

=ABSTRACT=

Establishment of Three Dimensional *in-vitro* Culture System with Human Endometrial Cells: Induction of Differentiation by Sex Steroid Hormone & Characterization

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Objective : The aim of this study was to establish three-dimensionally cultured endometrial cell model containing endometrial stromal cell (ESC), endometrial epithelial cell (EEC) and extracellular matrix (ECM) and to compare the morphological and biomolecular expression patterns of this model with mid-luteal endometrium *in vivo*.

Materials and Methods : The EEC and ESC was obtained from hysterectomy specimen and cultured separately. The EEC was overlaid in Matrigel layer on ESC embedded in collagen. The model had been cultured for 48 h in DMEM medium containing estrogen and progesterone. The ultrastructure was evaluated by electron microscopy. The expression of integrins, cyclooxygenases and matrix metalloproteinases were examined by immunohistochemistry and zymography.

Results : EEC in three-dimensional culture model grew with polarity and tight junction and desmosome between cells were found. The formation of pinopodes was also detected. In three-dimensionally cultured endometrial cell model, the expression of integrin α_1 , α_4 , α_3 , MMP-1, -2, -3 and 9 was detected which was not expressed in monolayer culture of EEC, ESC or ESC embedded in collagen.

Conclusion : The three-dimensionally cultured endometrial cell model possessed the morphological and biomolecular characteristics of *in vivo* endometrium of implantation period. These characteristics could be achieved by paracrine interactions between ESC and EEC. This model may contribute to the studies of differentiation of endometrium, process of implantation and pathophysiology of implantation-related diseases.

Key words : Three dimensional co-culture, Human endometrial cell, Integrin, MMPs

(mid secretory phase) COX, MMPs (immunohistochemistry)

(estrogen) (progesteron)

(sex steroid hormone)

(growth factor) (cytokine)

(epithelial cell, EEC) (stromal cell, ESC)

1. (

(EEC) 6 14

(ESC) integrin,³ Matrix metalloproteinase (MMPs),⁴ cyclooxygenase(COX)^{5,6} (Noyes et al., 1975)

pinopods가⁶

integrin 1, 4, 3, v 3가 (tissue remodeling) COX-2^{5,6} 2.

MMP-9⁸ 가 , PBS가 conical tube (Falcon, Becton Dickinson, New Jersey, USA)

PBS

Dulbeco's Modified Eagle's Media (DMEM; Gibco, Life Technologies, Roskilde, USA) 2-3 mL

가 1-2mm

conical tube trypsin-EDTA (Gibco, USA) 15 . 15

1% penicillin-streptomycin (Gibco, USA), 10% fetal bovine serum (Gibco, USA), 1nM estradiol (Sigma, USA)

DMEM 5mL 가 2 ESC

7mL EEC

3mL 60mm (Falcon, Becton Dickinson, New Jersey, USA) 24

EEC ESC

가 ,

EEC

EEC

conical tube

400rpm 10

1,000 units/mL collagenase (Sigma, St. Louis, MO, USA) 가 20

가 가

2

ESC trypsin-EDTA

2mL 가 5 conical tube

가 5mL 가 2

3 ESC collagen gel gel

Matrigel ECC

collagen gel (Biocoat, rat tail type I, 3.6mg/mL)

integrins,