저작자표시-비영리-변경금지 2.0 대한민국

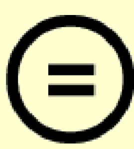
이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

전 용 필 교수 지도

석사학위 청구논문

Physiological statics specific expression of Lysyl Oxidase(LOX) during early pregnancy in mouse uterus

2017

성신여자대학교 대학원

미래응용과학학과

황 연 정

Physiological statics specific expression of Lysyl Oxidase(LOX) during early pregnancy in mouse uterus

Adviser: Yong-Pil Cheon, Ph.D.

Submitted in partial fulfillment of the requirements for the degree of master.

November, 2016

Sungshin University

Graduated School

Department of Next Generation Applied-Sciences

Hwang, Yeon Jeong

Certificate of Committee Approval

Be accepted partial fulfillment of the

requirements for the degree of:

Master of Science

Signatures:

Chairpersion Kim, Hae Kwon, Ph.D

Committee member Yang, Hyunwon, Ph.D

Committee member Cheon, Yong-Pil, Ph.D

Sungshin University

Graduate School

논 문 개 요

시스템 수준에서 스테로이드 호르몬 농도의 순차적 변동은 배아의 착상에 매우 중요한 요소로 알려져 있으며 이것은 성공적인 implantation window 의 개시를 이끌어 낸다. 이는 자궁 내 미세환경의 변화를 동반하는데 이러한 변화를 위해서는 스테로이드 호르몬의 직접적 또는 이를 매개하는 여러 성장인자, 그리고 사이토카인 등이 관여하는 것으로 알려져 있다. 착상전후로 관찰되는 자궁내막의 조직학적 변화는 내막 상피의 증식과 분화, 기질세포의 증식과 분화를 동반한다. 이러한 형태적, 기능적 변화는 포배의 착상, 침입을 조절하고 태반의 형성을 가능하게 하며 모체를 보호하는 기능을 수행한다. 이러한 탈락막화 현상에 대한 분자수준의 연구와 세포학적 연구가 다양한 측면에서 진행되어 왔다. Microarray 와 *Bax* 유전자 적중 t 생쥐를 이용한 사전연구에서 세포의 기질중 하나인 Lysyl oxidase(*Lox*) 를 포함한 몇몇 유전자가 임신 5 일째의 생쥐의 자궁에서 유의적인 발현 변이가 관찰되었다. LOX 는 copper-dependent 한 monoamine 산화효소로 아래와 같은 기능들이 알려져 있다: collagen 과 elastin 의 교차결합을 조절, 세포 기질 외 결합조직 기능과 구조적 안정을 조절하며 종양에서 전이의 억제 현상을 촉진하는 기능이 보고되었다. 이 연구에서는 *Lox* 가 생쥐의 자궁내 에서 스테로이드 호르몬 의존적 발현 여부, 임신 시기 및 조직 특이적 발현 양상을 알아보고자 하였다. *Lox* 는 난소제거술을 수행한 생쥐 모델에서 에스트로겐에 의해 발현량이 급증했고, 길항제인 ICI 182,780 에 의해 억제되었다. 지연착상 모델에서 에스트로겐 투여 후 0.5 시간이 경과했을 때 발현이 급증하였고 이후 감소하였다. 한편 초기 임신기간 동안 LOX mRNA 수준은 착상시기에 피크를 보였다. 또한 Westrern blotting 결과에서는 5 일째에 최고 발현을 나타냈다. LOX protein 의 위치는 착상 이전에는 자궁내 상피와 내막분비샘에 매우 약한 분포를 보였으나 임신 4 일째는 분비샘, 5 일째에는 맞자궁간막부위

에 강하게 표지되었다. 탈락막 반응이 왕성해 지는 시기에는 1 차 탈락막 부위를 중심으로 강하게 표지되었다. 이러한 결과들은 Lox 의 발현이 자궁에서 에스트로겐의 조절을 받아 발현하고 착상에서 중요한 역할을 할 것을 암시한다. 또한 더 많은 연구가 진행되어야 하겠으나 임신시기 조직특이적 발현 양상은 LOX 가 착상과정에서 자궁의 분화나 배아의 침입과 관련하여 중요한 역할을 할 것으로 사료된다.

CONTENTS

Abstract (Korean)

List of Tables

List of Figures

Introduction ································· **1**

Meterials and Methods ································· **3**

- Experimental animal ································· **3**
- Ovariectomy and steroid administration ································· **3**
- Delayed implantation model ································· **3**
- Uterus sampling ································· **4**
- Total RNA extraction ································· **4**
- First strand cDNA synthesis and PCR analysis ································· **5**
- Real-Time PCR ································· **5**
- Western blotting analysis ································· **6**
- Immunohistochemistry ································· **7**
- Statics ································· **8**

Result

- LOX mRNA expression was induced by estrogen ································· **12**
- Expression profiles of LOX mRNA during early pregnancy ································· **15**

Expression profiles of LOX mRNA during early pregnancy **17**

Immunohistochemistry of LOX in pregnant uterus **19**

Discussion **22**

Reference **23**

Abstract (English) **25**

List of Tables

Table 1. Real-time thermal cycler information for real-Time PCR ········ **9**

Table 2. Primer information for Real-Time PCR ········ **10**

Table 3. Antibody information for western blot and immunohistochemistry ········ **11**

List of Figures

Figure 1. Expression profiles of LOX mRNA in the uterus of ovariectomized mice ······ **13**

Figure 2. Expression profiles of LOX mRNA in uterus of delayed implantation mice ······ **14**

Figure 3. Expression profiles of LOX mRNA in the uterus of early pregnant mice ······ **16**

Figure 4. Western blotting analysis of LOX during early pregnancy ······ **18**

Figure 5. Photomicrograph of immunohistochemistry for LOX······ **20**

INTRODUCTION

In placenta vera species, embryo implantation is critical event for successful pregnancy. In mouse, blastocyst attach on luminal epithelium of uterus at day 4 of pregnancy (Cha et al., 2012). Nidation of embryo needs a specific uterine microenvironment, called decidualization which uterine stroma cell converted to polyploidy decidua cell (Ramathal et al., 2010). Estrogen and progesterone regulate a series of complex interaction in interface between a developing embryo and cells in the stromal compartment. These events lead to formation of decidua (Cheon et al., 2002).

In pregnant mouse uterus, estrogen level is increased on day1 of pregnancy, by preovulatory estrogen surge. Second day it is decrease and reaches approximately 15 pg/ml by third day (Das et al., 2009). On day 4 of pregnancy, can induce embryo attachment to luminal epithelium of uterus. During pregnant day 5-7, estrogen is synthesized in decidual tissue (Atkinson et al., 1945).

Our previous microarray analysis revealed several genes which are expected to be influential in decidualization, one of the genes is lysyl oxidase(LOX). LOX are copper-dependent monoamine oxidases secreted by fibrogenic cells including fibroblasts and smooth muscle cells. LOX is considered as a tumor suppressor gene. Expression of transfected *LOX* cDNA suppresses *Ha*-*ras*-induced cell transformation indicating a ras-suppressor effect of LOX(Barker et al.,2012). BAPN which LOX inhibitor, leads to abnormal cross-linking of collagens and elastin, and other varient disease (Maki et al.,2005).

LOX family members are LOX, LOXL (Lysyl oxidase like protein) 1, LOX 2, LOXL 3, and LOXL 4. They contain a highly conserved catalytic domain, copper-binding domain, lysyl-tyrosyl-quinone cofactor (LTQ) and cytokine receptor like (CRL) domain. All these factor compose conserved C-terminal (Lucero et al.,

2006). LOX and LOXL 1 contain pro-sequence and this sequence enable their secretion an inactive form of pro-enzymes. LOXL 2-4 contain scavenger receptor cysteine rich (SRCR) domain which involve in cell adhesion and protein-protein interaction (Trackman et al., 1992).

Most of the studies on LOX have focused on the specific cross-linking activity and catalytic mechanism of action of this enzyme on the extracellular matrix substrates, collagen and elastin. LOX participates in the critical post-translational modification essential to the biogenesis of connective tissue by deaminating the side chains of lysine residues in these proteins.

It is known that, during pregnancy day 8-10, expression levels of *Lox* mRNA decrease 8 to 10-fold in the vagina. Its level remain suppressed throughout gestation days. Changes in *Lox* mRNA level were accompanied by matrix remodeling in the vaginal wall and E2 treatment resulted in significant increases in *Lox* mRNA. This suggested that estrogen is a regulator of *Lox* in vagina wall and female reproductive tissue (Drewes et al., 2005). Although, *Lox* expression is under the control estrogen in vagina, it's expression or roles were not revealed.

In this study, the *Lox* expression regulation by estrogen was examined. To evaluate the possible roles in pregnancy, the expression profiles of mRNA and protein were analyzed in pregnant uterus.

MATERIALS AND METHODS

Experimental animals

Involving experiment animals were conducted according to the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health. CD-1mice were maintained under standard condition at Sungshin Women's University diurnal rhythm kept under the 14L : 10D schedule with light-on at 06:00 hr and clean room system. Animals were fed a standard rodent diet and water *ad libitum* from weaning at 21 days after birth.

Ovariectomy and steroid administration

6 weeks old CD-1 female mice were ovariectomized and administrated with sex steroid hormones after 2weeks later. 17β-estradiol(Sigma, Cat #: E8875, 2 μg/kg, Body weight) or progesterone(Sigma, Cat #: P0130, 40 μg/kg Body weight) were administrated into subcutaneous for 3days at 0830. E2+P4 were prepared one day E2 administration and two days more P4 administration. The mice were sacrificed at next day. And ICI182780 (Tocris, 0.0027 mg/0.1ml), RU486 (Tocris, 0.405 mg/ 0.1ml) were used for E2, P4 antagonist.

Delayed implantation model

Female CD-1 mice were superovulated by injection of 2.5IU PMSG and followed by injection of 2.5IU hCG after 48hr and then followed by mating with stud male mice. The next day morning, these mice were examined for the

check of vaginal plug, and this was defined day 1 of pregnancy, At day 4 of pregnancy, ovariectomized at the morning (0830) and day5, 6, and 7 of pregnancy were P4(2 mg/0.1 ml sesame oil) subcutaneous injected and followed E2 (25 ng/0.1 ml sesame oil) injected at the morning(0830). The mice were sacrificed to collect uteri on time dependent(P4 only (=0 hr), 0.5hr, 1hr, 1.5 hr, 3hr, 6hr, 9hr, and 12hr post E2).

Uterus sampling

Female CD-1 mice were mated with normal fertile male of the same strain. At the morning 9:00am. these mice were examined for vaginal plug check. And this was defined as day 1 of pregnancy. The mice were sacrificed to collect uterus on day 1-7, 9, and 12 of pregnancy. Each pregnancy day was confirmed by recovering embryos from the reproductive tracts at form 10:00 to 11:00 am. The embryos were removed from oviducts or uterus by flushing with DEPC-treated PBS. The uterus were frozen in liquid nitrogen and kept at -80℃until used for RNA and protein extraction

Total RNA extraction

Total RNAs of natural pregnant uterus were extracted using TRIzol reagent (Invitrogen, Cat #: TR 118, San Diego, CA, USA) according to manufacturer's instruction with modification. In short, the collected samples were homogenized with Trizol reagent (1 ml/100 mg) and stored for 10 min at room temperature (RT). The chloroform of 0.2 ml/ml TRIzol reagent were added to the homogenates and shaked vigorously for 15 sec. After then, the mixture kept for

15 min at RT.

and centrifuged 12000g for 15 min at 4℃. RNA in the aqueous phase was transferred into new tube, the RNA was precipitated by mixing 0.5 ml isopropyl alcohol, mixed gently, kept for 10 min at RT. After then, centrifuged 12,000g for 8 min at 4℃. After that, discard a supernatant, The RNA pellet was washed briefly in 0.8ml 75% ethanol, mixed by inverting, and centrifuged 7,500g for 5 min at 4℃. The supernatant was removed, dried to remove ethanol and added 50 µl DEPC treated water. Total RNA were assessed by NanoDrop 2000/2000c Spectrophotometer (Thermo, Cat #: ND-2000 Willington, DE,USA).

First strand cDNA synthesis

In order USA to perform reverse-transcription, 5µg total RNAs were used. First strand cDNAs were synthesized using Accuscript first strand cDNA synthesis kit (Stratagene, Cat #: 200436, CA, USA) according to the manufacturer's instruction. In short, reaction reagents were 5µg total RNA, 5.0 µl Accuscript buffer (10X), 1.0 µl oligo dT primer (0.5µg/µl), 1.0 µl random primers (0.1µg/µl), 2 µl dNTP mix (100mM), RNase- free water. Reaction mixture was incubated at 65℃ for 5min, placed the tube at RT to allow the primers to anneal to RNA for 10min, after then added 4.0 µl DTT(100mM), 2.0µl RNase block ribonuclease inhibitor (Agilent, Cat #: 300152-51, USA, 40U/ml), and 1.0µl Accuscript multiple temperature RT. The mixture was incubated at 42℃ for 1 hr and 70℃ for 15min to terminate cDNA synthesis.

Real-Time PCR

Real-Time PCR was performed using SYBR Premix Ex Taq™ (TaKaRa, Cat #: RR420, Japan) and Thermal Cycler Dice Real Time System TP800 (TaKaRa, Cat #: TP800, Japan). Each reaction was run in triplicate and consisted of 1 μl cDNA. Dissociation curves were run on all reactions to ensure amplification of a single product with the appropriate melting temperature. The fold change in gene with the appropriate melting temperature. The fold change in gene expression was calculated using the △△Ct method with the housekeeping gene, 36B4, as the internal control.

Protein extraction and Western blotting analysis

Before protein extraction, tissue were washed using cold Y-PBS (0.7 mM PMSF, 1 mM Benzamidine-HCl, 4μg/ml Leupeptin, 2 μg/ml Aprotinin, 2 mM EDTA). Uterine stroma cell were homogenized in cold homogenization buffer (50 mM Tris-Cl, 150 mM NaCl, 10 mM β-mercaptoethanol, 2 mM $CaCl_2$, 0.1 mM PMSF, 1μM Leupeptin, 1 μM Pepstatin, 0.5 mM EDTA, 15% Glycerol, 0.1% NP-40). The homogenates were centrifuged to remove insoluble materials. The protein concentration was determined using protein dye reagent (Bio-Rad Laboratories, Inc., Richmond, CA) by Bradford assay. 30㎍ /ml of protein were boiled in SDS/β-mercaptoethanol sample buffer, and loaded onto each lane of 10% SDS-PAGE. The proteins were separated by electrophoresis and then electrotransferred onto Immunobilon-P membranes (Merk KGaA, Damstaldt, Inc., Germany) in transfer buffer (25 mM Tris base, 192-

mM Glycine, 0.1% SDS, 20% Methanol, pH 8.3). The membranes were blocke din 5% skimmed dry milk in TBST buffer (10 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20) for 1 hr at RT, and washed three times with TBST. The membranes were incubated for 1 hr with rabbit polyclonal MISRII antibody (dilution 1:500); rabbit monoclonal beta actin antibody (dilution 1:500); rabbit ploycIonal progesterone receptor antibody (1:500). After incubation, membranes were washed three times and incubated for 30 min with horseradish peroxidase conjugated goat anti-rabbit IgG (dilution 1:1000). The bands were detected using ECL solution (GE Healthcare, Little Chalfont, UK) by Retina OXE Film (CARESTREAM HEALTH, INC, Cat #: 655 0701, New York, USA) in darkroom.

Immunohistochemistry

Dissected mouse uterine horns were cut into 0.5 segments, fixed in 4% formaldehyde for 24 hr and embedded in paraffin. 4μm sections were mounted on glass slides and subjected to antigen retrieval in boling 10mM citriate buffer (pH 6.0) for 13min. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in H_2O for 30min. LOX immunoreactivity was detected according to the Vectastain ABC kit method(Vector Laboratories, Inc., Burlingame, CA). Briefly, tissue were incubated with 1% normal blocking serum in PBS for 1hr and then incubated with goat monoclonal LOX antibody (dilution 1:1000); After washing in PBST, tissues were incubated with biotinylated anti-goat IgG; Tissues were washed with PBS and incubated with avidin-biotin-

complex reagent containing horseradish peroxidase for 30min. Slides were washed with PBS for 5min and color development was achieved using DAB substrate. The tissue section was counterstained with hematoxylin.

Statistics

All experiments were conducted at least in triplicate. The Student's t-test was performed to evaluate the statistical significance between control and experimental group. Results were presented as mean ± SEM. Values of $P < 0.05$ were considered to be significantly different.

Table 1. Thermal cycler schedule

step		Temperature (℃)	Time
Hold	Hold	95	30 sec
3 step PCR (45 cycle)	Denaturation	95	1 min
	Annealing	59	30 sec
	Extension	72	1 min
Dissociation	Denaturation	95	15 sec
	Annealing	60	30 sec
	Extension	95	15 sec
Hold		4	Indefinitely

Table 2. Sequence- specific of primers

Gene	Symbol	NCBI gene reference		Primer sequence(5'-3')
Lysyl oxidase	LOX	NM_010728	S AS	CAATCCCTACAAGTACTCCGACGAC TGACATCCGCCCTATATGCTGA
Ribosomal protein, large, P0	Rplp0	NM_007475	S AS	CGACCTGGAAGTCCAACTACTTCCT GCACCTTATTGGCCAACAGCAT

Table 3. Antibodies information

name		company
LOX	-Rabbit monoclonal	Abcam
β-Actin	-Mouse monoclonal	Sigma
Goat Anti-Rabbit IgG-HRP conjugate		Bio-Rad
Mouse Anti-Mouse IgG-HRP conjugate		Santa cruz

RESULTS

Estrogen treatment induced Lox expression in uterus

Steroid hormones regulate implantation specific genes. Lox is expected to have a role in implantation. Its expression regulate by steroid hormones was examined. of uterine genes. Ovariectomized mice were administrated with E2, P4 E2+P4, E2+ICI 182,780 and P4+RU486 as mentioned in Material and Method. Real-time PCR result showed that Lox expressed with basal levels in the uterus of OVX mice. Its expression was increased by E2 administered mice (Fig.1). In addition ICI 182,780 suppressed Lox expression (Fig.1). In other groups, P4 or E2+P4 did not induce Lox expression. In delayed implantation induced mice, the expression of Lox was induced by E2 administration at 0.5hr (Fig.2).

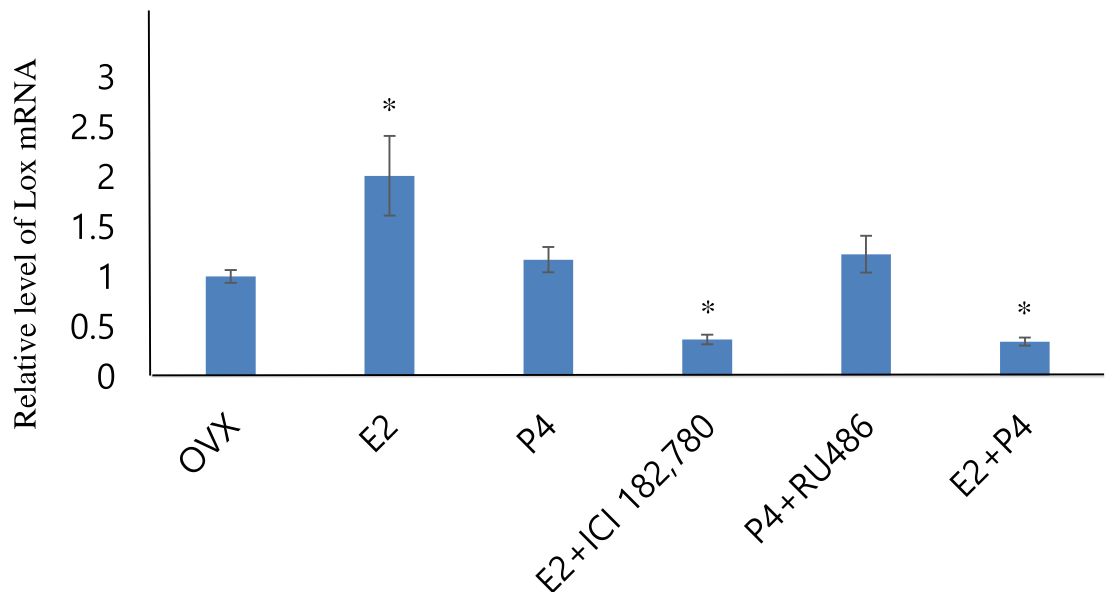


Fig 1. Expression profile of Lox mRNA level in ovariectomy mouse model

Lox mRNA expression level is increaseed by estrogen and suppressed by ICI 182,780

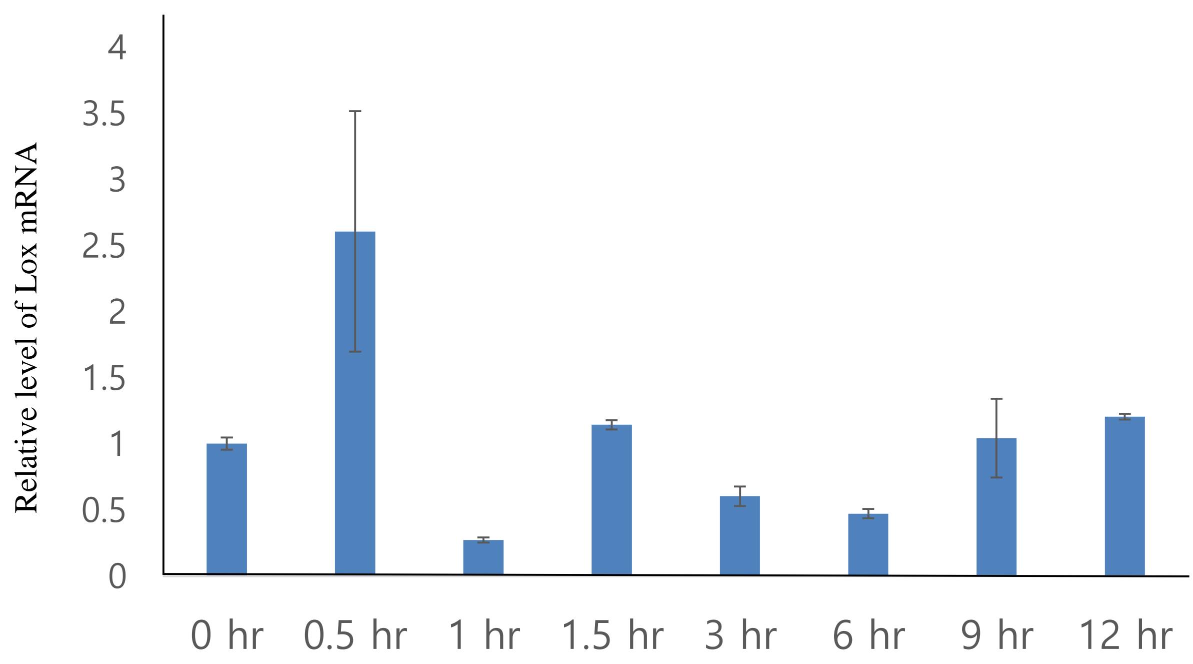


Fig 2. Expression profile of Lox mRNA level in delayed implantation mouse model.

Lox mRNA expression level is increased by E2 administration with 0.5hr

Lox mRNA level was dependent on implantation.

To evaluate the expression change during early gestational day, Real-Time PCR was performed, and 36B4 for used for internal control. Expression level were increased from day5 of gestation and decreased until day 12 of gestation (Fig.3).

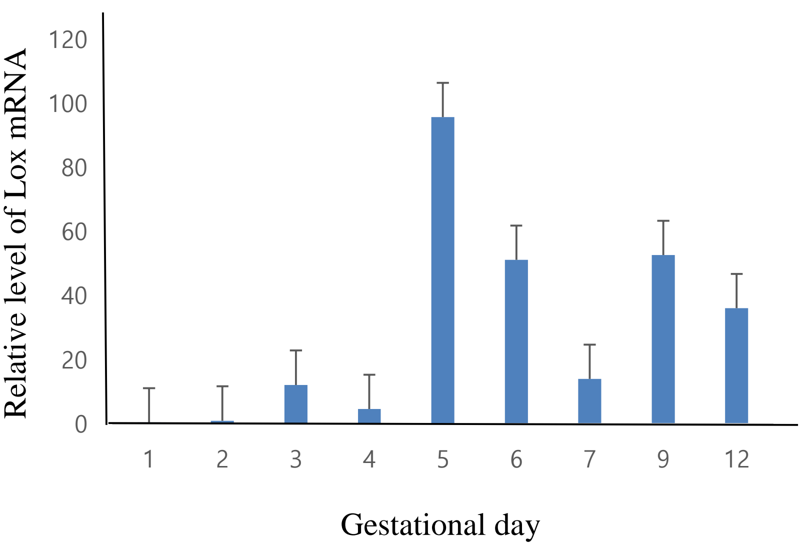


Fig 3. Lox mRNA level during early gestational day.

Lox mRNA expression level is increased on day 5

Profiles of Lox protein were similar with the patterns of mRNA expression level.

To evaluate the Lox protein levels during early pregnancy, Western blot assay was performed, as mentioned in Material and Method.

Lox protein levels were increased after day 4 of gestation, decreased until day7. Its level increased again on day12 of gestation (Fig. 4B).

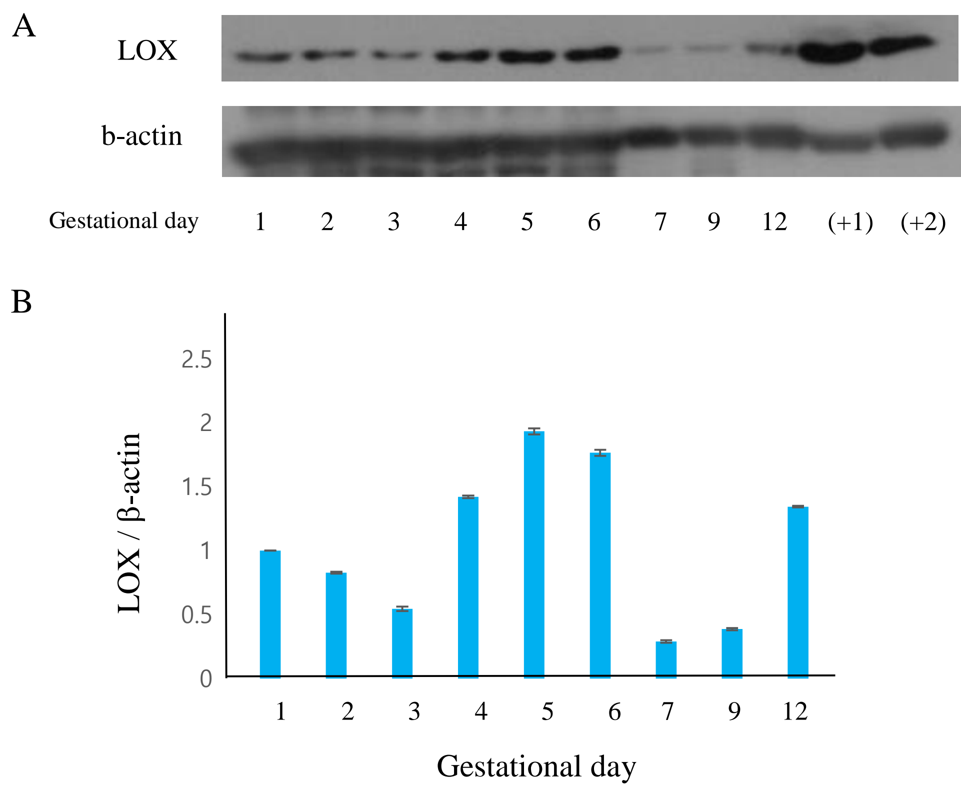


Fig 4. Profiles of Lox proteins, during pregnancy

Western blot analysis (A) was performed with . Its was normalized with b-actin (B). The levels of Lox protein were measured during implantation.

Immunohistochemical analysis in pregnant uterus

To evaluate the possible roles of Lox during implantation, IHC was performed with Lox specific antibody. From day 1 to 3, LOX was localized weakly in luminal epithelium and endometrial gland (Fig.5A,B,C). On day 4 of gestation, it was localized strongly in luminal epithelial and glandular epithelial cell (Fig.5D). On day 5 of gestation, Lox was localized in luminal epithelial cells and stromal cell next to implanting embryo (Fig.5E). Intensity was increased on day 6 at primary decidual zone (Fig.5F). On day 7, LOX was localized in cytoplasm and extracellular matrix (Fig.5G). On day 9 and 12 of gestation, LOX was localized in decidua (Fig.5H, I).

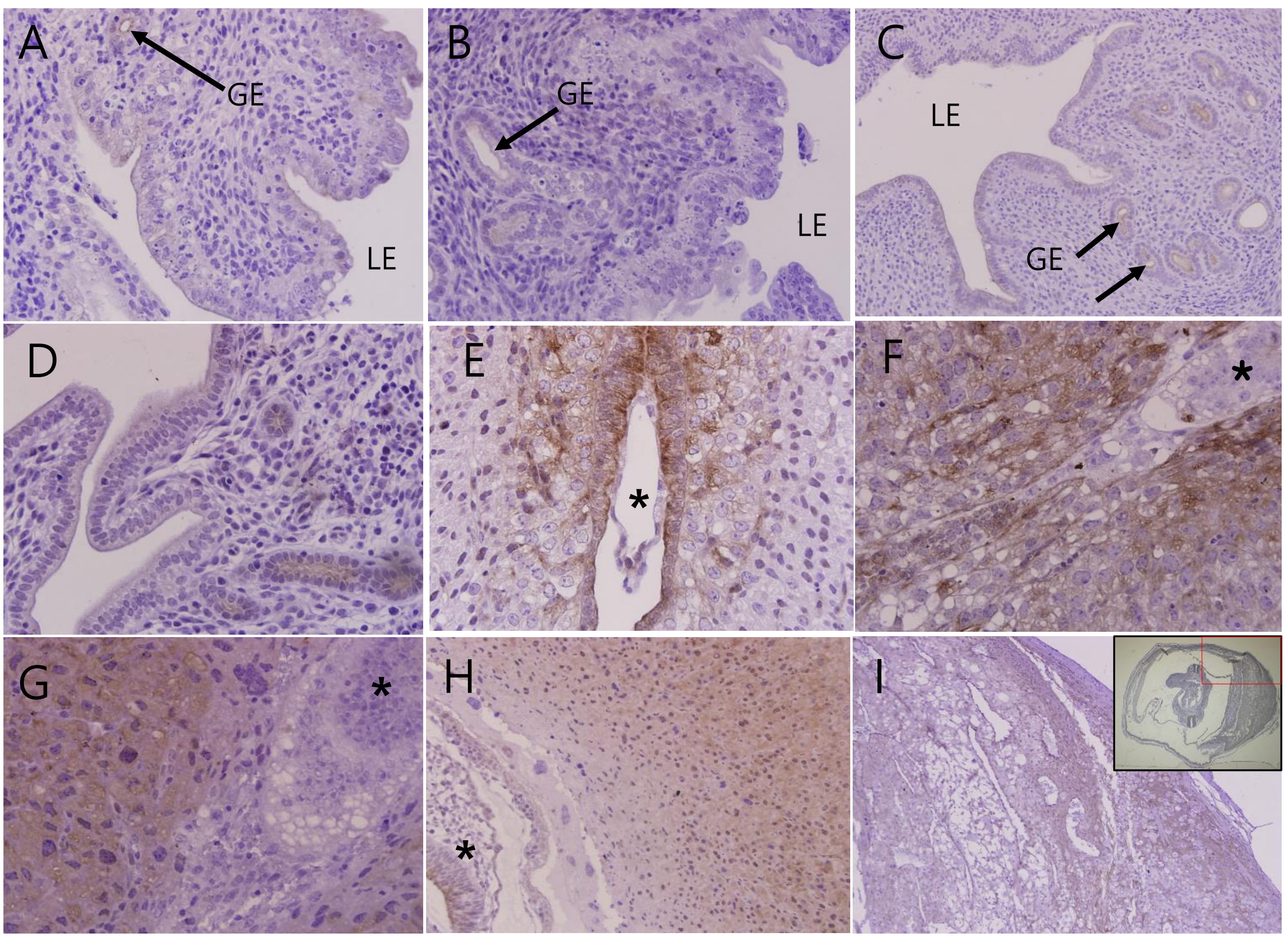

A
GE
LE
B
GE
LE
C
LE
GE
D
E
*
F
*
G
*
H
*
I

Fig 5. IHC of Lox during early pregnancy.

Immunohistochemistry was performed with DAB staininig. A) gestation day 1, (x400), B) day 2, (x400), C) day3, (x400), D) day 4, (x400), E) day 5, (x400), F) day 6, (x400), G) day 7, (x400), H) day 9, (x200), I) day 12, (x100). (box, x16) GE;glandular epithelium, arrow heads indicates GE, LE;luminal epithelium, *;embryo.

DISCUSSION

It has been suggested that estrogen is essential in preparation of pregnancy in mammals (Liang et al., 2010) and implantation. It is well explaned with delayed implanation model mice. In this model, dormant embryos can be implanted to the uterine luminal epithelial cells by E2 administraiton. Using high-throughput methodology, the E2 regulated genes have been explored but, so far, their role are not much uncovered. The remodeling of ECM is important to successful implantation. ECM components and structural integrity in the uterus during pregnancy, are changed by the various physiological status (Luce et al., 1964). A few of studies revealed that estrogen upregulate collagen synthesis in uterus (Woessner et al., 1972).

We performed ovariectomy and treated E2, P4 and their antagonists. *Lox* mRNA levels was dramatically induced by E2 but not P4 or E2+P4. In addition, ICI 182,780 suppressed the expression of *Lox* mRNA. In delayed implantation model, *Lox* expression was induce by E2 administration. E2 downstream genes such as *Msx1*, *Lif*, *Cox2*, *Bmp* are critical in blastocyst implantation (Daikoku et al., 2011). A well-known function of LOX is enzyme activity for polymerization collagen fibers and elstic fiber. These suggest that *Lox* may be important in blastocyst implantation.

LOX protein level was increased from day 4 and peaked on day 5 of gestation. This result is similar pattern to mRNA level of *Lox*. From day 1 to 3, it was faintly localized in luminal epithelia and glandular epithelium. On day 4 of gestation, it

was localized strongly in GE, and LE. On day 5 of gestation, LOX was mainly localized in ECM region of primary decidual zone. Such a localization patterns mean that LOX is involved in decidualization and implantation.

On summary, Lox expression is under the control of estrogen in uterus. *Lox* mRNA and protein levels were increased at the time of implantation. LOX was localized in decasualized zones. Put together, we know that *Lox* is one of the E2 downstream genes. In addition, it is suggested that LOX has a critical role in decidualization and early pregnancy.

REFERENCE

Atkinson WB, Hooker CW. 1945. The day to day level of estrogen and progestin throughout pregnancy and pseudopregnancy in the mouse. Anat Rec 93:75-95.

Barker HE, Cox TR, Erler JT. 2012. The rationale for targeting the LOX family in cancer. Nat Rev 12:540-552

Carson DD, Bagchi IC, Dey SK, Enders AC, Fazleabas AT, Lessey BA, Koji Y. 2000. Embryo Implantation. Dev Biol 223:217-237

Cha J, Sun X, Dey SK. 2012. Mechanisms of implantation: strategies for successful pregnancy.Nat Med 18:1754-1767

Csiszar K. 2001. Lysyl oxidases: A novel multifunctional amine oxidase family. Prog Nucleic Acid Res Mol Biol 70:1-32

Daikoku T, Cha J, Sun X, Tranguch S, Xie H, Fujita T, Hirota Y, Lydon J, DeMayo F, Maxson R, Dey SK. 2011. Conditional deletion of MSX homeobox genes in the uterus inhibits blastocyst implantation by altering uterine receptivity. Dev cell 21: 1014–1025.

Das A, Raju S, Kannan A, Dean B. Evans, Bagchi MK, Bagchi IC. 2009. De novo synthesis of estrogen in pregnant uterus is critical for stromal decidualization and angiogenesis. Proc Natl Acad Sci 106:12542-12547

Drewes PG, Marinis SI, Acevedo J, Keller P, Schaffer JI , Word RA. 2005. Regulation of lysyl oxidase in the vaginal wall: role of estrogen, progesterone, and pregnancy. J Pelvic Surg 11: S8-S9

Harper MJ. 1992. The implantation window. Baillieres Clin Obstet Gynaecol 6:351-371

Liang XH, Zhao ZA, Dery WB, Tian Z, Lei W, Xu X, Zhang XH, Su RW, Yang ZM. 2010. Estrogen regulates amiloride-binding protein 1 through CCAAT/enhancer-binding protein-beta in mouse uterus during embryo implantation and decidualization. Endocrinology 151:5007-5016.

Luce S, Hutton R. 1964. An electron microscopic study of the fate of collagen in the post partum rat uterus. Anat Rec 148:308-314.

Maki JM, Sormunen R, Lippo S, Kaarteenaho-Wiik R, Soininen R, Myllyharju J. 2005. Lysyl oxidase Is essential for normal development and function-of the respiratory system and for the Integrity of elastic and collagen fibers in various tissues. J Am Soc Cytopathol 167:927-936.

Paffaro VA Jr, Bizinotto MC, Joazeiro PP, Yamada T. 2003. Subset classification of mouse uterine natural killer cells by DBA lectin ractivity. Placenta 24: 479-488.

Ramathal CY, Bagchi IC, Taylor RN, Bagchi MK. 2010. Endometrial decidualization of mice and men. Semin Reprod Med 28:17-26

Rodriguez C, Rodriguez-Sinovas A, Martinez-Gonzalez J. 2008 . Lysyl oxidase as a potential therapeutic target. Drug News Perspect 24:218-224

Ryan JN, Woessner JF Jr, 1972. Oestradiol inhibits collagen breakdown in the involuting rat uterus. Biochem. J 127:705.

Trackman PC, Bedell-Hogan D, Tang J, Kagan HM. 1992. Post-translational-

glycosylation and proteolytic processing of a lysyl oxidase precursor. J Biol Chem 267:8666-8671.

Wang H, Dey SK. 2006. Roadmap to embryo implantation:clues from mouse models. Nat Rev Genet 7:185-199

Zong W, Jiang Y, Zhao J, Zhang J, Gao J. 2015. Estradiol plays a role in regulating the expression of lysyl oxidase family genes in mouse urogenital tissues and human Ishikawa cells. J Zhejiang Univ Sci B 10: 857-864

ABSTRACT

Role of Lysyl oxidase(LOX) in uterine receptivity and implantation

Hwang, Yeon Jeong
Department of Next Generation Applied-Sciences
Graduate School
Sungshin Women`s University

Steroid hormonal levels during early stage of pregnancy are critical in embryo implantation and it leads to open the successful implantation window. Uterine stroma cell undergoes specific changes, called decidualization during implantation. Using microarray methodology, we identified a few genes including LOX which are highly expressed in the 5 day of pregnant uterus of mouse. LOX are copper-dependent monoamine oxidases. The known functions of LOX including follows: regulating cross-linking between collagen and elastin, controlling the structural stability and function of connective tissues, and inhibiting the function of ras. The other known function of LOX is the dual role in suppressor of tumor gene expression and promotor of metastasis. In this study, the expression profiles of Lox and cell specific localization of it were examined using Real time PCR, IHC, and Western Blot assay. Expression profiles of LOX

mRNA were dramatically changed by pregnancy and E2 surge. Its expression levels were increased after implantation. LOX protein was localized at luminal and glandular epithelial cells before implantation. It was localized at decidualizing stromal cells after implantation. These results suggest that E2 is the main regulator of *Lox* expression in the uterus. LOX protein expression profiles showed specio-temporal expression and those results suggest that LOX has a funciton in implantation and uterine stromal cell differentiation.