—Research Note—

Expression Patterns of the Implantation-associated Genes in the Uterus during the Estrous Cycle in Mice

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Abstract. The mRNA expression patterns of EGF, HB-EGF, Amphiregulin, EGF receptor, IGF-1, CSF-1, IL-1 alpha, IL-1 beta, IL-1 receptor type 1, IL-1 receptor antagonist, LIF, COX-1, COX-2, Mucin-1, calcitonin, and rat USAG-1 mouse homologue, all of which are involved in the process of conceptus implantation to the endometrium, were examined during the estrous cycle by means of real-time quantitative PCR. COX-2, HB-EGF, LIF, Mucin-1, CSF-1, IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist were temporally regulated during the estrous cycle and highly expressed during the estrous stage. In the case of COX-1, EGF, IGF-1, and EGF receptor, the highest mRNA expression was during the diestrous stage. In contrast, the rat USAG-1 mouse homologue mRNA expression did not change during the estrous cycle. These results indicate that rat USAG-1 mouse homologue expression at implantation might be specifically regulated by embryonic factors rather than the maternal environment.

Key words: Estrous cycle, Implantation, Mice, Ovarian hormone

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Blastocyst implantation and successful establishment of pregnancy require delicate interactions between the embryo and maternal environments. Previous studies have indicated that the window of implantation is very narrow, and that it is under strict regulation by ovarian hormones such as estrogen and progesterone [1]. Although reproductive processes are dependent on multiple actions of these ovarian hormones, they influence the complex interplay of many mediators, such as prostaglandins, cytokines, leukotrienes, histamine, and growth factors in an endocrine,

autocrine, paracrine, and/or juxtacrine manner. Previous studies have identified the molecules associated with implantation, which appear to be regulated by ovarian hormones [2–5]. Although ovarian hormonal signaling is essential for proper uterine function prior to and during implantation, the changes in transcripts of these molecules have not been well characterized. In order to examine how the intrauterine mediators are expressed throughout the estrous cycle, we analyzed the mRNA expression patterns of sixteen molecules by means of real-time quantitative PCR. Factors examined were epidermal growth factor (EGF) [5], heparin-binding epidermal growth factor-like growth factor (HB-EGF) [6, 7], Amphiregulin [8],

EGF receptor [5, 9], insulin-like growth factor (IGF)-1 [10, 11], colony-stimulating factor (CSF)-1 [12], interleukin (IL)-1 alpha and beta [13], IL-1 receptor type 1 [14], IL-1 receptor antagonist [15, 16], leukemia inhibitory factor (LIF) [17–20], cyclooxygenase (COX)-1 and -2 [2], Mucin-1 [21, 22], calcitonin [23, 24], and rat uterine sensitization-associated gene (USAG)-1 mouse homologue [25].

Materials and Methods

Animals

Female ICR mice of 8–10 weeks of age, purchased from a commercial supplier (CLEA Japan, Tokyo, Japan), were maintained in the animal facility of the National Research Center for Protozoan Diseases at Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan. All animals were housed in polycarbonate cages, and maintained in a specific pathogen-free environment in light-controlled (lights-on from 07:00 to 19:00) and air-conditioned rooms (temperature: 24 ± 1C, humidity: 50 ± 10%). They had free access to standard laboratory chow (CE-2; CLEA Japan) and water *ad libitum*. The animals used in this study were cared and used under the Guiding Principles for the Care and Use of Research Animals promulgated by the Obihiro University of Agriculture and Veterinary Medicine.

Determination of the estrous cycle, ovariectomy and superovulation, and collection of uteri

The stage of the estrous cycle of mice was determined by cytological evaluation of vaginal smears. Vaginal smear inspection was performed daily between 15:30 and 16:00. After vaginal smear inspection for 10 days, females that showed two cycles of a typical estrous cycle were subjected to collection of their uterus. All mice were sacrificed by cervical dislocation. Uteri in mice exhibiting each stage of the estrous cycle, namely proestrus (n=4), estrus (n=6), metestrus (n=4), and diestrus (n=4), were then collected and immediately immersed into liquid nitrogen. Uteri from ovariectomized animals were collected and used as the control (n=6). In addition, uteri from supreovulated mice (n=7) were collected for similar examinations. One group of mice was bilaterally ovariectomized through a single dorsal incision under anesthesia and was sacrificed by cervical

dislocation 14 days after the surgery. The other group of mice was superovulated by intraperitoneal injection of 5 IU equine chorionic gonadotropin (eCG; Serotropin, Teikoku Hormone Mfg. Co., Tokyo, Japan) followed 48 hr later by 5 IU of human chorionic gonadotropin (hCG; Puberogen, Sankyo Co., Ltd., Tokyo, Japan), and sacrificed 16 h after the hCG injection.

Real-time quantitative PCR

Total RNA was extracted from frozen uterine tissues of the ovariectomized, superovulated, and cyclic mice by means of a TRI Reagent Kit (Sigma Inc., St. Louis, MO, USA) according to the manufacturer's protocol. Extracted total RNA was then subjected to real-time PCR analysis. Primers and TaqMan probe for each gene and beta-actin were designed using the primer design software, Primer Express version 1.5 (Applied Biosystems, Foster City, CA, USA). The GenBank Accession numbers of all cDNA sequences are summarized in Table 1. Quantification of all gene transcripts was carried with the ABI PRISM 7900 HT (Applied Biosystems, Foster City, CA, USA). Templates for real-time PCR were obtained by reverse transcriptase reaction of total RNA. For RT-PCR reaction, the TaqMan One-Step RT-PCR Master Mix Reagents Kit (PE Applied Biosystems, Foster City, CA, USA) was used at 20 μl/tube as follows: the template (20 ng) was mixed with 2 × Master Mix without UNG, 40 × MultiScribe and RNAse Inhibitor Mix, 200 nM TaqMan Probe, and 900 nM of each primer. Reaction conditions were 1 cycle at 48 C for 30 min and 1 cycle at 95 C for 10 min followed by 45 cycles of the amplification step (95 C for 15 sec and 60 C for 1 min). The gene expression levels of EGF, HB-EGF, Amphiregulin, EGF receptor, IGF-1, CSF-1, IL-1 alpha, IL-1 beta, IL-1 receptor type 1, IL-1 receptor antagonist, LIF, COX-1, COX-2, Mucin-1, calcitonin, and rat USAG-1 mouse homologue were calculated as gene expression rates, as previously reported [26]. Briefly, the amounts of each gene and beta-actin mRNA in samples were estimated with standard curves representing the log of the input amount (log starting cDNA molecules) as the X axis and the threshold cycle as the Y axis. A relative standard curve (SC) for real-time PCR was used as a common set of samples that linked the experimental PCR plates together and permitted overall analysis of the samples. Preparation and

Transcript		Primer/Probe sequence (5' to 3')	GenBank accession number
Amphiregulin	F:	GCGAATGCAGATACATCGAGAA	NM_009704
	R:	TCCACACCGTTCACCAAAGTAA	
		FAM-CATGCAATTGTCATCAAGA-MGB	
COX-1	F:	GACAGTATCACCTGCGGCTCT	NM_008969
	R:	GGTAGCGCATCAACACGGA	
		FAM-CTTAAGTACCAGGTGCTGGA-MGB	
COX-2	F:	GAACATGGACTCACTCAGTTTGTTG	NM_011198
	R:	CCACTGCTTGTACAGCAATTGG	
		FAM-AGACAGATTGCTGGCCGG-MGB	
EGF	F:	CCAAACGCCGAAGACTTATCC	NM_010113
	R:	TGATCCTCAAACACGGCTAGAGA	
		FAM-AACGACGTAGGTCACC-MGB	
HB-EGF	F :	TCCGTCTGTCTTCTTGTCATCGT	NM_010415
	R :	TAGCCACGCCCAACTTCACT	
		FAM-CTTCTCATGTTTAGGTACCACAG-MGB	
LIF	F :	ACGGCAACCTCATGAACCA	NM_008501
	R :	GGAAACGGCTCCCCTTGA	
		FAM-CTCTTCATTTCCTATTACACAGC-MGB	
Mucin-1	F:	CCATCCTATGAGTGAATACCCTACCT	NM_013605
	R:	AAGAGAGACTGCTACTGCCATTACC	
		FAM-CCTATGAGGAGGTTTCGG-MGB	
IGF-1	F :	GCTGGTGGATGCTCTTCAGTT	NM_010512
	R:	TCCGAATGCTGGAGCCATA	
		FAM-ACTTCAACAAGCCCACAGG-MGB	
Rat USAG-1 mouse homolog	F:	GAGGCAGGCATTTCAGTAGCA	NM_025312
	R:	TGTATTTGGTGGACCGCAGTT	
		FAM-TCGAAACAGTCGAGTTCA-MGB	
Calcitonin	F :	CAGGACTATATGCAGATGAAAGCC	NM_007587
	R:	ATGCAGGTACTCAGATTCCCACA	
		FAM-CTCTAGCTTGGACAGCC-MGB	
CSF-1	F :	GGCATCATCCTAGTCTTGCTGACT	NM_007778
	R:	CCCCACAGAAGAATCCAATGTC	
		FAM-AGGAGCCATCGAGACC-MGB	
EGF receptor	F :	CATCTGTGCCCAGCAATGTT	NM_007912
	R:	TTGCATGTGGCCTCATCTTG	
		FAM-TGACTGTCTGGTCTGCCAA-MGB	
IL-1 alpha	F:	AAGACCAGCCCGTGTTGCT	NM_010554
	R:	TTCCAGAAGAAAATGAGGTCGG	
		FAM-AGGAGTTGCCAGAAAC-MGB	
IL-1 beta	F:	GGACCCATATGAGCTGAAAGCT	NM_008361
	R:	GTCGTTGCTTGGTTCTCCTTGTA	
		FAM-ACCAACAAGTGATATTCT-MGB	
IL-1 receptor antagonist	F :	AGACCTTTTACCTGAGAAACAACCA	NM_031167
	R :	GGTCAATAGGCACCATGTCTATCTT	
		FAM-AGGACCAAATATCAAACTAGAAGA-MGB	
IL-1 receptor type 1	F :	CGGCGCATGTGCAGTTAA	NM_008362
	R:	GCCCCCGATGAGGTAATTCT	
		FAM-ATACCCAG TCCCTGACTT-MGB	
beta-Actin	F:	GCTCTGGCTCCTAGCACCAT	NM_007393
	R:	GCCACCGATCCACACAGAGT	
		FAM-ATCAAGATCATTGCTCCTC-MGB	

utilization of this SC as a quality control of the efficiency of amplification of the PCR plate is described elsewhere [27]. The gene expression rate was obtained by normalizing the amount of each gene cDNA with that of beta-actin.

Statistical analysis

All data are expressed as means ± standard error

(SE). The statistical comparisons of relative mRNA expression of each gene between experimental groups were analyzed by one-way analysis of variance followed by *post hoc* test using StatView 4.5 (Abac Concepts, Inc., Berkley, CA, USA). In all statistical tests, the difference was considered significant when p was <0.05.

Results

Expression of mRNA of the EGF superfamily, EGF receptor, and IGF-1 in the mouse uterus during the estrous cycle

As shown in Fig. 1-a, although EGF mRNA was

stably detected in all stages of the estrous cycle, the expression levels of EGF in the uterus during diestrus were significantly higher than those in the other experimental groups, respectively ($P<0.05$). The highest level of HB-EGF mRNA expression was found during estrus in the mouse uterus (Fig. 1-b). Expression levels of HB-EGF mRNA during estrus were significantly higher than those in ovariectomized mice ($P<0.01$). As shown in Fig. 1-c, amphiregulin mRNA was expressed at high levels during the estrous cycle with a transient reduction in the metestrous stage. The EGF receptor mRNA expression during diestrus was significantly higher than that of ovariectomized animals ($P<0.05$, Fig. 1-d). IGF-1 mRNA expression
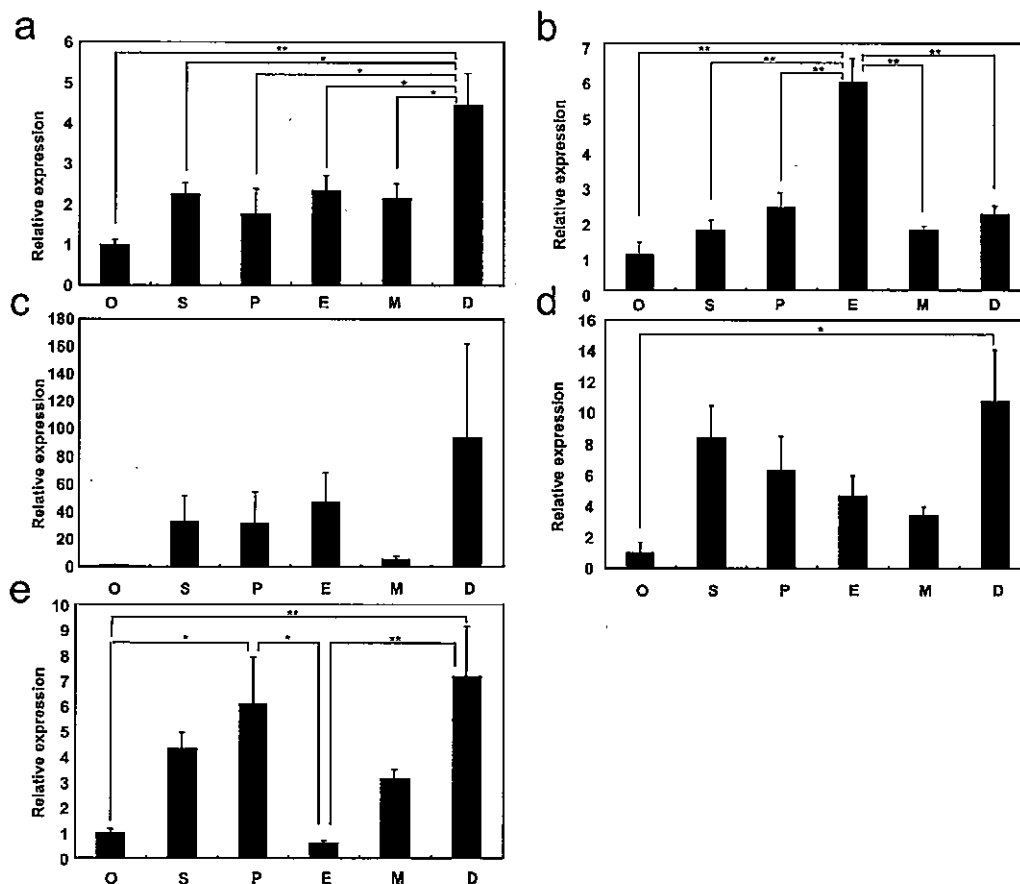

Fig. 1. Expression pattern of mRNA of the EGF superfamily genes, EGF receptor, and IGF-1 in the mouse uterus during the estrous cycle. Total RNA was extracted from the uteri of ovariectomized, superovulated, and cyclic mice and subjected to real-time PCR analysis as described in materials and methods. The mRNA is expressed relative to the control group, O. The results are shown as the mean ± S.E. a, EGF; b, HB-EGF; c, amphiregulin; d, EGF receptor; e, IGF-1; O, ovariectomized; S, superovulated; P, proestrus; E, estrus; M, metestrus; D, diestrus. *$P<0.05$ for significant differences between experimental groups. **$P<0.01$ for significant differences between experimental groups.
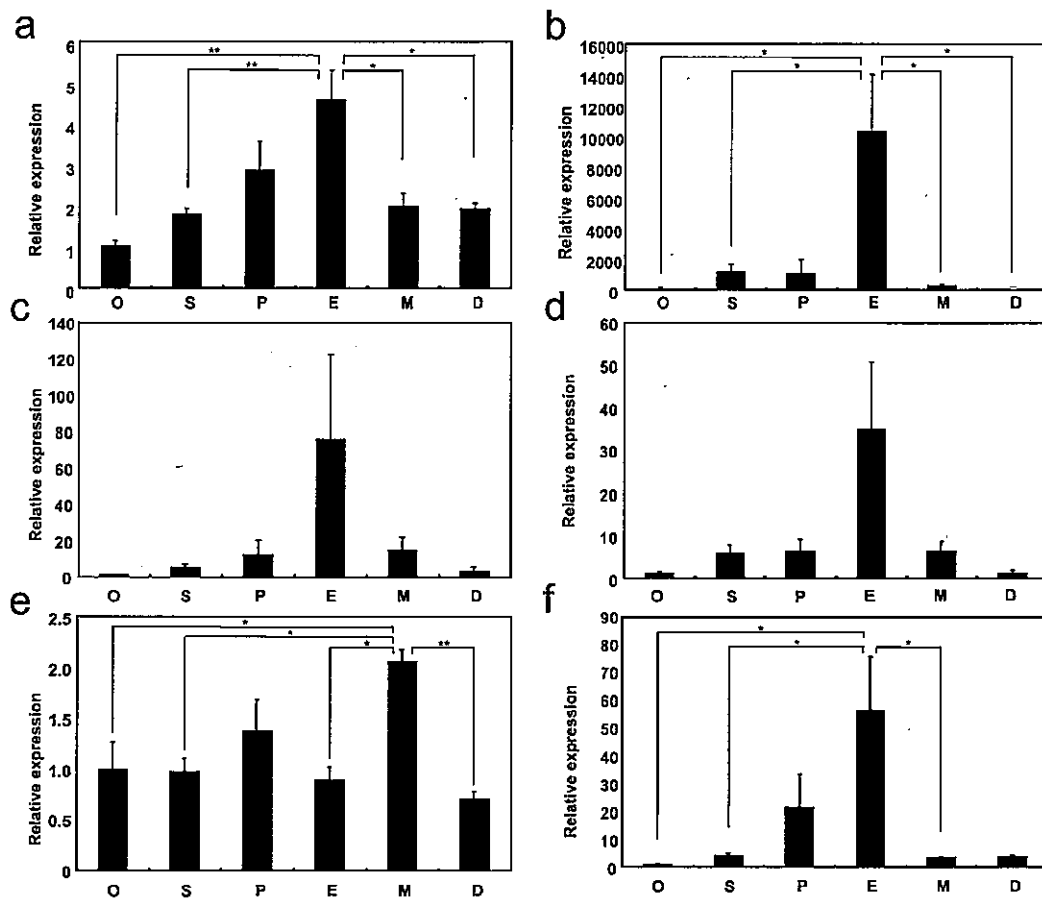
Fig. 2. Expression pattern of cytokines, such as CSF-1, IL-1 alpha, IL-1 beta, IL-1 receptor type 1, IL-1 receptor antagonist, and LIF mRNA in the mouse uterus during the estrous cycle. The mRNA levels are expressed relative to the control group, O. The results are shown as the mean ± S.E. a, CSF-1; b, IL-1 alpha; c, IL-1 beta; d, IL-1 receptor antagonist; e, IL-1 receptor type 1; f, LIF; O, ovariectomized; S, superovulated; P, proestrus; E, estrus; M, metestrus; D, diestrus. *P<0.05 for significant differences between experimental groups. **P<0.01 for significant differences between experimental groups.

was highly detected during all stages of the estrous cycle except the estrous stage (Fig. 1-e). Changes in all of the genes examined above appeared to be estrous cycle dependent.

Cytokine expression of CSF-1, IL-1 alpha and IL-1 beta, IL-1 receptor type 1, IL-1 receptor antagonist, and LIF mRNA in the mouse uterus during the estrous cycle

As shown in Fig. 2-a, CSF-1 mRNA expressed throughout the estrous cycle. Amount of CSF-1 mRNA were the highest during estrus, and were 5 times higher than that of ovariectomized mice. The expression level of CSF-1 mRNA significantly decreased in the metestrous and diestrous stages

(P<0.05). IL-1 alpha expression was very high throughout the estrous cycle (Fig. 2-b). During proestrus, the IL-1 alpha mRNA expressed approximately 1,000 times higher than that of ovariectomized mice, and then peaked during the estrous stage. The degree of increase in IL-1 alpha mRNA was 10,000 times higher than that of ovariectomized mice (P<0.01). It decreased sharply during the metestrous and diestrous stages, but was still 200 and 11 times higher than that of ovariectomized mice, respectively. As shown in Fig. 2-c and -d, respectively, the expression patterns of IL-1 beta and IL-1 receptor antagonist mRNA were similar to those of IL-1 alpha mRNA, although the expression levels of both IL-1 beta and
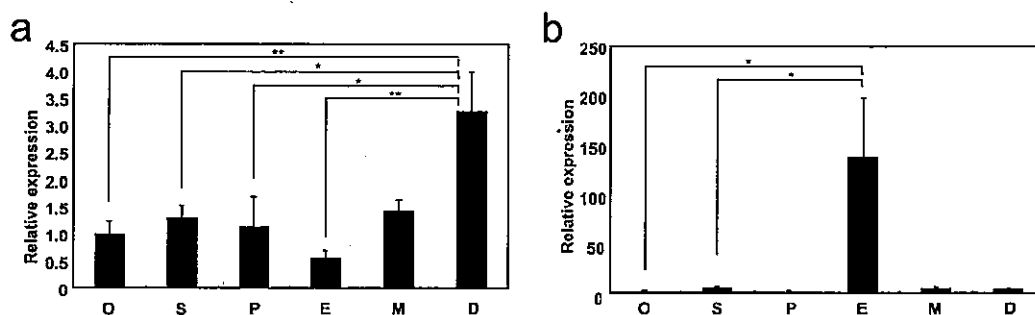
a

b

Fig. 3. COX-1 and COX-2 mRNA expression in the mouse uterus during the estrous cycle. The mRNA is expressed relative to the control group, O. The results are shown as the mean ± S.E. a, COX-1; b, COX-2; O, ovariectomized; S, superovulated; P, proestrus; E, estrus; M, metestrus; D, diestrus. *P<0.05 for significant differences between experimental groups. **P<0.01 for significant differences between experimental groups.
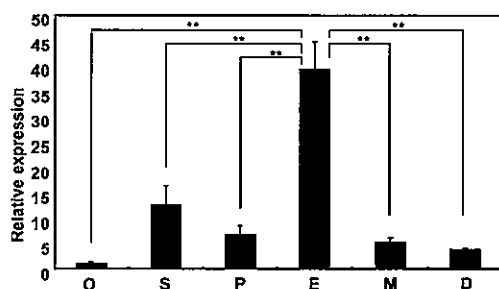

Fig. 4. Mucin-1 mRNA expression in the mouse uterus during the estrous cycle. The mRNA is expressed relative to the control group, O. The results are shown as the mean ± S.E. O, ovariectomized; S, superovulated; P, proestrus; E, estrus; M, metestrus; D, diestrus. **P<0.01 for significant differences between experimental groups.

IL-1 receptor antagonist were lower than those of IL-1 alpha throughout the estrous cycle. IL-1 receptor antagonist during estrus tended to express with a higher level than that of ovariectomized mice (P=0.07). Expression of IL-1 receptor type 1 mRNA significantly increased and peaked during metestrus with an expression level approximately 2 times higher than that of the other experimental groups including the ovariectomized mice, except during proestrus (P<0.05, Fig. 2-e). As shown in Fig. 2-f, LIF mRNA was highly expressed during proestrus and estrus with approximately 20 and 60 times higher levels than that of ovariectomized mice. Although LIF mRNA expression decreased dramatically from estrus to diestrus (P<0.05), the expression level during estrus was still significantly higher than that of ovariectomized animals (P<0.05). Changes in all of the cytokines mRNA examined in these experiments appeared to be estrous cycle dependent.

COX-1 and COX-2 mRNA expression in the mouse uterus during the estrous cycle

COX-1 mRNA expression was lowest in the

estrous stage, and then it increased to the diestrous stage (Fig. 3-a). The expression level of COX-1 mRNA during diestrus was significantly higher than that of ovariectomized animals and the other experimental groups except during metestrus (P<0.05). In contrast, the highest expression of COX-2 mRNA was detected during estrus, and it was significantly higher than that of ovariectomized animals (P<0.05, Fig. 3-b). These results indicate that the expression of both COX-1 and COX-2 mRNA depended on the estrous cycle.

Mucin-1 mRNA expression in mouse uterus during the estrous cycle

As shown in Fig. 4, Mucin-1 mRNA in the mouse uterus was detected throughout the estrous cycle. The expression of Mucin-1 mRNA increased remarkably during estrus with an expression level approximately 40 times higher than that of ovariectomized animals (P<0.01). However, the expression sharply decreased towards the metestrous and diestrous stages.
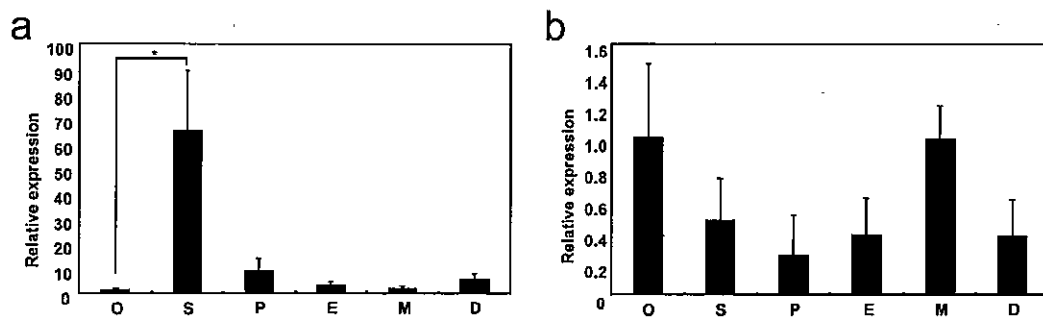
Fig. 5.   Calcitonin and rat USAG-1 mouse homologue mRNA expression in the mouse uterus during the estrous
cycle. The mRNA is expressed relative to the control group, O. The results are shown as the mean ± S.E.
a, calcitonin; b, rat USAG-1 mouse homolog; O, ovariectomized; S, superovulated; P, proestrus; E, estrus;
M, metestrus; D, diestrus. *P<0.05 for significant differences between experimental groups.

*Calcitonin and rat USAG-1 mouse homologue
mRNA expression in the mouse uterus during the
estrous cycle*

As shown in Fig. 5-a, the highest expression level
of calcitonin mRNA during the estrous cycle was
detected during proestrus at amounts 8 times
higher than that of ovariectomized mice.
Interestingly, the expression level of calcitonin
mRNA in the uterus after superovulation was
remarkably higher than that at any stage in the
spontaneous estrous cycle. Although expression of
rat USAG-1 mouse homologue mRNA could be
detected during the estrous cycle, the expression
level was definitely low throughout the cycle (Fig.
5-b). There were no significant differences in the
expression levels between the stages of the estrous
cycle. Even during metestrus, which displayed the
highest expression during the estrous cycle, the
expression level of rat USAG-1 mouse homologue
mRNA was lower than that of ovariectomized
mice. These results indicate that the expression of
rat USAG-1 mouse homologue mRNA is not
present in an estrous cycle dependent manner.

Discussion

*Expression of the mRNA of the EGF superfamily of
genes, as well as EGF receptor and IGF-1 in the
mouse uterus during the estrous cycle*

It has been shown that EGF localises in uterine
luminal and glandular epithelial cells during late
proestrus and estrus [28, 29] or in uterine stroma
during the estrous cycle [30]. The present results
showed the highest expression level of EGF during

the diestrous stage (Fig. 1-a). Paracrine actions
between EGF expressed predominantly in the
epithelium, and IGF-1 from stromal cells may
facilitate the epithelial-stromal interactions, which
are thought to be essential in endometrial
proliferation and differentiation induced by
estrogen [31]. However, the significance of the
highest expression of EGF or IGF mRNA shown
during diestrus but not during estrus was not
elucidated in our experiment. The highest
expression of HB-EGF mRNA was detected during
estrus in the mouse uterus (Fig. 1-b). It has been
shown in rats that HB-EGF is expressed in most
granulosa cells of early follicles and all the
developing follicles, but not in preovulatory
follicles. Furthermore, a strong staining pattern of
HB-EGF in corpora lutea was also found in a
immunohistochemical study [32]. These results
suggest that HB-EGF expression during estrus
might be related to the initiation of luteinization.
Although amphiregulin mRNA accumulated in the
luminal epithelium exclusively at the sites of
blastocyst attachment prior to implantation [8], it is
not essential for implantation because mice
deficient in amphiregulin are fertile [33]. Our data
showed that amphiregulin mRNA was expressed
throughout the estrous cycle in thé mouse uterus
(Fig. 1-c). Although amphiregulin is known to be
regulated by progesterone and LIF induced by
estrogen [34], the highest expression level of
amphiregulin during diestrus was not consistent
with the LIF mRNA expression pattern during the
estrous cycle (Fig. 1-c and 2-f). Previous studies
have indicated that estrogen, but not progesterone,
up-regulates uterine expression of EGF receptor in

immature rats [35, 36]. The transition in EGF receptor mRNA levels (Fig. 1-d,) paralleled changes in the plasma estrogen concentration [37]. It has been shown that IGF-1 mRNA was detected during all stages of the estrous cycle [38]. Our results also indicate that IGF-1 mRNA is detectable during all stages of the estrous cycle, with a lower expression level at estrus (Fig. 1-e).

Expression of cytokines, such as CSF-1, IL-1 alpha and IL-1 beta, IL-1 receptor type 1, IL-1 receptor antagonist, and LIF mRNA in the mouse uterus during the estrous cycle

It has been shown that the uterine CSF-1 concentration is regulated by the synergistic action of estrogen and progesterone [39] and that estrogen and progesterone stimulation for CSF-1 production by mouse uterine epithelial cells controls recruitment and distribution of macrophages in the uterus during the estrous cycle in mice [40]. CSF-1 protein and mRNA were elevated during estrus (Fig. 2-a) and on day 1 of pregnancy in the uterus [39, 41]. It has been reported that IL-1 production increases from diestrus to proestrus and estrus [42], and that it is dependent on the levels of circulating estrogen and progesterone [43–45]. Since progesterone directly induces uterine cells to produce IL-1 alpha and IL-1 beta, IL-1 has been detected at diestrus [42]. In the present study, however, IL-1 alpha or beta was highly expressed, with the maximum level during estrus (Figs. 2-b and -c), suggesting that both IL-1 alpha and beta might be induced by estrogen. Although previous studies have reported that IL-1 receptor type 1 is elevated after hCG stimulation [46], the level of IL-1 receptor type 1 mRNA was not differentially expressed during the estrous cycle except for during metestrus (Fig. 2-e). In this study, the expression pattern of IL-1 receptor antagonist mRNA was similar to that of IL-1 alpha and beta (Figs. 2-b, -c and -d). Real-time PCR analysis showed that the expression level of LIF mRNA increased from the proestrous stage onward and reached its highest level during estrus in cycling mice (Fig. 2-f). It has been reported that LIF is transiently expressed in the glandular epithelium of mice at ovulation and on Day 4 of pregnancy [18, 19].

COX-1 and COX-2 mRNA expression in the mouse uterus during the estrous cycle

Previous reports have demonstrated that COX genes are differentially regulated during the preimplantation period in the mouse uterus [2]. It has been shown that combined treatment with progesterone and estrogen induces COX-1 gene in the epithelium in ovariectomized mice [2]. On the other hand, our results showed that COX-1 mRNA was suppressed during estrus and then up-regulated thereafter, with the highest expression during diestrus (Fig. 3-a). A previous study has shown that COX-2 is elevated during the proestrous and estrous stages in rat uterine epithelial cells, with the highest expression during proestrus and estrus and the lowest during diestrus [47]. On the other hand, COX-2 mRNA was not detected in the proestrous stage, but dramatically increased during estrous in the present experiment (Fig. 3-b). However, these results are not inconsistent with the fact that the ovulatory action of IL-1 beta is mediated through the induction of COX-2 (Figs. 2-c and 3-b, [48]).

Mucin-1 mRNA expression in the mouse uterus during the estrous cycle

Mucin-1 was down-regulated in the epithelium during implantation. It has been thought that Mucin-1 interrupts implantation [21, 49]. The strongest Mucin-1 positive immunostaining of the uterine glands has been shown in the estrous stage [21]. Increases in Mucin-1 mRNA (Fig. 4) and protein levels during proestrus and estrus are coincident with the highest levels of estrogen in the plasma [50].

Calcitonin and rat USAG-1 mouse homologue mRNA expression in the mouse uterus during the estrous cycle

Calcitonin is expressed in the human endometrium during the implantation window [23]. Although it has been shown that calcitonin gene expression was induced by progesterone [51], as shown in Fig. 5-a, expression of calcitonin mRNA was not significantly different between any stages of the estrous cycle. Interestingly, there was dramatically higher expression in the superovulated uterus. Since calcitonin is one of the progesterone-responsive uterine genes [51], it appeared that the acute luteinization after superovulation may be related to the strong

expression of calcitonin mRNA.

It has been shown that USAG-1 is preferentially expressed in the maximally sensitized/receptive rat endometrium and that it may be involved in the onset of endometrial receptivity for implantation [25]. USAG-1 has a 98% sequence homology with the predicted amino acid sequence for the mouse cDNA 0610006G05Rik, rat USAG-1 mouse homologue [52]. It has been reported that rat USAG-1 mouse homologue is expressed in the adult testis and kidney [53] and in developing ectodermal organs in mice [54]. However, the expression pattern and function of rat USAG-1 mouse homologue in mouse reproductive organs have not been elucidated yet. In contrast to the other genes examined in the present study, rat USAG-1 mouse homologue mRNA did not appear to be temporally regulated during the estrous cycle (Fig. 5-b). We detected rat USAG-1 mouse homologue mRNA in very low amounts within the basal level of the ovariectomized uterus throughout the estrous cycle by real-time PCR analysis. Rat USAG-1 mouse homologue appeared to be expressed in an estrous cycle-independent manner.

In conclusion, our present study showed evidence for the differential expression of amphiregulin, COX-1, COX-2, EGF, HB-EGF, LIF, Mucin-1, IGF-1, rat USAG-1 mouse homologue, calcitonin, CSF-1, EGF receptor, IL-1 alpha, IL-1 beta, IL-1 receptor antagonist, and IL-1 receptor

type 1 in the mouse uterus throughout the estrous cycle. COX-2, HB-EGF, LIF, Mucin-1, CSF-1, IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist were temporally regulated during the estrous cycle and were highly expressed during estrus. In terms of the mRNA expression of COX-1, EGF, IGF-1, and EGF receptor, the highest expression of these genes was during the diestrous stage. In contrast, rat USAG-1 mouse homologue appeared to be unchanged throughout the estrous cycle. However, our preliminary experiments indicated that this gene is expressed in the mouse uterus during the peri-implantation period, especially 3.5 to 5.5 days post coitum (data not shown). Recently, it has been demonstrated that the implantation window can be extended when advanced stage embryos are transferred into recipient mice [55]. Although the trigger molecule/s for this potential extension of the implantation window has not been identified, molecules with expression that is not dependent on the estrous cycle might participate in the initiation of uterine receptivity.

Acknowledgements

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