap with an increased CBG glycoform acidity compared with controls although there was no evidence of the solely tri-antennary-glycosylated glycoforms. These results indicate that there may be additional factors to oestrogen that may be involved in the modulation of CBG glycosylation during pregnancy.