

## PLACENTA PROGRAMMING OF FETAL HPA AXIS

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**Background:** Maternal stress and prenatal alcohol exposure (PAE) alter fetal programming of hypothalamic-pituitary-adrenal (HPA) axis, including glucocorticoid regulation of the stress response in placenta, leading to lifelong health issues. While preclinical studies began underlining the importance of placenta-mediated programming of the glucocorticoid signaling system in PAE, clinical studies are extremely limited.

The amount of cortisol that crosses the placenta is a function of relative expression of 11- $\beta$  hydroxysteroid dehydrogenases (11 $\beta$ -HSD): 11 $\beta$ -HSD2 oxidizes active maternal cortisol into inactive 11-dehydrocortisone, while 11 $\beta$ -HSD1 acts as a reductase and converts inactive 11-dehydro-corticosterone to active cortisol. Down regulation of 11 $\beta$ -HSD2 is associated with altered glucocorticoid programming.

**Methods:** In the prospective cohort study, conducted at the University of New Mexico, we evaluated the effect of maternal stress, mental health, and PAE on key placenta targets of HPA axis and corresponding downstream markers in umbilical cord blood. PAE was assessed by prospective repeated TLFB interviews and a battery of ethanol biomarkers. Maternal stress and mental health were assessed by the Perceived Stress Scale (PSS), Generalized Anxiety Disorder-7 screener (GAD-7), PTSD symptoms (PCL-5), Edinburgh Postnatal Depression Scale (EPDS), and Adverse Childhood Experience (ACE-Q). Placenta specimens were collected within 5 hours of delivery; tissue excised from grossly normal areas of the villous parenchyma, excluding the decidua basalis and chorionic plate, flash frozen, and stored at -80°C. Placenta protein was isolated via fractional centrifugation, treated with protease/phosphatase inhibitors, producing lysates enriched with cytosolic proteins, and analyzed by ELISA for 11 $\beta$ -HSD type 1, 11 $\beta$ -HSD-type 2, and their ratio. Cortisone, cortisol, and cortisone/cortisol ratio was evaluated in umbilical cord blood, collected at birth, as a downstream measure of fetal HPA axis dysregulation. T-test was conducted to assess differences in the mean expression of the HPA axis biomarkers among PAE and control participants and those with  $\geq 2$  binge drinking episodes vs.  $< 2$  binge episodes. Spearman correlation examined an association between biomarkers and continuous scores on maternal stress and mental health scales.

**Results:** In 85 placenta samples (27 with PAE and 58 controls) analyzed to date, higher 11 $\beta$ -HSD2/11 $\beta$ -HSD1 ratio was observed in participants with PAE ( $p=0.02$ ), ACE $\geq 4$  ( $p=0.03$ ), and those with  $\geq 2$  binge drinking episodes ( $p=0.07$ ). Additionally, significant correlation was observed between 11 $\beta$ -HSD2 and PSS ( $\rho=0.277$ ;  $p=0.01$ ), GAD-7 ( $\rho=0.273$ ;  $p=0.01$ ), and PCL-5 ( $\rho=0.224$ ;  $p=0.04$ ). In analysis of 101 samples of umbilical cord plasma, higher cortisone/cortisol ratio was observed in participants with  $\geq 2$  binge drinking episodes ( $p=0.04$ ).

**Conclusion:** These data demonstrate the importance of placenta-mediated programming of the fetal glucocorticoid signaling system, and the effect of maternal stress and alcohol exposure on HPA axis. The focus on placenta, as a key interactive endocrine entity linking maternal and fetal HPA axes is expected to lead to identification of early markers of impaired stress reactivity/regulation in affected children.