

## Investigating the Enzymatic Activity of Endometrial DPPIV in the Molecular Adhesive Pathways at Embryo Implantation Using an *in vitro* Co-culture Model

Nathan John Parks; Mohamed Shuaib; Stéphane C. Berneau

School of Pharmacy and Biomedical Sciences, THRIVE research centre, University of Central Lancashire, Preston, United Kingdom

In the UK, 1 out of 6 couples are infertile and 10% of them suffer from recurrent implantation failures. The clinical success rate has stagnated at 25-30% per cycle for the last decade. The knowledge of molecular events at the embryo implantation remains limited, hindering further increase in clinical pregnancy success rates.

The endometrial dipeptidyl peptidase IV (DPPIV) was identified to be upregulated during the window of implantation in fertile women and downregulated in women experiencing recurrent implantation failure. However, its enzymatic function remains unclear at implantation, and we aim to investigate it at the embryo-endometrial interface.

In endometrial cells, DPPIV was heterogeneously expressed in endometrial cell membranes using immunocytochemistry, with highly DPPIV-positive cells detected at the site of BeWo spheroid attachment onto endometrial Ishikawa monolayer in our co-culture model. The role of DPPIV enzymatic function in cell adhesion was tested *in vitro* using sitagliptin and vildagliptin. Using bovine serum albumin-, collagen-1-, poly-L-lysine- or fibronectin- coatings, BCECF-AM-treated endometrial cells were plated for single-cell attachment assays. Fluorescent endometrial cells were pre-treated with inhibitors concentration range (diprotin A: 1µg/ml-100µg/ml; sitagliptin: 10µM-150µM; vildagliptin: 10µM-100 µM).

The enzymatic activity of DPPIV was measured *in vitro* and significantly inhibited by a potent DPPIV inhibitor, diprotin A. A 50µg/mL diprotin A pre-treatment of endometrial cells significantly decreased their cell attachment to fibronectin and in our co-culture model, significantly triggered a reduction in spheroids attachment.

Using prescribed DPPIV inhibitors, only sitagliptin concentrations caused a significant reduction in endometrial cell attachment in fibronectin- and collagen-1-coated wells compared to vildagliptin. Using an *in vitro* model of implantation, monolayers of Ishikawa cells were pre-treated or not with sitagliptin or vildagliptin and thus, trophoblastic BeWo spheroids were co-cultured onto the monolayer. A decreasing trend in spheroid attachment was observed using 10µM and 150µM sitagliptin compared to control and vildagliptin concentrations.

In this study, the inhibition of endometrial DPPIV by diprotin A impaired its adhesive function *in vitro*. However, the prescribed DPPIV inhibitors acted differently: sitagliptin impaired endometrial cell attachment and had a limited effect on spheroid attachment, whereas vildagliptin had no impact. These preliminary data suggest the need for further investigations on the inadvertent use of prescribed gliptins at implantation for couples trying to conceive.