Article: Human Primary Trophoblast Cell Culture Model to Study the Protective Effects of Melatonin Against Hypoxia/reoxygenation-induced Disruption

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ABSTRACT: This protocol describes how villous cytotrophoblast cells are isolated from placentas at term by successive enzymatic digestions, followed by density centrifugation, media gradient isolation and immunomagnetic purification. As observed in vivo, mononucleated villous cytotrophoblast cells in primary culture differentiate into multinucleated syncytiotrophoblast cells after 72 hr. Compared to normoxia (8% O2), villous cytotrophoblast cells that undergo hypoxia/reoxygenation (0.5% / 8% O2) undergo increased oxidative stress and intrinsic apoptosis, similar to that observed in vivo in pregnancy complications such as preeclampsia, preterm birth, and intrauterine growth restriction. In this context, primary villous trophoblasts cultured under hypoxia/reoxygenation conditions represent a unique experimental system to better understand the mechanisms and signalling pathways that are altered in human placenta and facilitate the search for effective drugs that protect against certain pregnancy disorders. Human villous trophoblasts produce melatonin and express its synthesizing enzymes and receptors. Melatonin has been suggested as a treatment for preeclampsia and intrauterine growth restriction because of its protective antioxidant effects. In the primary villous cytotrophoblast cell model described in this paper, melatonin has no effect on trophoblast cells in normoxic state but restores the redox balance of syncytiotrophoblast cells disrupted by hypoxia/reoxygenation. Thus, human villous trophoblast cells in primary culture are an excellent approach to study the mechanisms behind the protective effects of melatonin on placental function during hypoxia/reoxygenation.

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