Mitochondrial function in cell lineages of the placenta: does function alter between gestational disorders?

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Optimal pregnancy is critical to providing the best start to life. Suboptimal pregnancy conditions correlate with poor health outcomes and are a strong risk factor for the development of adult cardiovascular disease and diabetes. Important pregnancy disorders include gestational diabetes mellitus (GDM) and preeclampsia (PE) and are increasing in prevalence in Australia. The placenta is a critical organ during pregnancy, forming the interface between maternal and foetal systems. Placental dysfunction is central to pregnancy disorders as the placenta drives maternal metabolic adaptations to meet foetal demands. In GDM, maternal organs may not properly adapt to pregnancy, inducing glucose intolerance and insulin resistance. In PE, placental-driven remodelling of maternal uterine vasculature may be inadequate, leading to reduced perfusion, hypoxia, and perturbed nutrient supply. Although placental dysfunction is associated with gestational disorders, the mechanistic causes behind these remains largely unknown. Mitochondrial dysfunction has been suggested in many of these disorders, with alterations in mitochondrial morphology and content identified in affected placentae. Despite these established yet contentious differences, studies into placental mitochondrial function in gestational disorders remain limited. Furthermore, the placenta contains cell lineages (cytotrophoblast and syncytiotrophoblast) with distinct mitochondrial types (reference), but investigation into potential alterations in mitochondria between the placental cell lineages as a result of GDM and PE is relatively novel. The aim of this study was to advance our understanding of the physiology and progression of gestational disorders at a mitochondrial level, optimizing a protocol to assess mitochondrial respiration between cytotrophoblast and syncytiotrophoblast cell lineages in healthy placentae and placentae affected by PE and GDM.

Placental samples were collected immediately following delivery; mitochondria were isolated by differential centrifugation following homogenization of placental tissue in isolation media. Centrifugation at 1,500g for 10 min to remove cellular debris before transferring the supernatant for further centrifugation at 4,000g for 15 min produced a cytotrophoblast (heavy) enriched mitochondrial pellet. Supernatant was centrifuged at 12,000g for 15 min to produce a syncytiotrophoblast (light) enriched mitochondrial fraction. Enriched fractions where then suspended in mitochondrial isolation buffer. Protein estimation was used to confirm relative levels of protein, with confocal imagining staining for mitochondrial membrane potential using tetramethylrhodamine, ethyl ester (TMRE) and nuclear debris using HCS stain, confirming the presence of mitochondria in both fractions. Mitochondrial respiration was analysed in heavy and light fraction using an O2K oxygraph (Oroboros). Western blotting was used to ascertain protein expression levels of mitochondrial electron transport chain complexes of both the cytotrophoblast and synctiotrophoblast fractions.

Mitochondrial respiration (n=7) through complexes I, (P=0.03) and II (P=0.0004) were lower in syncytiotrophoblast when compared to cytotrophoblast mitochondria. However, syncytiotrophoblast mitochondria had a higher spare respiratory capacity. Confocal microscopy performed on fractions confirmed the presence of mitochondria with intact membrane potential, and indicated significantly smaller mitochondria in the syncytiotrophoblast fraction. Western blot analyse found no significant differences in expression of mitochondrial complexes between the syncytiotrophoblast and cytotrophoblast fractions. Finally, analyses of control (n=7), PE (n=7) and GDM (n=7) fractions demonstrated decreased respiration in complex I and II of both cytotrophoblast and syncytiotrophoblast mitochondria in gestational disorders compared to controls. GDM mitochondria respired the lowest across both complexes and fractions with control samples respiring the highest. This study validated a suitable methodology to assess morphology, respiration, and expression of complexes in mitochondria from cytotrophoblast and syncytiotrophoblast cell lineages of the placenta in both healthy and pathological samples. We were able to demonstrate significant lineage dependant differences in cellular respiration; syncytiotrophoblast mitochondria had lower respiration but higher spare respiratory capacity, potentially enabling them to mediate cellular stress more successfully than cytotrophoblast mitochondria. This may have implications in the progression of pregnancy disorders, although this is yet to be investigated. Despite differences in respiration, no changes were found in the expression of mitochondrial complexes between the fractions, indicating respiratory differences were not due to differences in complexes expression. Preliminary investigation separating samples by gestational disorders suggests altered respiration in cell lineages of gestational disorders. These changes may indicate lineage specific mitochondrial mechanisms which result in mitochondrial dysfunction and cause placental dysfunction. These finding may be crucial in physiology and progression of GDM and PE. This technique has great potential to be disseminated throughout multiple disciplines and research areas which focus on mitochondria in disease.