87. IGF-2 MEDIATES THE EFFECT OF HYPOXIA ON HUMAN CYTOTROPHOBLAST OUTGROWTH

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Poor placental development has been associated with a variety of pregnancy-associated disorders, including preeclampsia. Placentation involves spatial and temporal regulation of cytotrophoblast (CTB) proliferation, differentiation and invasion. These processes, during early pregnancy occur in a relatively hypoxic environment and insulin-like growth factor (IGF)-2 is abundantly expressed by the invading extravillous CTBs. Therefore, this study aimed to investigate the interaction of O_2 concentration and IGF-2 on CTB morphology and IGF-2 and IGF-2 receptor (IGF2R) gene expression *in vitro*. First trimester (7-8 weeks gestation) human placental villous explants were cultured in serum-free media with and without the addition of 125 ng/mL IGF-2, in 1%, 5%, or 20% O_2 (6 groups). On day 6, explants were photographed and RNA was extracted and pooled (8 wells/treatment) for quantification of IGF-2, IGF2R and 18S RNA using real time RT-PCR. Photographs were scored for CTB column formation, the number of tips growing/explant and extravillous CTB phenotype (rounded or dendriform). Villous explants cultured in 1% and 5% O_2 formed 55% and 40% more CTB cell columns than those cultured in 20% O_2, respectively ($P<0.001$). Exogenous IGF-2 enhanced CTB outgrowth by 35% in 20% O_2 explants ($P=0.001$), but not in those cultured in 1% or 5% O_2. Less than half of extravillous CTBs exhibited a dendriform migratory phenotype in 20% O_2 compared to 1% O_2 ($P=0.02$). IGF-2 expression relative to 18S RNA, was increased 3-fold and 2.5-fold in explants cultured in 1% O_2 compared with 5% and 20%, respectively, but this was not significant. IGF-2 mRNA was positively correlated with extravillous CTB migration ($r=0.38$, $P=0.009$). There was no effect of treatment on IGF2R transcription in placental villous explants, but IGF2R expression was positively correlated with cell column formation ($r=0.37$, $P=0.01$) and extravillous CTB migration ($r=0.56$, $P<0.000$). In conclusion, culture in low O_2 promotes early CTB outgrowth and addition of IGF-2 mimics this effect. Our previous work found a positive correlation between IGF-2 mRNA synthesis and CTB cell column formation on an individual well basis. These data suggest that the effect of hypoxia may be mediated by IGF-2.

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