P-1 생쥐자궁조직의 ADAM-9, 12, 15, 17 mRNA의 발현

서울여자대학교 생명공학과[1], 미래와희망산부인과[2],
서울대학교 의과대학 산부인과학교실[3]

김지영[1] · 허주영[1] · 김해권[1] · 이승재[2] · 최영민[3]

목 적: Metalloprotease/disintegrins는 transmembrane glycoprotein으로서 이들은 N-terminal signal sequence를 갖는 conserved domain, prodomain, metalloprotease/disintegrin domain, cystein-rich region, transmembrane domain 그리고 cytoplasmic domain으로 이루어져 있고 지금까지 30개 이상의 ADAM 및 10개 이상의 ADAMTS 단백질이 알려져 있다. 이들의 기능은 포유동물의 수정시 sperm-egg binding과 fusion, myoblast fusion, integrin과의 결합 등에 직접 관여하거나, TNF-alpha 등의 생체신호전달물질이 세포로부터 분비될 때에 이들의 구조를 변화시켜 활성화시키는 효소작용을 하거나, dendritic cell differentiation 등에 관여하는 것으로 알려져 있다. 그러나 ADAM 및 ADAMTS의 경우 자궁내막조직에서의 유전자 및 단백질 발현여부에 관해서는 전혀 보고되어 있지 않고 있다. 본 연구에서는 생쥐의 자궁조직을 대상으로 estrous cycle 동안 RT-PCR 방법을 이용하여 ADAM-9, 12, 15, 17 mRNA 발현여부를 조사하였다.

대상 및 방법: 본 실험에서는 생 후 8주 이상된 생식 능력이 있는 생쥐 암컷 ICR을 사용하였다. 발정주기는 Rugh (1990)의 방법에 따라 vaginal smear 방법을 이용해 diestrus, proestrus, estrus, metestrus의 네시기로 구분하였고 각 시기마다 자궁조직을 얻어 tri reageant 용액에 담아 -20℃에서 보관하였다. 시료로부터 RNA을 추출하여 역전사 중합효소반응 (RT-PCR)을 실시하였고 그 결과를 densitometry를 이용하여 비교하였다.

결 과: 생쥐 발정주기에 따라 diestrus, proestrus, estrus, metestrus로 나누어 자궁에서의 mRNA의 양을 β-actin의 양에 대하여 상대적으로 측정한 결과 ADAM-9의 경우 각각 30.6%, 52.3%, 85.3%, 43.4% 발현되었으며 ADAM-12는 각각 38.2%, 63.2%, 60.6%, 53.8% 발현되었다. 또한 ADAM-15는 32.2%, 57.2%, 62.1%, 44.4% 발현되었고 ADAM-17은 47.4%, 56.7%, 71.4%, 66.5% 발현되었다.

결 론: ADAM-9, 15, 17의 mRNA는 estrus시기에서, ADAM-12는 proestrus시기에서 가장 많이 발현되었다. Estrous cycle에 따라 생쥐의 자궁조직의 ADAM-9, 12, 15, 17의 mRNA의 발현이 달라지는 것으로 미루어 이들 ADAM은 자궁조직의 구조 변화에 중요한 역할을 하는 것으로 생각된다.

P-2 Expression Pattern of Smad Family in the Preimplantaion Mouse Embryos and Uterus

Nah HY, Hong SH, Lee JY, Kim JH, Kim CH

Department of Obstetrics and Gynecology, College of Medicine, Ulsan University, Asan Medical Center, Seoul, 138-736, Korea

Transforming growth factor β (TGF-β) family is multifunctional such as cell proliferation, development, differentiation, motility, adhesion and cell death. It is comprised large number of polypeptide growth factors. Although TGF-β may have an important effect throughout embryonic development, Smad related

TGF-β signal transduction is poorly understood. Eight different Smads have been in mammals; there are primarily three kinds of Smad protein as receptor-Smad (R-Smad), common partner Smad (Co-Smad) and inhibitory Smad (I-Smad). Each of them was controlled differentially through TGF-β family.

The purpose of our study is to detect Smads and TGF-β receptors mRNA in the preimplantation mouse embryos and uterus using RT-PCR.

Smad famiy and TGF-β receptors generally increased in uterus on day 4 pregnant, and especially TGF-β R-II mRNA was greatly increased. While Smad 1, 2 and 5 mRNA highly was expressed in unfertilized oocytes, PN and blastocyst stage embryos, Smad 3 just detected in unfertilized oocytes and PN stage embryos. In particular, Smad 7 diversely was expressed in the preimplantation mouse embryo.

Therefore, we suggest that Smad family may act differential modulator of signal transduction of TGF-β family in the preimplantation mouse embryos and uterus.

For further study, we are to be planing localization of Smad proteins in preimplantaion mouse embryos and uterus.

P-3 Improved Post-thawed Preimplantation Development after Vitrfication using Taxol™, a Cytoskeleton Stabilizer

Park SE[1], Ko JJ[2], Chung HM[2], Shin TE[1], Cha KY[1]

Infertility Medical Center of CHA General Hospital[1], College of Medicine, Phochon CHA University[2], Seoul, Korea

Objectives: Selection of oocyte cryopreservation method is a prerequisite factor for developing an effective bank system. Compared with slow freezing method, the vitrification has various advantages such as avoiding intracellular ice crystal formation. We previously reported that mouse mature oocytes can survive and develop to the blastocyst stage after vitrfication and thawing using ethylene glycol (EG) and an electron microscope (EM) grid. However, a high incidence of spindle and chromosome abnormalities was detected in thawed oocytes after vitrification. We examined whether the addition of a cytoskeleton stabilizer, Taxol™, to the vitrification solution could promote the post-thawed survival and subsequent development of stored oocytes.

Materials and Methods: Cumulus-enclosed oocytes (CEOs) were collected from ICR mice superovulated by PMSG and hCG injections. CEOs were pre-equilibrated in Dulbecco,s phophate buffered saline (DPBS) with 1.5 M EG with and without 1 μM Taxol™. Oocytes were vitrified with 5.5 M EG and 1 M sucrose containing DPBS with or without 1 μM Taxol™. CEOs were then loaded onto EM grid for storing in liquid nitrogen. Stored oocytes were thawed by a five-step method. Vitrified-thawed oocytes were then fertilized in vitro with epididymal semen and cultured in a chemically defined, modified preimplantation-1 medium up to 124h after IVF. Some blastocysts were transferred to synchronized recipients.

Results: More oocytes developed to the 4-cell (44.7% vs. 69.7%), 8-cell (31.8% vs. 64.2%), morula (24.7% vs. 54.3%), and blastocyst (20.3% vs. 49.2%) stages after the addition of Taxol™ to the cryopro-