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Development of an In Vitro Model to Study Uterine Functions and Early Implantation

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(子宮機能および初期着床を解析するための生体外モデルの開発)

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Thesis Summary

The uterine endometrium is composed of luminal and glandular epithelial cells, stromal components, and a closely associated extracellular matrix. Uterine endometrium recognized as a complex tissue, which experiences significant remodeling in response to the initiation of implantation and further pregnancy progressions. But the intricate mechanism of implantation remains imprecise due to the involvement of a number of elements like cytokines, growth factors, hormones, adhesion molecules etc. In that connection an *in vitro* model will help to uncover the complex uterine functions and further implantation mechanism, although no appropriate *in vitro* model is available so far.

Endometrial modulation is essential for the preservation of normal uterine physiology, and this modulation is driven by a number of growth factors. Thus the first objective of this study was to investigate the mitogenic, motogenic, and morphogenic effects of epidermal growth factor (EGF) and hepatocyte growth factor (HGF) on rat endometrial epithelial (REE) cells. The isolated and cultured REE cells were characterized based on morphological features and indirect immunofluorescence staining using specific epithelial cell markers. Then the EGFR and HGFR (c-Met) mRNA were identified in REE cells. Then the biological effects of EGF and HGF were investigated in terms proliferation, migration and lumen formation of REE cells. The MTT assay revealed that EGF and HGF induce proliferation of REE cells. Consistent with increased proliferation, it was found that the cell cycle regulatory factor Cyclin D1 was also upregulated upon EGF and HGF addition. REE cell migration was prompted by EGF, as observed with the Oris Cell Migration Assay. The morphogenic impact of growth factors on REE cells was studied in a three-dimensional BD Matrigel cell culture system, wherein these growth factors also increased the frequency of lumen formation. The study showed that EGF and HGF have a stimulatory effect on REE cells, promoting proliferation, cell migration, and lumen formation. These findings provide important insights that further the understanding of endometrial regeneration and its regulation.

Over past few decades, several *in vitro* models were developed to better understand the uterine functions and early implantation mechanism. However, years of effort to culture either the whole or some part of the uterine horn have been in vain. This might be due to the heterogeneous cell types of the uterine horns, which mediate structural and functional integrity, whereas, the *in vitro* models fail to support this condition. From the above perspective, the second objective of this study was aimed to develop uterine explants and study of their morphology, hormonal regulation and

remodelling in terms of decidualization. Rat uterine explants (1-2 mm) were isolated, cultured and further characterized by phase contrast microscopy, histological analysis and indirect immunofluorescence staining using specific antibodies. Then explants were treated with steroid hormones and the regulation of MUC1, PR, AREG and IGFBP1 were investigated. RT-qPCR data revealed that MUC1 and PR was upregulated significantly by E2. On the other hand, AREG was upregulated significantly by P4. Surprisingly, IGFBP1 was upregulated significantly by E2 and P4, although in rat IGFBP1 is E2 dependent. Furthermore, the remodelling ability of the uterine explants in terms of *in vitro* decidualization was also investigated emphasizing on PRL8a2 and BMP2. RT-qPCR data revealed that the PRL8a2 and BMP2 were significantly ($P<0.05$) up regulated in treated explants compared to non-treated explants. In this study, cultured uterine explants exhibited comparable characters of the *in vivo* uterine conditions, which suppose to mimic the morphology and physiology of the uterus and can be utilize to study implantation as an *in vitro* model system.

Although a number of studies describe the *in vitro* co-culture model, still the positioning of embryos to the uterine lumen and examination of blastocysts attached to the endometrial tissue was not so easy due to the small size of blastocysts compared to the endometrial tissues. But, the development of uterine explants (second objective), which possessed comparable structure of uterus and suppose to mimic the morphology and physiology of *in vivo* uterus make it imperative to study the early implantation in a co-culture model system. Thus, the third objective was to co-culture of hatched blastocyst and cultured uterine explants in view of study the early implantation. Rat uterine explants (1-2 mm) were isolated, cultured and further characterized by phase contrast microscopy. Then morphologically normal embryos were flushed from uterine horns and hatching was induced by Acidic Tyrode's solution (pH-2.5) for 15-30 second to remove the zona pellucida. Individual hatched blastocyst and cultured explant was placed in a 96U well plate. After 24 hours of co-culture the attachments of embryos to the explants were evaluated based on the stable attachments of embryos to the explants after mild shaking and/or pipetting, where the non-attached embryos showed no stable attachment and easily dislodged. Then the stable attachments were observed after 48 hours of co-culture, where embryos were stably attached to the explants and could not be dislodged after mild shaking and/or pipetting. Furthermore, steroid hormones are critical for endometrial receptivity and further implantation process. The steroid hormone treatment revealed that the rate of attachment of embryos to the explants were significantly increased in P4 treated group (63.63%) compared to the control or non-treated group (35.48%). On the other hand, attachments of embryos to the explants were significantly reduced in E2 treated group compared to the control group, where no stable attachments were observed in E2 treated group (0.0%). In this study, the early implantation i.e. attachment of embryos to the explants as well as the effect of steroid hormones on the rate of attachments were investigated in an *in vitro* co-culture model system.

Overall, the *in vitro* model developed using rat endometrial epithelial cells will provide new insights into mechanisms that may be critical for the regulation of endometrial regeneration. Furthermore, the cultured rat uterine explants, which suppose to mimic the morphology and physiology of the *in vivo* uterus, will provide a new platform to study uterine functions and early implantation.