View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Publications from Karolinska Institutet

Karolinska Institutet

Department of Women´s and children´s Health

Endometrial Receptivity and Human Embryo Implantation: *In vivo* and *In vitro* studies

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Skandiasalen, Astrid Lindgrens Barnsjukhus, Karolinska Universitetssjukhuset

Torsdagen den 28 November, 2013, kl 09.00

av
Sujata Lalit Kumar
Leg Läk

Huvudhandledare:
Professor Kristina Gemzell Danielsson
Karolinska Institutet
Department of Women´s and Children´s Health
Division of Obstetrics and Gynecology

Bihandledare:
Lalit Kumar PGL
Karolinska Institutet
Department of Women´s and Children´s Health
Division of Obsterics and Gynecology

Fakultetsopponent:
Professor Nick S Macklon
University of Southampton
Complete Fertlity Center
Southampton, United Kingdom

Betygsnämnd:
Professor Stefan Hansson
University of Lund
Clinical Sciences
Department of Obstetrics and Gynecology

Docent Helena Åkerud
Department of Women´s & Children´s Health
Uppsal Universitet,
Uppsala

Docent Kenny Rodriguez-Wallberg
Karolinska Institutet
Department of Clinical Sciences, Intervention and Technology

Stockholm 2013

ABSTRACT

Background: Infertility, one of the common gynaecological disorders, affects 10-15% women in their reproductive years. Despite the advances in assisted reproduction techniques and the best efforts of infertility specialists, implantation rates do not exceed more than 28-30%. Implantation failure is one of the major causes of infertility of couples with unexplained infertility. Understanding the mechanisms of human embryo implantation is important in the attempt to alleviate infertility, improve pregnancy rates and foetal health. This knowledge could also be further explored to develop new modalities for fertility regulation.

Objective: To understand endometrial receptivity and the human embryo implantation process through experimental and translational research using *in vivo* and *in vitro* approaches.

In ***Paper I,*** an *in vitro* three-dimensional embryo-endometrial cell culture model expressing receptivity markers ER-α, ER-β, PR-(A+B), PR-B, VEGF, LIF, IL-1β and COX-2 and αVβ3 and MUC1 was developed and tested for its progesterone regulation using anti-progestin mifepristone and gestagen levonorgestrel on the human embryo implantation process. It was found that none of the 15 embryos in the cultures exposed to mifepristone attached to the endometrial construct, whereas 10/17 in control and 6/14 in levonorgestrel groups did attach. This model was further utilised in ***Paper II*** to study the role of Leukemia inhibitory factor (LIF) in human embryo implantation by using a potent LIF inhibitor, PEGLA (PEGylated LIF inhibitor). Inhibition of LIF by PEGLA, inhibited blastocyst attachment to the *in vitro* model, down-regulated its expression of AKT and triggered apoptosis in the inner cell mass, as studied by immunofluorescence and real-time PCR. ***Papers III and IV*** explored large-scale progesterone regulated human endometrial receptivity markers during the implantation window in stromal and epithelial compartments using a laser capture microdissection and microarray analysis. Interestingly, the expression of both the mRNA and the protein for ectonucleoside pyrophosphatase/phosphodiestrase 3 (ENPP3) in the epithelial compartment were not detectable in the progesterone-depleted group, as studied by microarray, real-time PCR and immunohistochemistry. The major canonical pathways altered in epithelial compartment were oxidative phosphorylation and the mitochondrial dysfunction pathway. In the stromal compartment, 101 genes were potentially differentially regulated (FC > 2; p-value < 0.05). Real-time PCR analysis showed significant differences in the expression of SFRP4 (6.73; p=0.005), CTSC (2.3; p=0.04), SMARCA 1 (1.66; p=0.02), CPM (16.37; p=0.03), HMGN5 (1.82; p=0.03), MT1G (-333; p= 0.003) and MT2A (-4.67; p=0.03) with progesterone. The major canonical pathways differentially regulated with progesterone in stromal cells, as analysed by IPA, were EIF2 signalling and the mitochondrial dysfunction pathway.

Conclusion: In this study, an *in vitro*-three dimensional embryo-endometrial cell culture model to investigate the human embryo implantation process was established and this shed more light onto the role of LIF in the embryo implantation process. This model could be used to examine the embryo-endometrial interaction that leads to successful implantation, as well as to develop new contraceptive agents. A novel endometrial epithelial progesterone-regulated receptivity marker, ENPP3, was identified and its potential application in ART needs to be explored further. The knowledge gained from the expression of progesterone-regulated genes in the endometrial, glandular and stromal compartments could assist in understanding the molecular mechanisms involved in endometrial receptivity and would be beneficial for improving fertility rates in women, as well as paving way to the development of new endometrium-based fertility regulating agents.
Key words: *3D-endometrial cell construct, human blastocyst implantation, receptivity markers, LIF, PEGLA, stromal cells, epithelium, progesterone regulation, ENPP3, endometrial receptivity.*

ISBN: 978-91-7549-337-4