Proceedings Paper:
Timms, K, Day, A, Mclaughlin, J et al. (2 more authors) (2019) Plant Extracellular Vesicles Influence Trophoblast Differentiation. In: Reproductive Sciences. Society for Reproductive Investigation 2019, 12-16 Mar 2019, Paris. SAGE Publications , 352A-353A.

https://doi.org/10.1177/1933719119834079

352A Reproductive Sciences Vol. 26, Supplement 1, March 2019

S-160

Plant Extracellular Vesicles Influence Trophoblast Differentiation.

Kate Timms[1†], Anil Day[1], John Mclaughlin[1], Karen Forbes[2*], Melissa Westwood1[*].

[1]University of Manchester, Manchester, United Kingdom; [2]University of Leeds, Leeds, United Kingdom

[†]presenting author; ***** *senior author*

Introduction: Abnormalities in trophoblast differentiation contribute to both fetal growth restriction and preeclampsia. The incidence of both complications is reduced in women consuming high levels of fruits & vegetables (F&V), but cannot be explained wholly by their known nutritional constituents. We hypothesise that extracellular vesicles (EVs) from F&V, which can be internalised by the intestine, can contribute to the beneficial effects of F&V by influencing gut-placental communication and therefore trophoblast behaviour.

Methods: EVs isolated from watermelon by differential centrifugation were applied to the apical (i.e. luminal) compartment of differentiated intestinal epithelial (Caco-2) transwell cultures (n=13). The resultant conditioned basal media (CBM; collected after 4 hours) was then applied to trophoblast (BeWo or primary cells/explants) for 4-48h. Protein profiles of both CBM and BeWo cells cultured in CBM, were determined using mass spectrometry (n=3) and analysed by hierarchical clustering, isomap and ingenuity functional analyses. Trophoblast differentiation was assayed by area of extravillous trophoblast (EVT) outgrowth from first trimester placental explants (n=10; Two-Way ANOVA), primary term cytotrophoblast syncytialisation (nuclei/cell; n=7; Mann-Whitney test) and qPCR (n=7; Kruskal-Wallis test).

Results: Proteomic analysis of CBM revealed that exposure to watermelon EVs alters the intestinal (Caco-2) secretome (45% of proteins up/ downregulated). In-silico analysis revealed that CBM from watermelon EV-exposed Caco-2 cells induced changes to the BeWo cell proteome consistent with enhanced trophoblast differentiation into migratory EVT and syncytium. In keeping with the bioinformatic predictions, the area of EVT outgrowth from explants was increased by incubation in CBM from watermelon EV-exposed Caco-2 cells (24h: 212%, $p<0.05$; 48h: 294%, $p<0.0001$). Such treatment also upregulated the expression of key genes associated with syncytialisation (e.g. syncytin-1: 883%; $p<0.01$) in BeWo and promoted syncytialisation (175%; $p<0.001$) in primary term cytotrophoblasts.

Conclusion: These results suggest that by altering the gut secretome, intestinal absorption of watermelon EVs influences the communication