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Expression of ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1 Genes in Mouse Uterus During Periimplantation Period

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착상 전후시기의 생쥐 자궁조직에서의 ADAM-8, 9, 10, 12, 15, 17과 ADAMTS-1 유전자의 발현

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연구목적: ADAMs은 metalloprotease/disintegrin domain을 가진 transmemebrane glycoprotein으로써 지금까지 30개 이상의 ADAM 및 10개 이상의 ADAMTS가 알려져 있다. 이들의 기능은 포유동물의 수정 시 sperm-egg binding과 fusion, myoblast fusion, integrin과의 결합 등에 직접 관여하거나, TNF-alpha 등의 생체신호전달물질이 세포로부터 분비될 때에 이들의 구조를 변화시켜 활성화시키는 효소작용, 그리고 dendritic cell differentiation 등에 관여하는 것으로 알려져 있다. 그러나 자궁내막 조직에서의 유전자 및 단백질 발현 여부에 관해서는 거의 보고되어 있지 않고 있다. 본 연구에서는 착상 전후 시기의 생쥐 자궁조직에서 ADAM-8, 9, 10, 12, 15, 17 그리고 ADAMTS-1의 유전자가 발현하는 지를 알아보았다.

연구 재료 및 방법: 본 연구에서는 생쥐의 자궁조직을 대상으로 ADAM-8, 9, 10, 12, 15, 17 그리고 ADAMTS-1을 선정하여, 초기 임신 기간에서의 유전자 발현 여부를 조사하였고 이 결과를 바탕으로 자궁조직에서의 이들 유전자들의 생리적인 기능을 규명하고자 하였다.

결 과: 임신한 생쥐 자궁조직에서의 ADAM-8, 9, 10, 12, 15, 17 그리고 ADAMTS-1의 유전자 및 단백질의 발현 양상을 RT-PCR 방법을 이용하여 알아본 결과, 조사된 ADAM 종류와 임신 날짜별로 다르게 나타났다. ADAM-8의 유전자 전사체는 임신 1일째 매우 강하게 발현되었으나 임신 3일째로 진행되면서 감소하다가 이후 다시 임신 5일째가 되면서 증가하는 양상을 보였다. ADAM-9, 10, 17 그리고 ADAMTS-1의 경우는 임신 1일째에서 5일째까지 유전자의 발현 양상이 크게 변하지 않았고 ADAM-12와 ADAM-15의 유전자 전사체는 임신 1일에서 5일로 진행되면서 현저하게 증가되는 양상을 보였다. 이후 임신 6일에서 8일에서는 생쥐 배아가 착상된 부위와 비 착상부위로 나누어 유전자의 발현 양상을 관찰한 결과, 조사된 ADAM 모두 비착상 부위보다 착상부위에서 유전자 전사체의 발현이 크게 증가되는 것으로 나타났다.

결 론: 이상의 결과로 미루어 ADAM 유전자는 임신초기 착상과정과 임신 단계에 따른 자궁의 조직 재구성에 중요한 역할을 할 것으로 생각된다.

Key Words: ADAM, Gene, Uterus, Implantation

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Mammalian uterus is a dynamic tissue that undergoes a cyclic degradation and renewal during the reproductive cycle and a drastic remodeling during pregnancy. At the beginning of implantation, endometrial fibroblasts surrounding the embryo transform into an epithelioid type, a reaction called decidualization, which is dependent on the priming by ovarian steroid hormones. Decidualization in murine endometrium involves cell growth and a severe reduction of the extracellular spaces accompanying modifications of many extracellular matrix (ECM) components. In mice, progressive loss of laminin and type IV collagen in the uterine luminal epithelial basement membrane occurs in a consistent spatiotemporal pattern following the onset of blastocyst implantation which is closely correlated with the area occupied by decidualized endometrial stroma and occurs in areas not yet in contact with trophoblast cells.[1] Among factors involved in the rearrangement of ECM components accompanying decidualization, matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinase (TIMPs) have been studied well and are known to play important roles in the uteri of many mammals.[2] In mice, early pregnancy is associated with the expression of mRNA and positive immunoreactivity for multiple MMPs, including MMP-2, 3, 7, 9, 11, 13 and membrane type-MMP-1.

ADAMs (a disintegrin and metalloproteas) are a unique family of protein members consisting of a prodomain, metalloprotease, disintegrin-like and cysteine-rich domains, and, in most cases, epidermal growth factor-like, transmembrane, and cytoplasmic domains. To date more than 30 ADAM genes have been found in mouse genome, and about 39 ADAM family members have been reported until recently. 17 members of the ADAM family have an active metalloprotease site as deduced by amino acid sequencing and enzymatic activity assay, while other members lack functional protease activity.[3] With respect to ADAMs having a metalloprotease active site, ADAM-17 cleaves 26 kDa membrane-anchored tumor necrosis factor α (TNF-α), which proteolytically releases an active soluble 17 kDa extracellular domain.[4,5] Moreover, ADAM-10/Kuzbanian is responsible for the release of a soluble form of Delta, a Notch ligand.[6] ADAM-9/metrin-γ/MDC9 has been reported to shed membrane-anchored heparin-binding epidermal growth factor-like growth factor.[7] Some of the ADAM members that are believed to lack protease activity exhibit cell adhesion activity. ADAM-2/fertilin-β and ADAM-3/cyritestin play a pivotal role in sperm-egg binding and fusion in many mammals.[8] ADAM-12 and -15 have been reported to interact with integrin $\alpha_9\beta_1$[9] and human ADAM-23 has been suggested to interact with integrin $\alpha_v\beta_3$.[10] ADAM-9, in addition to its protease activity, has also been reported to interact with integrin $\alpha_v\beta_5$.[11] ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) is a new family of the ADAM-related proteins. They differ from other ADAMs by having a unique characteristic - a thrombospondin type 1-repeat motif lying between the disintegrin and the cystein-rich domain. ADAMTS-1/METH-1 is the prototype of the currently known 30 members, and plays an important role in normal growth, fertility and organogenesis.[12] ADAMTS-5/aggrecanase-2 and ADAMTS-6 are exclusively expressed in the placenta suggesting their possible role during implantation in mice.[13] In light of these findings and others, the proteolytic and/or cell adhesion activities of ADAM and ADAMTS proteins are viewed as being required for the remodeling of various tissues. However, the role of ADAM proteins in mouse uterine tissue remodeling, particularly regarding implantation, is poorly understood.

In the present study, whether genes of ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1 might play a role during early pregnancy in mouse uterus has been investigated. By using RT-PCR techniques,

we have observed that these selected ADAM genes are involved in the remodeling events of the mouse uterus around the time of implantation.

MATERIALS AND METHODS

1. Animals

ICR mice were supplied from Daehan Biolink (Daejeon, Korea). Animals were kept under conditions following the institutional guidelines for the care and use of experimental animals. They were housed under controlled lighting of 12 h light and 12 h dark cycle. Sexually mature, 6- to 8-wk-old female mice were used for the study. Females were mated with fertile males of the same strain and checked for vaginal plugs on the following morning. The day of vaginal plug formation was regarded as day 1 of pregnancy. The whole uteri were collected from pregnant mice on days 1~5. From day 6 to 8 of pregnancy when implantation and interimplantation sites were visualized, uterine tissues of each site were separately collected. The experiment has been approved by the Institutional Review Board of Seoul Women's University.

2. Total RNA isolation and reverse transcription - polymerase chain reaction (RT-PCR)

All solutions used were prepared using distilled water treated with 0.1% diethylpyrocarbonate (DEPC, Sigma). Uterine tissues were washed with Ca^{2+}, Mg^{2+}-free phosphate-buffered saline (PBS) and then transferred to a chilled eppendorf tube on ice. Five hundred μl of Tri-reagent (Sigma) was immediately added to the tube and tubes were stored at -20℃. Total RNA was isolated according to the manufacturer's instructions. The RNA was allowed to stand at 65℃ for 5 min in heating block before chilling on ice and was quantified spectrophotometrically. The purity of RNA was assessed by measuring the ratio of the absorbance at 260 nm to that at 280 nm (> 1.8). RT-PCR was carried out using a GeneAmp PCR system 2400 (Perkin Elmer). Fifteen μg of total RNA was reverse transcribed using the following RT mixture: 25 mM $MgCl_2$, 10X PCR buffer, 2.5 mM dNTP mixture, 0.5 mg/ml oligo $(d)T^{15}$, 40 U RNase inhibitor (Takara) and 20 U Avian myeloblastosis virus reverse transcriptase (Promega). RT reaction was carried out for 60 min at 42℃. The cDNA of ADAM and β-actin was submitted to PCR amplification using gene-specific upstream and downstream primers shown in Table 1. PCR was performed in a 50 μl reaction mixture containing 25 mM $MgCl_2$, 10X PCR buffer, 2.5 U Taq polymerase (Takara), 100 pM of each gene-specific upstream and downstream primers and nuclease-free water. PCR of ADAM-8, -10, -12 and -17 was performed in the following conditions: initial denaturation at 95℃ for 5 min; 30 cycles of denaturation at 95℃ for 30 sec, annealing at 60℃ for 30 sec, extension at 72℃ for 30 sec; and a final extension at 72℃ for 10 min. For ADAM-9, -15, ADAMTS-1 and β-actin, PCR conditions were the same as mentioned above except for the annealing temperature of 55℃ for ADAM-15 and ADAMTS-1 and 50℃ for ADAM-9 and β-actin. PCR products were mixed with 6X loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose) and separated on 2% agarose gel. After staining the gel with ethidium bromide (Sigma), DNA signals on the gel were imaged under ultraviolet light and the intensity was measured using a Bioprofile image analysis system (Viber Lourmat). The amount of cDNA product from each ADAM mRNA was calculated as a relative percentage ratio against their respective β-actin values. Each value was obtained from three independent experiments and expressed as means ± SEM.

3. Statistical Analysis

Results are shown as the means ± SEM. Statistical significance was determined by one-way

Table 1. ADAMs cDNA pairs used in this study

	Primer pairs	Primer sequence	Size	Origin
β-actin	5'-primer	5'-GTGGGCCGCTCTAGGCACAA-3'	539 bp	mouse
	3'-primer	5'-CTCTTTGATGTCACGCACGATTTC-3'		
ADAM-8	5'-primer	5'-TTGCCCCATGTGAAACAGTA-3'	408 bp	mouse
	3'-primer	5'-GATGTTTGCCTGATACATCGC-3'		
ADAM-9	5'-primer	5'-TTGCTCATGAATTGGGGCATAAC-3'	425 bp	mouse
	3'-primer	5'-CAGTACTCAGGAACATCACA-3'		
ADAM-10	5'-primer	5'-CCATCAACTTGTGCCAGTAC-3'	421 bp	mouse
	3'-primer	5'-CCCATTTGATAACTCTCTCG-3'		
ADAM-12	5'-primer	5'-CTTGACTGTAGGAATCCTGG-3'	494 bp	mouse
	3'-primer	5'-CTCACCAAGGCACTAGTGAG-3'		
ADAM-15	5'-primer	5'-GGAGAGCAGTGTGACTGTGGC-3'	186 bp	mouse
	3'-primer	5'-GCAGAACTCAGGCAGATCACA-3'		
ADAM-17	5'-primer	5'-CACTTTTGGGAAGTTTCTGG-3'	492 bp	mouse
	3'-primer	5'-CTCTGTCTCTTTGCTGTCAAC-3'		
ADAMTS-1	5'-primer	5'-CAAACGAGTCCGCTACAGGT-3'	500 bp	mouse
	3'-primer	5'-AGCTGCCATTGTTTCTGGAC-3'		

ANOVA test using SPSS 10.0 statistical software (Data solution). p value of less than 0.05 was considered to be significantly different from each other.

RESULTS

Expression of ADAM genes in mouse uterus during early pregnancy was examined using RT-PCR. During days 1~5 of pregnancy, the whole uterus was examined for the expression of mRNA of ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1 genes. During days 6~8 of pregnancy, implantation and interimplantation sites of uterus were distinguished and examined. Messenger RNAs of all ADAM genes examined were consistently detected throughout day 1~8 of pregnancy although their expression levels were variable depending on the species of ADAMs and the pregnancy stage.

1. Expression of ADAM-8

During progression from day 1 to day 5 of pregnancy, mRNA amount of ADAM-8 in whole uterine tissue decreased on days 1~3 but increased on day 4 until day 5 (day 1, 119.5±3.3%; day 2, 54.8±8%; day 3, 29.1±1.1%; day 4, 38.2± 10.5%; day 5, 72.8±5.7%). During days 5~6 of pregnancy, the mRNA level in both implantation and interimplantation sites was constant. However, the level at the implantation site was about 5-fold higher than that of the interimplantation site (Figure 1B).

2. Expression of ADAM-9

ADAM-9 mRNA expression was consistently observed with a minor variation throughout days 1~5 of pregnancy. However, on days 6~8 of pregnancy, the transcription level became significantly higher as pregnancy progressed. Particularly

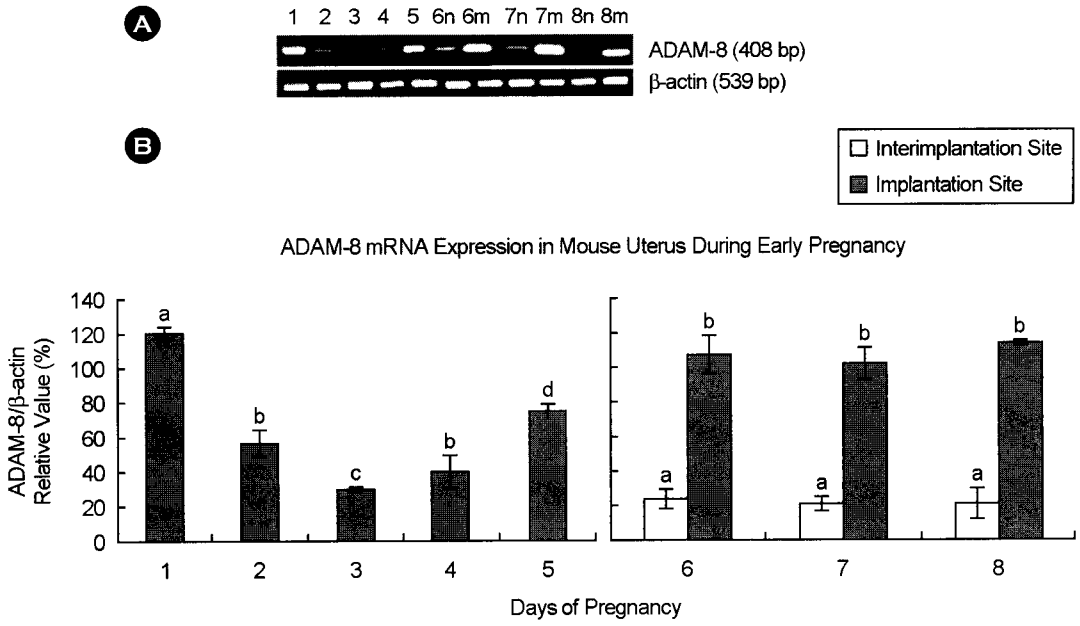

Figure 1. Gene expression of ADAM-8 in mouse uterus on days 1~8 of pregnancy. **A,** RT-PCR products of ADAM-8 (408 bp) and β-actin (539 bp) during days 1~8 of pregnancy. Numerical numbers indicate the day of pregnancy; m and n indicate the type of uterine tissues obtained from implantation (m) or interimplantation (n) sites. **B,** densitometric analysis of ADAM-8 mRNA expression. Data are means ± SEM of three independent experiments. a vs. b-d; c vs. d, $p<0.05$.

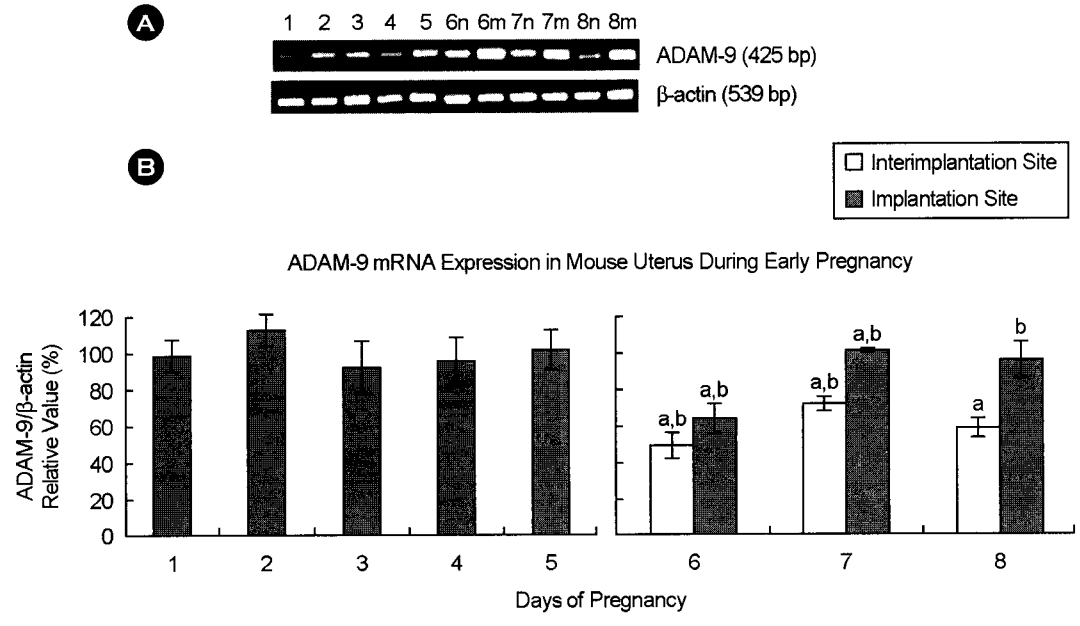

Figure 2. Gene expression of ADAM-9 in mouse uterus on days 1~8 of pregnancy. **A,** RT-PCR products of ADAM-9 (425 bp) and β-actin (539 bp) during days 1~8 of pregnancy. Numerical numbers indicate the day of pregnancy; m and n indicate the type of uterine tissues obtained from implantation (m) or interimplantation (n) sites. **B,** densitometric analysis of ADAM-9 mRNA expression. Data are means ± SEM of three independent experiments. Different superscript letters indicate a significant difference ($p<0.05$).

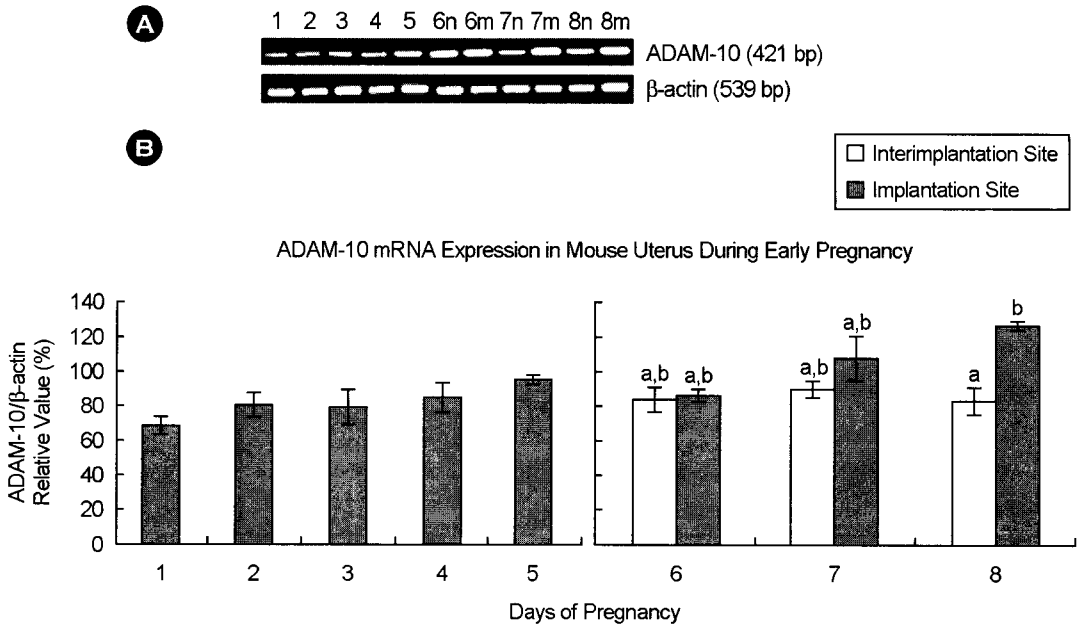

Figure 3. Gene expression of ADAM-10 in mouse uterus on days 1~8 of pregnancy. **A,** RT-PCR products of ADAM-10 (421 bp) and β-actin (539 bp) during days 1~8 of pregnancy. Numerical numbers indicate the day of pregnancy; m and n indicate the type of uterine tissues obtained from implantation (m) or interimplantation (n) sites. **B,** densitometric analysis of ADAM-10 mRNA expression. Data are means ± SEM of three independent experiments. Different superscript letters indicate a significant difference ($p<0.05$).

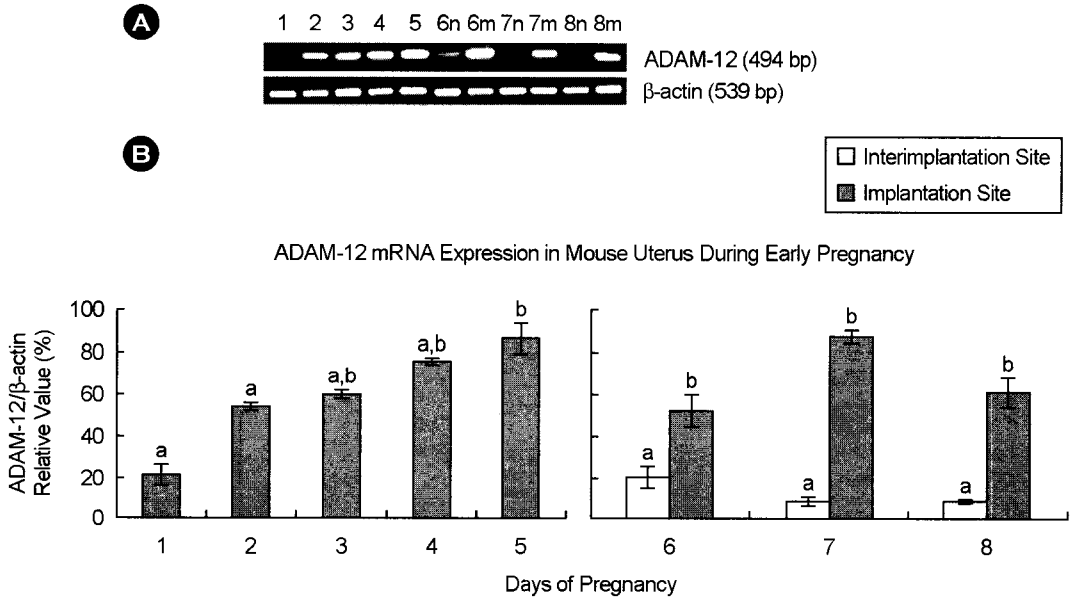

Figure 4. Gene expression of ADAM-12 in mouse uterus on days 1~8 of pregnancy. **A,** RT-PCR products of ADAM-12 (494 bp) and β-actin (539 bp) during days 1~8 of pregnancy. Numerical numbers indicate the day of pregnancy; m and n indicate the type of uterine tissues obtained from implantation (m) or interimplantation (n) sites. **B,** densitometric analysis of ADAM-12 mRNA expression. Data are means ± SEM of three independent experiments. Different superscript letters indicate a significant difference ($p<0.05$).

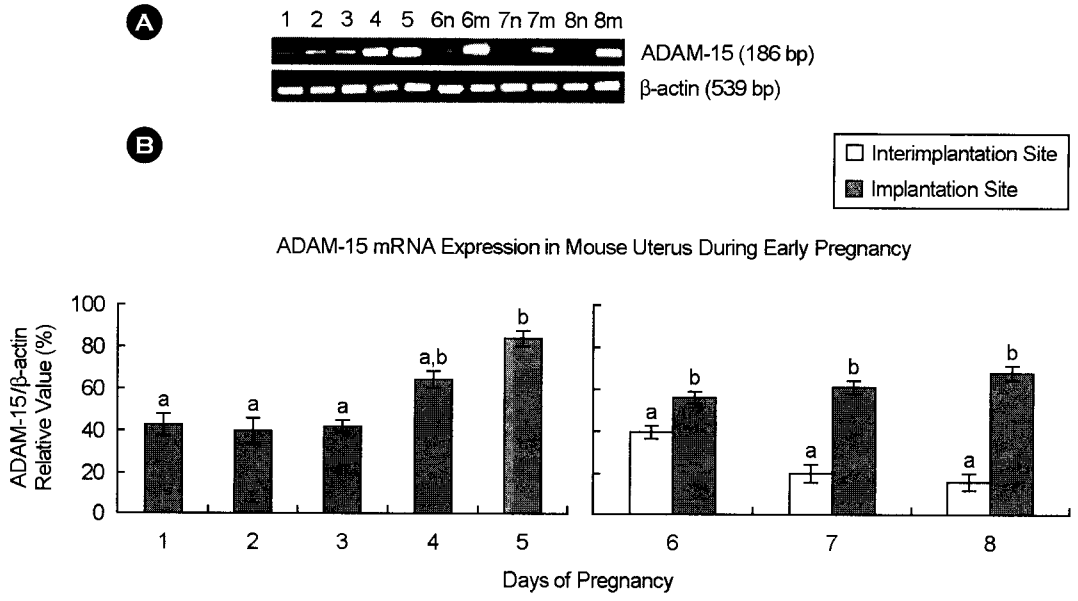

Figure 5. Gene expression of ADAM-15 in mouse uterus on days 1~8 of pregnancy. **A,** RT-PCR products of ADAM-15 (186 bp) and β-actin (539 bp) during days 1~8 of pregnancy. Numerical numbers indicate the day of pregnancy; m and n indicate the type of uterine tissues obtained from implantation (m) or interimplantation (n) sites. **B,** densitometric analysis of ADAM-15 mRNA expression. Data are means ± SEM of three independent experiments. Different superscript letters indicate a significant difference ($p<0.05$).

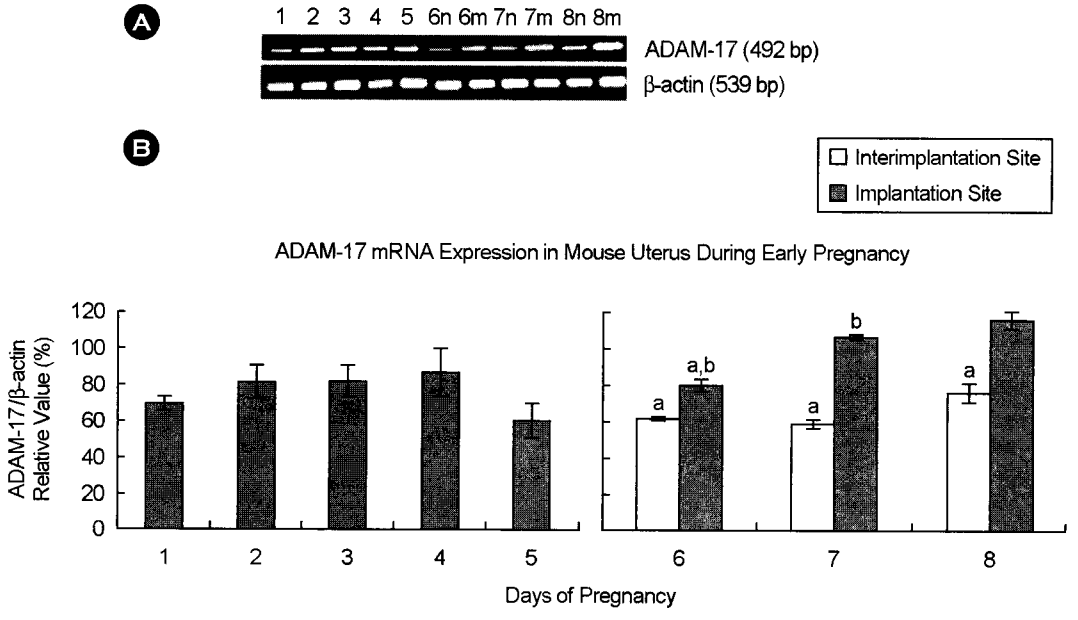

Figure 6. Gene expression of ADAM-17 in mouse uterus on days 1~8 of pregnancy. **A,** RT-PCR products of ADAM-17 (492bp) and β-actin (539bp) during days 1~8 of pregnancy. Numerical numbers indicate the day of pregnancy; m and n indicate the type of uterine tissues obtained from implantation (m) or interimplantation (n) sites. **B,** densitometric analysis of ADAM-17 mRNA expression. Data are means ± SEM of three independent experiments. Different superscript letters indicate a significant difference ($p<0.05$).

the level was considerably higher at the implantation site compared to the interimpantation site (Figure 2B).

3. Expression of ADAM-10

During progression from day 1 to day 5, level of ADAM-10 mRNA gradually increased (day 1, 68.7±2.3%; day 2, 80.2±5.1%; day 3, 79.4± 7.7%; day 4, 84.8±9.5%; day 5, 96.1±9.5%), though not with significance (Figure 3A). On days 6~8, there was no difference between mRNA levels of the implantation site and the interimplantation site. However, on day 8, the level at the implantation site was significantly greater than that of the interimplantation site (Figure 3B).

4. Expression of ADAM-12

As seen in Figure 4A and 4B, level of ADAM-12 mRNA was initially low on day 1 and progressively increased to about 4-fold on day 5 (day 1, 21.5±4.8%; day 2, 54.4±1.4%; day 3, 59.4± 2.7%; day 4, 75.4±1.8%; day 5, 87.2±7.6%). On days 6~8, the level at the implantation site was significantly greater compared to the interimplantation site (day 6, 2.7-fold; day 7, 10-fold; day 8, 7-fold).

5. Expression of ADAM-15

The messenger RNA level of ADAM-15 remained constant until day 3 and gradually increased thereafter (day 1, 42.4±4.8%; day 2, 38.7±7.0%; day 3, 41.2±2.7%; day 4, 64.2±3.7%; day 5, 82.7 ±4.8%) as seen in Figure 5A. The expression continued during days 6~8, however, the level of the implantation site was significantly greater than that of the interimplantation site (Figure 5B).

6. Expression of ADAM-17

While the uterus on days 1~5 of pregnancy consistently expressed ADAM-17 gene, the mRNA

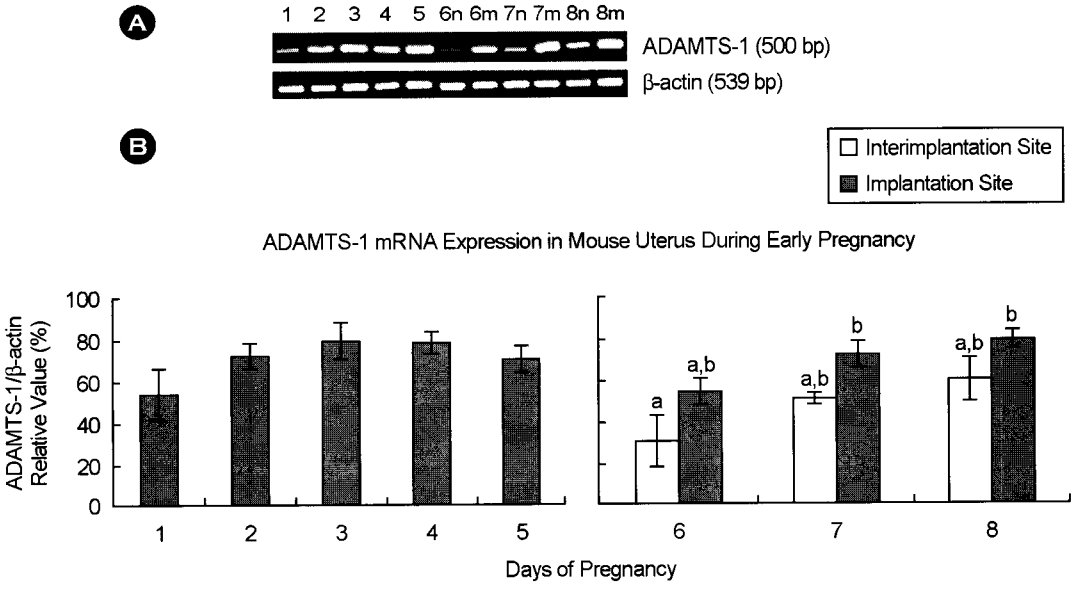

Figure 7. Gene expression of ADAMTS-1 in mouse uterus on days 1~8 of pregnancy. **A,** RT-PCR products of ADAMTS-1 (500 bp) and β-actin (539 bp) during days 1~8 of pregnancy. Numerical numbers indicate the day of pregnancy; m and n indicate the type of uterine tissues obtained from implantation (m) or interimplantation (n) sites. **B,** densitometric analysis of ADAMTS-1 mRNA expression. Data are means ± SEM of three independent experiments. Different superscript letters indicate a significant difference ($p<0.05$).

level did not vary regardless of pregnancy stage. Similarly during days 6~8 of pregnancy, the mRNA level remained constant at the interimplantation site. However, the level gradually increased during the same period at the implantation site, the level of which was significantly greater than that of the interimplantation site (Figure 6B).

7. Expression of ADAMTS-1

Continuous expression of ADAMTS-1 was observed throughout day 1 to day-5 with the highest level on day 3 (79.8±5%) and the lowest on day 1 (56.4±10.3%). However, the difference in level was not significant between the highest and the lowest. During days 6~8, the mRNA level in the implantation site was much higher than that of the interimplantation site (Figure 7B).

DISCUSSION

The present study demonstrates that the ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1 genes are continuously expressed throughout day 1 to day 8 of pregnancy predominantly in implantation site suggesting their possible role in the mechanism of uterine remodeling during the periimplantation period in mice. Particularly the ADAM-8, 12 and 15 genes show differential expression during preimplantation period implying that they are more likely to participate in the remodeling of the uterus in preparation for the implantation.

Increasing evidences have provided the importance of the role of ADAM family in various tissues. ADAM-8 is involved in neurite outgrowth and the suppression of neuronal death,[14] and in the up-regulation of IgE production and the induction of inflammatory cytokines in B cell lines.[15] While its role in these cells is mediated by proteolytic activity, it also displays adhesive activity. The importance of adhesive activity has been shown in the neurodegeneration observed in wobbler mutant mice.[16] In mouse uterus, ADAM-8 mRNA exhibited differential expression from day 1 throughout day 5 of pregnancy. After the onset of implantation, its expression was significantly higher in implantation site than ininterimplantation site. These results imply that ADAM-8 expression might be related to the initiation and maintenance of implantation. From these observations, it is suggestable that ADAM-8 might play a role in remodeling of the mouse uterus before and after implantation and might act via an adhesive interaction with apposed epithelial cells, stromal cells and/or embryos.

Gene expression products of ADAM-12 in mouse uterus remarkably increased from the beginning of pregnancy. And, like a ADAM-8, expression of ADAM-12 was significantly higher in implantation site than ininterimplantation site. Therefore it is very likely that ADAM-12 might be intimately related to uterine remodeling accompanied by implantation. Thus in addition to their differential expression, the fact that mouse uterine tissues actively synthesize ADAM-12 protein leads to suggest that ADAM-12 might be deeply involved in the proliferation and differentiation of mouse uterine cells. For the differentiation of early adipocytes and osteoblasts,[17] ADAM-12 acts via its cell-cell and/or cell-matrix adhesion activity. In cardiomyocytes,[18] it exhibits proteolytic activity releasing HB-EGF, insulin-like growth factor-binding protein (IGFBP)-3 or IGFBP-5. Thus by using either one or both of these activities, ADAM-12 is believed to participate in uterine remodeling from the beginning of pregnancy.

Since ADAM-15 can cleave type IV collagen and gelatin,[19] it might play a role in the reconstruction of ECM components. During early pregnancy in mice, progressive loss of laminin and type IV collagen in the uterine luminal epithelial basement membrane occurs in the area occupied by decidualized endometrial stroma and occurs in areas not

yet in contact with trophoblast cells.[1] Potential role of ADAM-15 in these events appears to be mediated via its specific interaction with integrin αvβ3 in an RGD-dependent manner,[20] with integrin α9β1 in an RGD-independent manner[9] or ectodomain sheddase activity. Revealing the action mode of ADAM-15 will give an insight how it could play a role in mouse uterus during the peri-implantation period.

In many mammals, the apical surface of the uterine epithelium is covered by a mucin glycocalyx. In rabbits, the presence of blastocysts in the uterine lumen resulted in a localized reduction of Muc1 at the implantation site of the luminal epithelium.[21] Higher expression of ADAM-9 mRNA was shown to correlate with the implantation site by in situ hybridization study.[21] Thus ADAM-9 was suggested to play a role as a Muc1 sheddase during the implantation window for rabbit embryos. Human uterine epithelial cells cultured in vitro also exhibited a local loss of MUC1 at the site of blastocyst adhesion.[22] In the receptive phase of human endometrium, the luminal and glandula uterine epithelial cells was the predominant site of MUC1 localization and ADAM-17 protein has been localized to this area.[23] These findings suggested that in human, ADAM-17 appears to be responsible for the shedding of MUC1.[23] In mice, Muc-1 expression is high in the proestrous and estrous stages and the protein declines to barely detectable levels by day 4 of pregnancy, i.e. before the time of blastocyst attachment.[21~23] Our results showed that genes of ADAM-8, 12 and 15 began to increase their expression from the beginning of pregnancy or on day 3 until day 5 around the time of implantation mostly at the epithelial layers. Thus one or more of these ADAMs can be candidates for mouse MUC1 sheddase.

Although genes of ADAM-9, 10, 17 and ADAMTS-1 were consistently expressed in the uterus from day 1 until day 5, there was no significant change in the level of mRNA expression during this period. However, after initiation of implantation, differential expression was observed that strong expression was seen on day 8 at the implantation site and significantly lower expression occurred at the interimplantation site. Therefore, rather than participating in the remodeling during the preimplantation period, these ADAMs seem to be involved in the later remodeling events after the initiation of implantation.

ADAM-9 has been shown to play either adhesive or proteolytic activity. The disintegrin domain of ADAM-9 can function as an adhesion molecule by interacting with an αvβ5 integrin in an RGD-independent manner[11] or an α6β1 integrin.[24] Its metalloprotease domain has an alpha-secretase-like activity cleaving amyloid precursor protein, acts as an insulin-like growth factor binding protein-5 protease in human osteoblasts[25] or degrades gelatin, β-casein, and fibronectin.[26] It would be interesting to determine which type of ADAM-9 activity is involved in the remodeling of uterine tissues. Many studies have attributed the role of ADAM-10 to an ectodomain sheddase releasing a soluble fragment from Delta1 ligand.[6] In addition, ADAM-10 is also capable of cleaving type IV collagen and gelatin.[27] Considering no known adhesive activity of ADANM-10, it is presumded to function in mouse uterus via proteolytic activity. Similar to ADAM-10, ADAM-17 is well known to act as a sheddase for various cell signaling molecules and their receptors.[28] Thus roles of ADAM-10 and 17 in the remodeling of mouse uterus might occur via their proteolytic activity.

Unlike other ADAMs in the present study, ADAMTS-1 does not contain a transmembrane domain and after synthesis, it is secreted into the extracellular matrix. As a metalloprotease, it can cleave versican or aggrecan, resulting in the impaired ovulation.[29] Since ADAMTS-1 depends on a progesterone receptor in response to luteinizing

hormone in periovulatory follicles of mouse,[30] it is reasonable to suggest that ADAMTS-1 might play a role in the uterus, a major target of progesterone, acting on ECM molecules and/or growth factors.

While our studies suggest that all of these ADAM genes might play a role in the tissue remodeling of mouse uterus during periimplantation period, birth and/or normal development of knockout mice of ADAM-9,[31] 12,[32] 15,[33] 17[34] or ADAMTS-1[29] have shown that these genes do not play a critical role in uterine function. One possible explanation for the discrepancy is that the lack of one gene function could be overcome by other related genes. Many studies have suggested the compensation of loss of function in knockout mice. For example, expressions of MMP-3/stromelysin-1 and MMP-10/stromelysin-2 were dramatically up-regulated in MMP-7/matrilysin-deficient mice and expressions of MMP-7 and MMP-10 were also up-regulated in MMP-3-deficient mice.[35] In these contexts, normalities reported in knockout mice of ADAM-9, 12, 15, 17 and ADAMTS-1 gene could be due to compensation by other ADAM genes or related genes.

In the present study, we have examined the expression of selected ADAM genes in mouse uterine tissues during the periimplantation period. Our results from RT-PCR suggest the involvement of ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1 genes in the remodeling of uterus accompanied by implantation. However, normalities of knockout mice of these ADAM genes except ADAM-8 and 10 indicate that they are not critical the uterine function. Further studies will clarify whether the compensation by other genes might indeed take place in these knockout mice of ADAM gene.

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