Journal of Cellular Biochemistry 58:269–278 (1995)

Mouse Uterus Peptidylarginine Deiminase Is Expressed in Decidual Cells During Pregnancy

Tomoji Arai, Masashi Kusubata, Tetsuya Kohsaka, Masakazu Shiraiwa, Kiyoshi Sugawara, and Hidenari Takahara

Laboratories of Biochemistry and Animal Reproduction, School of Agriculture, Ibaraki University, Ami-machi, Inashiki-gun, Ibaraki 300-03, Japan

Abstract Peptidylarginine deiminase is localized in the cytosol of the luminal and glandular epithelia of the nonpregnant murine uterus and its expression is regulated by sex hormones [Takahara et al., [1989]: J Biol Chem 264, 13361–13368; Takahara et al. [1992]: J Biol Chem 267,520–525]. Here, we demonstrate that changes occur in the enzyme level in the mouse uterus during pregnancy and parturition. After a rapid decrease in enzymatic activity from day 1 to day 5 of pregnancy, the activity sharply increased during the middle stage of pregnancy (day 8 to day 10) and then gradually decreased during late pregnancy. Expression of the enzyme occurred only in the decidual cells that had differentiated from endometrial stroma cells surrounding the implantation site. The immunochemical properties of the enzyme expressed in the decidualized cells was indistinguishable from those in the uterine epithelia. These results suggest that peptidylarginine deiminase has important roles in decidual cells and not just in the epithelia of the nonpregnant uterus. Moreover, the level of enzyme activity increased slightly just before parturition (day 17), and then decreased during the 12 h period after parturition. The tissue localization of the enzyme expressed around the time of parturition changed from decidua to the luminal and glandular epithelia. Semiquantitative analyses of the enzyme mRNA content in the pregnant uteri showed a remarkable increase from day 7 leading to the onset of the enzyme synthesis in the decidual cells. After reaching the maximal level at day 12, small peaks in the mRNA level were observed at two times during late pregnancy. Since these serial changes in the mRNA level did not correlate with changes in sex hormones, the expression of decidual peptidylarginine deiminase seemed to be controlled by factors other than sex hormones.

Key words: peptidylarginine deiminase, mouse decidual cells, pregnancy, gene expression, immunohistochemistry

Peptidylarginine deiminase (protein-L-arginine iminohydrolase, EC 3.5.3.15) catalyzes the conversion of arginyl residues of proteins to citrullyl residues in the presence of Ca^{2+}. This enzyme was first found in hair follicles of the guinea pig [Rogers and Taylor, 1977] and has since been show to be widely distributed among vertebrates [Fujisaki and Sugawara, 1981; Kubilus and Baden, 1985; Takahara et al., 1986]. Some physicochemical and enzymatic properties of the enzyme purified from skeletal muscle [Takahara et al., 1983; Watanabe et al., 1988; Takahara et al., 1989], epidermis [Kubilus and Baden, 1985], and brain [Kubilus and Baden, 1983; Takahara et al., 1986; Lamensa and Moscarello, 1993] have been reported. Recently, the amino acid sequences of the enzyme in the rat [Watanabe and Senshu, 1989] and mouse [Tsuchida et al., 1993] were predicted from cDNA nucleotide sequences. The gene structure of rat peptidylarginine deiminase has also been reported by Watanabe et al. [1992]. In our previous studies [Takahara et al., 1985; Kurokawa et al., 1987; Takahara and Sugawara, 1987; Inagaki et al., 1989; Azuma et al., 1994], we demonstrated that the enzyme specifically deiminated the functional arginine residues of proteins in vitro and caused a change in their biological activities. However, the physiological role of the enzyme is not yet known.

Recently, we have found that a high activity of peptidylarginine deiminase occurred in the luminal and glandular epithelia of the mouse uterus

Abbreviations used: PBS, phosphate-buffered saline; buffer A, 20 mM Tris–HCl, pH 7.6, containing 10 mM 2-mercaptoethanol, 1 mM EDTA and 0.43 mM phenylmethylsulfonyl fluoride.

Received September 16, 1994; accepted October 24, 1994.

Address reprint requests to Hidenari Takahara, Laboratory of Biochemistry, Department of Resource Biology, School of Agriculture, Ibaraki University, Ami-machi, Inashiki-gun, Ibaraki 300-03, Japan.

and that the enzyme activity in those cells changed markedly during the estrous cycle [Takahara et al., 1989]. In addition, we elucidate that the expression of the enzyme in the uterus was controlled by the steroid hormones estrogen (agonist) and progesterone (antagonist) at the pretranslational level [Takahara et al., 1992]. Thus, it is of particular interest not only to clarify the physiological role of the enzyme in the uterus, but also to determine the mechanism by which its gene expression in that tissue is regulated.

One of the remarkable events in the uterus is the decidualization of stromal cells in response to implantation. A series of experiments was therefore performed in which changes in the level of peptidylarginine deiminase activity and its localization in the uterus during pregnancy was determined, using enzyme- and immunohistochemical methods. In this paper, we report that the enzymatic activity in the uterus dramatically increased during the middle stage of pregnancy and that the specific expression of the enzyme occurred in decidual cells. We also show evidence that the regulation of peptidylarginine deiminase at the pretranslational level correlated with the differentiation of decidualized cells.

MATERIALS AND METHODS

Animal and Tissue Preparations

Ddy sexually mature mice (10–25 weeks old) were purchased from Saitama Experimental Animal Co. (Saitama, Japan). They were kept at a room temperature of 21–22°C and with lights on from 06:00 to 20:00. Food and water were made available ad libitum. Female mice were paired with males of proven fertility in the evening and the morning on which a vaginal plug or spermatozoa were found in the vagina was designated as day 1 of pregnancy. On each day of pregnancy, females were anesthetized with ethyl ether and killed by bloodletting from the heart. The uteri were quickly removed, rinsed in phosphate-buffered saline (PBS), frozen in liquid nitrogen, and kept at −70°C until use. Up to day 9 of pregnancy, whole uterine tissues were used. From day 10 to parturition, unless specified, the uteri were stripped off from the fetus and fetal elements, including fetal placentae. For immunohistochemical studies, whole tissues were fixed in Bouin's fixative without freezing.

Preparation of Uterine Extracts

Each uterus was homogenized with 9 vol of 20 mM Tris–HCl, pH 7.6, containing 10 mM 2-mercaptoethanol, 1 mM EDTA, and 0.43 mM phenylmethylsulfonyl fluoride (buffer A) with an Ultra Turrax homogenizer. After the homogenate was centrifuged at 15,000*g* for 20 min, the supernatant was dialyzed against buffer A overnight. The dialysates were assayed through enzymatic activity measurements and immunoblotting.

Peptidylarginine Deiminase Activity and Protein Assays

Peptidylarginine deiminase activity in the extract was assayed by measuring the formation of citrullyl residues in protamine as described previously [Takahara et al., 1983]. One unit of the enzyme is defined as the amount of enzyme that catalyzes the formation of 1 μmol of citrullyl residues from arginyl residues in 1 h at 55°C. The concentration of protein was determined by the method of Read and Northcote [1981], using bovine serum albumin (BSA) as a standard.

Western Immunoblotting Analysis

Each sample (20 μg of protein) was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (9% gel) [Laemmli, 1970] and transferred to a PVDF membrane (Nihon Millipore Kogyo K.K., Yonezawa, Japan) by the method of Towbin et al. [1979]. Pretreatment of the blotting membrane, immunochemical reaction with the primary and secondary antibodies, and color development were carried out as reported previously [Takahara et al., 1989].

Immunohistochemistry

Immunochemical procedures were much the same as those described previously [Takahara et al., 1992]. Briefly, tissues fixed in Bouin's fixative were dehydrated with a graded series of ethanol, infiltrated with xylene and embedded in paraffin wax. The sections (4 μm thick) were deparaffinated with xylene, treated with PBS containing 3% hydrogen peroxide to block endogenous peroxidase activity, and then rinsed with PBS. After incubation with 1:20 diluted normal goat serum to block nonspecific binding, the sections were incubated successively with 1:1,500 diluted rabbit antimouse peptidylarginine deiminase, 1:80 diluted $F(ab')_2$ fragment of goat anti-rabbit IgG, and 1:60 diluted peroxidase–rabbit antiperoxidase complex. The reaction product of

the peroxidase was visualized by using 3,3′-diaminobenzidine and hydrogen peroxide. The sections were counterstained with Mayer's hematoxylin.

Immunoelectron Microscopy

To determine the intracellular localization of peptidylarginine deiminase in decidual cells, immunoelectron microscopy was carried out as described previously [Takahara et al., 1989; Kohsaka et al., 1992]. In brief, the uterus was first fixed by perfusion with PBS containing 4% paraformaldehyde and 0.3% glutaraldehyde. The selected tissue was cut into small pieces (1 cm^3) and immersed in the above fixative solution. The prefixed tissue was further fixed with PBS containing 1% osmium tetraoxide, dehydrated in a graded series of ethanol and acetone, and then embedded in epon resin. A thin section was cut with a glass knife and mounted on a nickel grid. The section on the grid was incubated successively in saturated metaperiodic acid, PBS containing 1% BSA and 1:500 diluted rabbit antimouse peptidylarginine deiminase. After rinsing with PBS, the section was incubated with protein A–gold solution, and then negative staining was performed with uranyl acetate. Localization of the enzyme was observed with a JEOL JEM-100 electron microscope.

Dot RNA Hybridization

For a semiquantitative analysis of uterine peptidylarginine deiminase mRNA, dot RNA hybridization was performed as described previously [Takahara et al., 1992]. In brief, poly(A)$^+$RNA was isolated from the uterus (0.7–0.8 g) at each stage using a Fast Track mRNA Isolation Kit (Invitrogen) and RNA concentration of each sample was estimated by measuring absorbance at 260 nm. Prior to hybridization analysis, each sample was serially diluted to be 6.0, 3.0, 1.5, 0.75, 0.38, 0.19, and 0.09 μg/100 μl in 20 mM morpholino propanesulfonic acid buffer, pH 7.0, containing 5 mM sodium acetate, 0.5 mM EDTA, 50% formamide, 2.2 M formaldehyde, denatured for 15 min at 65°C; 100-μl aliquots were then immobilized on Hybond-N$^+$ membrane (Amersham) using a dot-blot apparatus (ATTO Co., Tokyo, Japan). The membrane was prehybridized for 12 h at 63°C in prehybridization solution, consists of 6× SSC (1× SSC: 150 mM NaCl, 15 mM sodium citrate), 0.5% SDS, 5× Denhardt's solution, 50% formamide, and 0.1 mg/ml denatured salmon sperm DNA. *Eco*RI–*Fsp*I fragment (598-bp) derived from the 5′-terminal tract of mouse peptidylarginine deiminase cDNA [Tsuchida et al., 1993] was labeled using Randam Priming DNA Labeling Kit (Boehringer Mannheim) and [α-^{32}P]dCTP (ICN), and used for hybridization probe. Hybridization was performed for 12 h at 63°C in the prehybridization solution with the ^{32}P-labeled DNA probe (specific radioactivity = 6.5×10^8 cpm/μg DNA) added. The membrane was washed successively with 1× SSC containing 0.1% SDS and 0.2× SSC containing 0.1% SDS for 30 min at 63°C. Autoradiography was carried out at −80°C using Fuji RX film.

RESULTS

Stage-Dependent Changes of Uterine Peptidylarginine Deiminase Activity During Pregnancy

The activity of peptidylarginine deiminase in the mouse uterus during pregnancy was determined per g of tissue and per mg of protein in the extract. As reported previously, enzymatic activity during the estrous cycle was highest at the estrus stage [Takahara et al., 1989; Tsuchida et al., 1993]. Since the uterus can be fertilized only in the estrus, the biological condition of the uterus on day 1 is thought to be almost same as that in the estrus stage. Indeed, as shown in Figure 1, the uterus on day 1 revealed a high level of peptidylarginine deiminase activity, which was consistent with high activities in the estrus stage [Takahara et al., 1989; Tsuchida et al., 1993]. From day 1 to day 3, the enzymatic activity rapidly decreased, and after remaining at a low level for 4 days, the activity increased dramatically from day 8 to day 10. There was about a 10-fold difference in the enzyme activity per g of tissue between the minimum (day 5) and the maximum (day 10). After attaining maximal activity, the enzymatic activity decreased again until day 17. Figure 1 also shows a slight, but obvious peak in activity just at the onset of parturition. In order to distinguish whether the time-dependent changes in enzymatic activity were due to regulation of the enzymatic activity or to changes in the actual amount of the enzyme, we performed an immunoblotting analysis with extracts from the different stages. As shown in Figure 2, a single band with the same mobility as that of the authentic peptidylarginine deiminase could be seen in the extracts. The density of each band

correlated with the enzymatic activity. These results indicated that the changes in activity during pregnancy were due to changes in the amount of the enzyme present in the extracts. As mentioned below, the tissue localization of the enzyme at mid-pregnancy was quite different from that at early pregnancy. Therefore, we compared the immunological properties of the enzymes obtained from the uteri in early and mid-pregnancy. A double immunodiffusion analysis demonstrated that the antibodies against peptidylarginine deiminase from skeletal muscle reacted equally well with enzymes from both early and mid-pregnancy and the precipitin lines were completely fused (Fig. 3). These results indicated that the enzyme expressed in middle pregnancy was immunologically indistinguishable from that of early pregnancy.

Specific Expression of Uterine Peptidylarginine Deiminase in the Decidualized Cells at Mid-pregnancy

By using immunohistochemical methods, we examined the tissue localization of uterine peptidylarginine deiminase at different times of pregnancy and parturition. On day 1 of pregnancy, the morphology and the enzyme distribution were quite similar to those of the estrus stage [Takahara et al., 1989]; the enzyme localized in the luminal and glandular epithelia (data not shown). From day 2 to day 4, the immunoreactive staining of the enzyme decreased with the regression of both types of epithelial cells (data not shown).

In the mouse, implantation of the embryo is initiated at the antimesometrial luminal epithelium on day 5, and endometrial stromal cells surrounding the implanted embryo, in response to the implanting blastocyst, undergo proliferation and differentiation resulting in the formation of decidual cells [Defeo, 1967]. Figure 4 shows typical immunohistochemical stainings at low and high magnifications of the implantation zone between day 5 and day 15. As shown in Figure 4A and 4a, decidualization of the endometrial stromal cells was initiated around the implantation site. Immunohistochemical staining of the enzyme was scarcely detectable in this section, except that a trace of the enzyme remained in the luminal and glandular epithelial cells. On day 7, extensive decidualization was observed in both mesometrial and antimesometrial zones (Fig. 4B and 4b). At this stage, the uterine gland had disappeared and the luminal epithelium was short. The immunoreactive staining of the enzyme was evident in the decidual cells within the central zone (Fig. 4B and 4b). On day 9, the antimesometrial decidua started to regress and formed the decidua capsularis, while the embryo and the extraembryonic tissues, such as the trophoblastic giant cells and

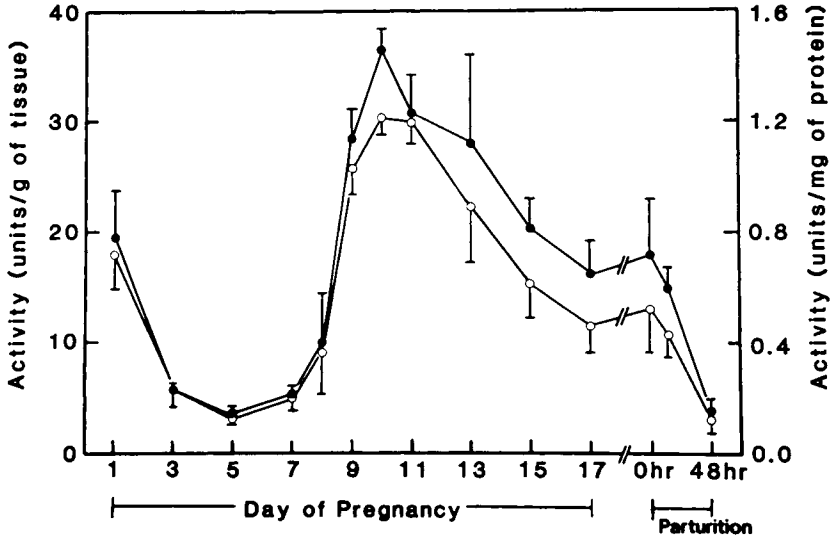

Fig. 1. Changes of peptidylarginine deiminase activity in mouse uterus during pregnancy and in parturient period. Uterine extracts of each stage were prepared, and peptidylarginine deiminase activity and protein contents in the extracts were assayed, as described under Materials and Methods. Closed and open circles indicate the activity per g of tissues and per mg of proteins, respectively. Each value is the mean ± SD of triplicate determinations done with the extracts composed from five animals.

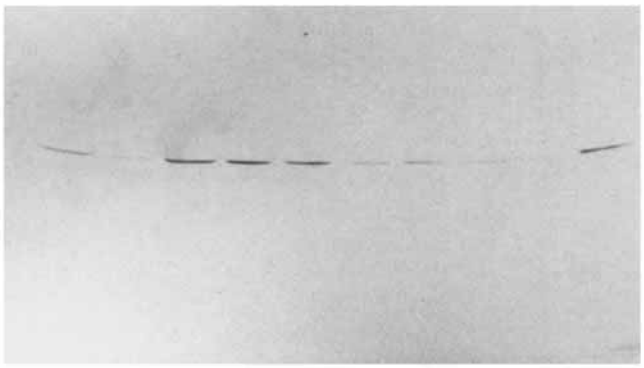

Fig. 2. Western immunoblotting analysis of peptidylarginine deiminase in uterine extracts during pregnancy and in parturient period. The extracts (20 μg) were electrophoresed on SDS–polyacrylamide gel, transferred, and immunochemically stained using rabbit antimouse peptidylarginine deiminase serum. **Lanes 1–6,** day 1, 5, 9, 11, 13, 17 of pregnancy, respectively; **lane 7,** onset of parturition; **lanes 8, 9,** 12 and 48 h after parturition, respectively; **lane 10,** control mouse skeletal muscle peptidylarginine deiminase (3 μg).

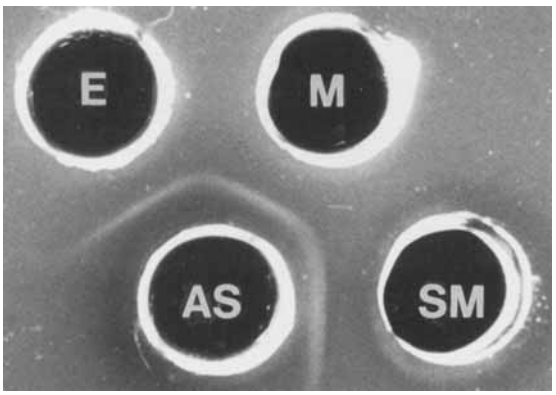

Fig. 3. Ouchterlony double immunodiffusion test of uterine peptidylarginine deiminase from early and middle stage of pregnancy. Uterine peptidylarginine deiminases at early (day 1) and middle (day 11) stages of pregnancy were subjected to immunodiffusion test in 0.1% agarose gel described as previously [Takahara et al., 1989]. E, peptidylarginine deiminase (1.2 units) at day 1 of pregnancy; M, the enzyme (1.2 units) at Day 11 of pregnancy; SM, control mouse skeletal muscle peptidylarginine deiminase (1.1 units); AS, rabbit antimouse peptidylarginine deiminase.

the cytotrophoblast-labyrinth structure, began to proliferate (Fig. 4C). As shown in Figure 4C and 4c, widespread immunoreaction to the enzyme was observed in the decidual cells surrounding the embryo. On day 10, when peptidylarginine deiminase activity reached the highest level (Fig. 1), intense immunoreaction to the enzyme occurred in the decidua capsularis and the decidual cells along the cytotrophoblast-labyrinth structure (Fig. 4D and 4d). The giant cells have proliferated since day 9, but these cells showed no immunoreaction (Fig. 4D and 4d). Furthermore, no immunochemical staining was observed in the luminal and glandular epithelial cells around the far implantation zone, in which cells the enzyme was induced by estrogen at the estrus stage of nonpregnancy (data not shown). From day 11 to day 15, the decidua capsularis was reduced to a fine layer of cells between the fetal cavity and the uterus. The antibodies against the enzyme reacted with the decidual cells bordering the trophoblast giant cell layer on both sides. These cells probably correspond to remnants of the antimesometrial decidua on the side of the mesometrial decidua. No immunoreaction was seen in the labyrinthine placenta, which had proliferated since day 11 (Fig. 4E, 4e, 4F, and 4f).

During the parturient period, peptidylarginine deiminase was expressed again in the uterine epithelium. Figure 5A and 5a shows the immunohistochemistry of the enzyme just after parturition. The enzyme was localized in both epithelial cells of the uterine gland and the lumen. As suggested by the level of enzymatic activity in Figure 1, this expression was temporary. The intensity of the immunochemical staining in the epithelia decreased 12 h after parturition (data not shown).

Immunocytochemical Localization of Peptidylarginine Deiminase in Decidual Cells

A previous immunocytochemical study of peptidylarginine deiminase in the nonpregnant murine uterus indicated that the enzyme was distributed throughout the cytoplasm of the

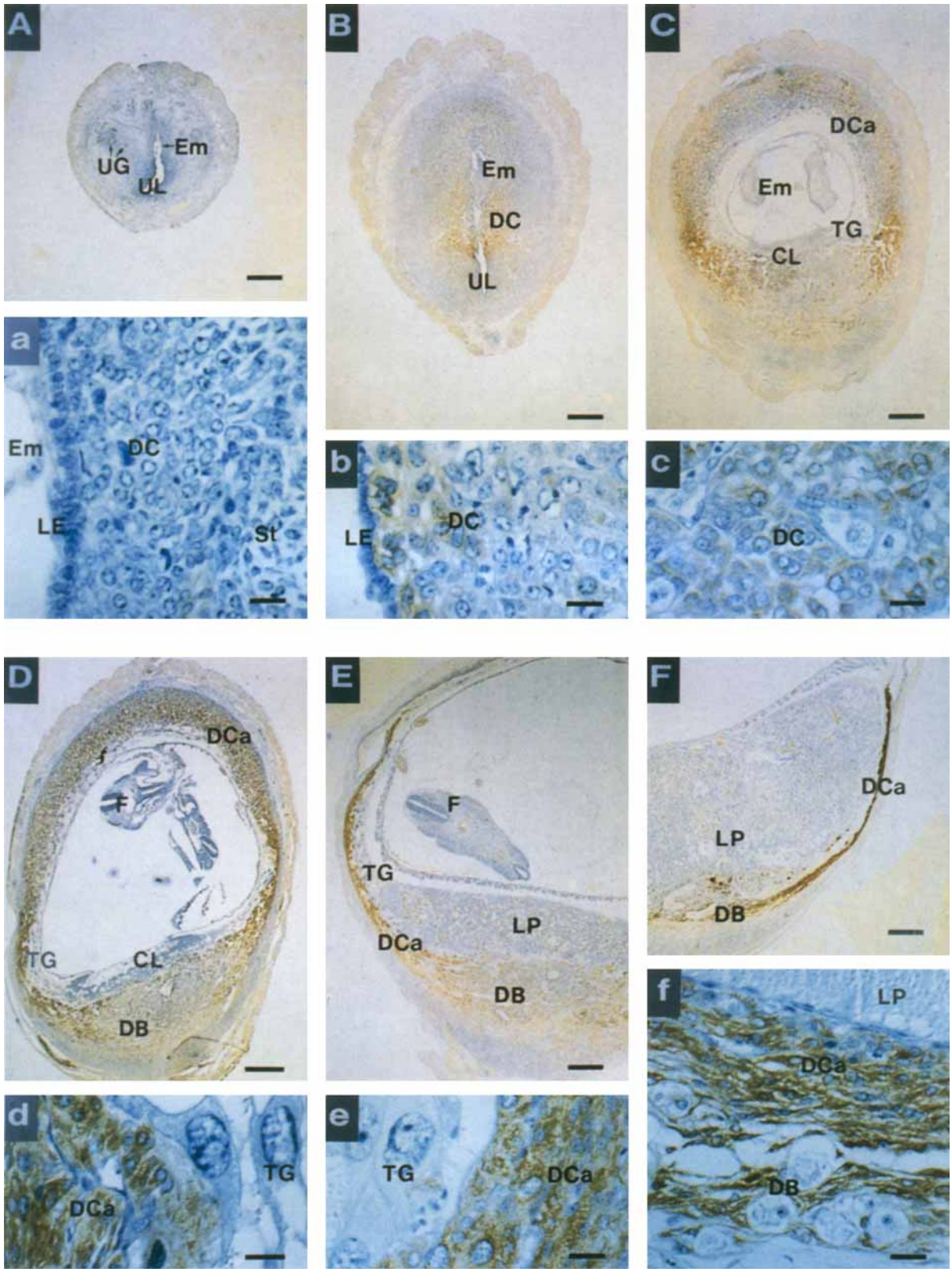

Fig. 4. Immunohistochemical stains of peptidylarginine deiminase in murine uterus during pregnancy. Sections of the uterus on day 5 (**A, a**), day 7 (**B, b**), day 9 (**C, c**), day 10 (**D, d**), day 11 (**E, e**), and day 15 (**F, f**) were immunostained as described under Materials and Methods. A–F: Low magnification; a–f, high magnification by Nomarski differential interference contrast. Bars in A–F and in a–f indicated 0.4 and 0.02 mm, respectively. The upper direction of each low magnified photography shows antimesometrial side. UL, uterine lumen; UG, uterine gland; LE, luminal epithelium; St, stromal cell; Em, embryo, F, fetus; P, placenta; TG, trophoblastic giant cell; DC, decidual cell; DCa, decidua capsularis; DB, decidua basalis; CL, cytotrophoblast-labyrinth; LP, labyrinthine placenta.

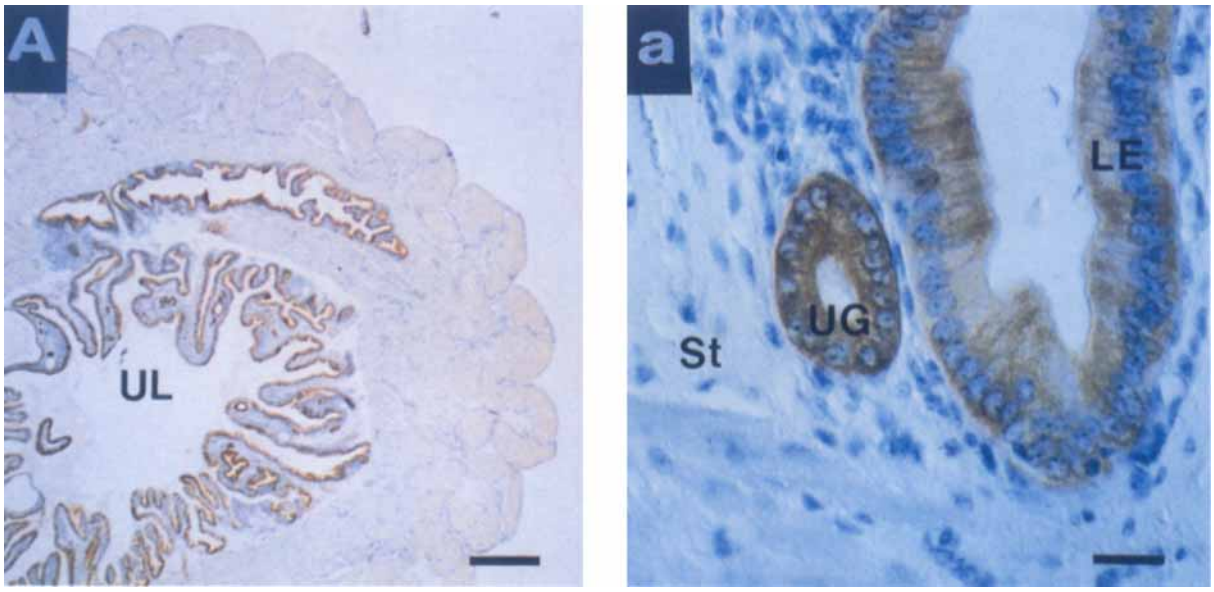

Fig. 5. Immunohistochemical stains of peptidylarginine deiminase in murine uterus just after parturition. Section of the uterus just after parturition was subjected to immunohistochemical staining as described under Materials and Methods. **A**, low magnification (bar = 0.4 mm); **a**, high magnification (bar = 0.02 mm) by Nomarski differential interference contrast. UL, uterine lumen; LE, luminal epithelium; UG, uterine gland; St, stromal cell.

epithelial cells [Takahara et al., 1989]. To investigate the intracellular localization of the enzyme in decidual cells, we carried out an immunoelectromicroscopic analysis of the uterus from day 10 of pregnancy. As shown in Figure 6, gold particles, which represented the location of the enzyme, were diffused throughout the cytoplasm.

Changes of the Uterine Peptidylarginine Deiminase mRNA Level During Pregnancy

To determine whether the expression of the uterine peptidylarginine deiminase was regulated at the translational or pretranslational level during pregnancy, semiquantitative analysis of the mRNA was carried out by Northern RNA dot-blot hybridization at various times of the pregnancy. Serially decreasing amounts of poly(A)$^+$ RNA (6.0–0.09 μg) were blotted on a nylon membrane and hybridized with the radioactive cDNA fragment derived from mouse uterus peptidylarginine deiminase cDNA [Tsuchida et al., 1993]. As shown in Figure 7, the RNAs gave little or no signal, even at the maximum input (6 μg), up to day 6 of pregnancy. From day 7, the RNAs showed a markedly increased signal that remained high for 2 days. On day 11, the level of the mRNA decreased to one-half of that of day 10, and then increased again, reaching the highest level on day 12. The hybridization signal of the RNA on day 12 was detectable with the minimum amount of the RNA (0.09 μg) (Fig. 7, lane 12). Although the mRNA level decreased gradually after day 12, one additional increase was observed between day 15 and day 16 of pregnancy.

Figure 7 also demonstrates the changes in the level of the mRNA in the parturition period. The level of mRNA increased on the border of parturition, and then fell sharply. Two days after parturition, the enzyme mRNA had almost disappeared.

DISCUSSION

Our previous studies revealed that peptidylarginine deiminase was localized in the luminal and glandular epithelia of the nonpregnant murine uterus and that the level of the enzyme varied with the estrous cycle, with the maximum occurring at estrus and the minimum at diestrus [Takahara et al., 1989; Tsuchida et al., 1993]. Furthermore, the expression of the enzyme in the epithelia was induced by estrogen [Takahara et al., 1992]. In this study, we found further changes in the level and the tissue localization of the uterine enzyme during pregnancy and parturition. The most remarkable finding of this study was that peptidylarginine deiminase was expressed in decidual cells in middle and late pregnancy. The decidua are recognized as

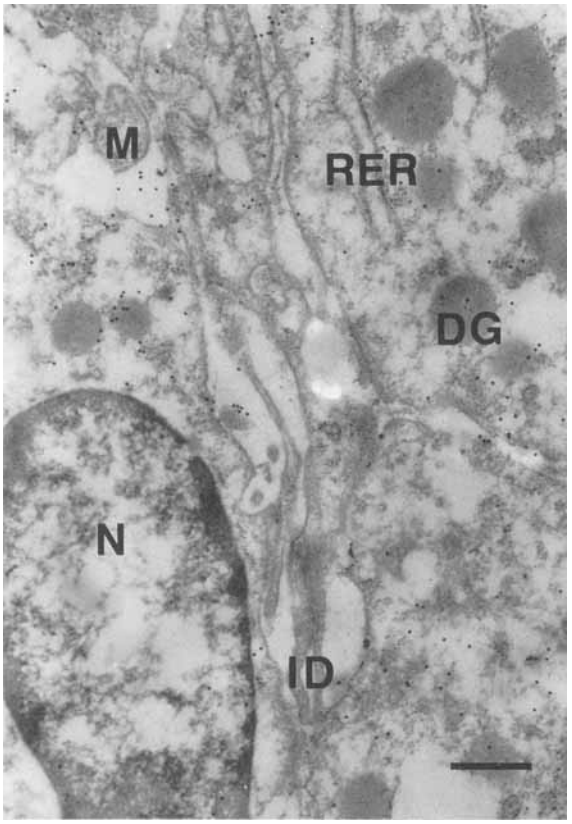

Fig. 6. Immunocytochemistry of peptidylarginine deiminase in decidualized cells. Thin section of decidualized cells were immunostained by protein A–gold method described under Materials and Methods. Dark dots are gold particles indicating localization of the enzyme. Original magnification is ×10,000 and the bar shows 500 nm. N, nucleus; M, mitochondria; RER, rough endoplasmic reticulum; DG, dense granule; ID, interdigitation.

endocrine tissues that synthesize many peptide hormones, such as prolactin [Markoff et al., 1983], prolactin-like hormone [Jayatilak et al., 1985; Croze et al., 1990], and decidual calcyclin [Thordarson et al., 1991]. In addition to these peptide hormones, alkaline phosphatase [Manning et al., 1969] and histaminase [Weisburger et al., 1978; Lin et al., 1978] were also expressed with decidualization of the stroma cells. Two hypotheses about the role of decidual cells are that they provide a supplement of nutrients to the embryo until the completion of blood vessels between the mother and fetus [Finn, 1971] and that they provide the mother with immunosuppression against fetal antigens [Clark et al., 1990]. Since peptidylarginine deiminase modifies biologically active arginine residues of proteins [Takahara et al., 1985; Kurokawa et al., 1987; Takahara and Sugawara, 1987; Inagaki et al., 1989; Lamensa and Moscarello, 1993; Azuma et al., 1994], in the decidual cells, the function of the enzyme may be to regulate the activity of peptide hormones and other enzymes produced in these cells.

The decidua were differentiated from the endometrial stroma surrounding the embryo. Immunohistochemical and immunoelectromicroscopic analyses demonstrated that peptidylarginine deiminase was distributed in the cytosol of the decidual cells. Decidualization started at the implantation zone on day 5 of pregnancy and the maximal proliferation was observed on day 7, whereas the expression of the enzyme in the decidua was delayed for about 3 days and the amount of enzyme maintained until the degeneration (Fig. 4). Therefore, the occurrence of the enzyme does not seem to be correlated with the initial phase of the decidualization. Previously we reported that peptidylarginine deiminase modified the intermediate filament protein in a micromolar Ca^{2+}-dependent manner, causing the disassembly of the polymerized filaments and the inhibition of polymerization of the soluble intermediate proteins [Inagaki et al., 1989]. Recently, Senshu et al. [1992] have demonstrated the presence of citrullyl residues in the intermediate filament proteins. These results indicate that peptidylarginine deiminase may take part in the morphological changes or degeneration of decidual cells mediated through the disassembly of the intermediate filaments.

Increased level of peptidylarginine deiminase occurred not only in the middle stage of pregnancy, but also on day 1 of pregnancy and before the onset of parturition. Immunohistochemical studies of both stages showed that the enzyme exists in the luminal and glandular epithelial cells. This tissue localization has also been observed in the estrus of the nonpregnant mouse uterus. Our previous studies demonstrated that the expression of the enzyme in the uterine epithelia was regulated by estrogen (agonist) and progesterone (antagonist) [Takahara et al., 1992]. Peripheral estradiol-17 levels showed an inverse relationship with respect to progesterone levels during the estrous cycle and pregnancy [Shaikh, 1971; Morishige et al., 1973; Kalra and Kalra, 1974]. Since the estrogen levels were high on day 1 and around the time of parturition, concomitant with a low level of progesterone [Shaikh et al., 1971; Morishige et al., 1973], the expression of the enzyme in the uterine epithelial cells at the above two stages might

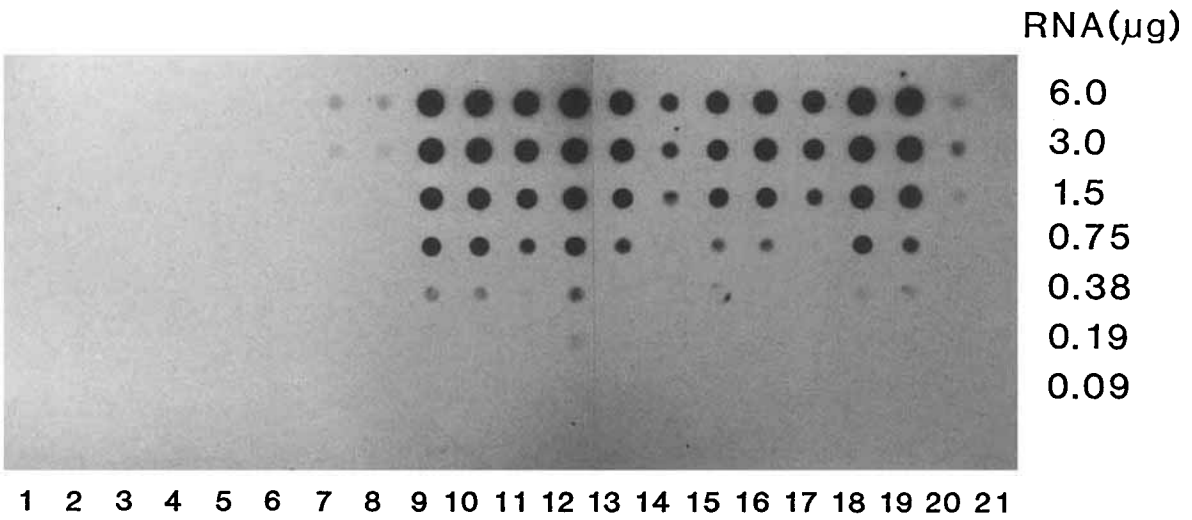

Fig. 7. Dot RNA hybridization of peptidylarginine deiminase mRNA in uterus during pregnancy and in parturient period. Poly(A)$^+$RNA (6.0–0.9 μg) isolated from uterus at each stage were blotted onto nylon membrane, hybridized with [^{32}P]-labeled cDNA fragment, washed, and autoradiographed by the method as described under Materials and Methods. **Lanes 1–18,** the RNAs from day 1–day 18; **lane 19,** onset at parturition; **lanes 20, 21,** 12 and 48 h after parturition, respectively.

be regulated by the steroid hormones. The expression of the enzyme mRNA around the time of parturition, when estrogen levels increase, supports the above speculation. On the other hand, expression of the enzyme mRNA in the decidua seems to be controlled by a mechanism different from that in the epithelial cells. The enzyme mRNA level during the first half of pregnancy started to increase on day 7 and rose to a peak on day 10, with a slight decrease on day 11. The concentration of the mRNA during the second half of pregnancy rose on day 12 with a significant decrease on days 13–14, and increased slightly again on days 15–16. These serial changes in mRNA level could not be implicated in the control by the estrogen and progesterone. McCormack and Greenwald [1974] measured the concentration of estrogen and progesterone in peripheral plasma on each day of pregnancy in the mouse. They demonstrated that the high level of estrogen on day 1 was followed by a precipitous decline to a low level on day 2 and day 3. After a slight rise in the estrogen concentration occurred on day 4, the level remained low from day 5 to days 15–16, when a significant increase began along with a drop in progesterone and continued to the parturition. Recently, Thrailkill et al. [1988] reported that expressions of some peptides in the uterus were regulated under an autocrine or paracrine mechanism in decidual cells. The expression of peptidylarginine deiminase in decidua may be controlled under those mechanisms. Peptidylarginine deiminase expressed in the uterine epithelia and decidua were indistinguishable from one another with respect to immuno- and proteochemical properties. The peptidylarginine deiminase genes of rat [Watanabe et al., 1992] and mouse [Ichi et al., submitted for publication] were found to be single copy genes. Thus, the mechanism that regulates expression of the enzyme in the uterus is very interesting. Studies on the peptidylarginine deiminase gene expression mechanism and the physiological function of the enzyme in the uterus are in progress in our laboratory.

ACKNOWLEDGMENTS

This work was supported in part by a Scientific Research grant (02660077) from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

Azuma N, Oikawa K, Furuuchi S, Takahara H, Sugawara K, Kanno C (1994): Role of the arginyl residues of κ-casein in micelle formation—Effect of deimination on α_{s1}–κ-casein complex formation. Int Dairy J 4:193–204.

Clark DA, Flanders KC, Banwatt D, Millar-Book W, Manuel J, Stedronska-Clark J, Rowley B (1990): Murine pregnancy decidua produces a unique immunosuppressive molecule related to transforming growth factor β-2. J Immunol 144:3008–3014.

Croze F, Kennedy TG, Schroedter IC, Friesen HG (1990): Expression of rat prolactin-like protein B in deciduoma of pseudopregnant rat and in dicidua during early pregnancy. Endocrinology 127:2665–2672.

Defeo VJ (1967): Decidualization. In Wynn, RM (ed): "Cellular Biology of the Uterus." New York: Appleton-Century-Crofts, pp 191–290.

Finn CA (1971): The biology of decidual cells. Adv Reprod Physiol 5:1–26.

Fujisaki M, Sugawara K (1981): Properties of peptidylarginine deiminase from the epidermis of newborn rats. J Biochem (Tokyo) 89:257–263.

Inagaki M, Takahara H, Nishi Y, Sugawara K, Sato C (1989): Ca^{2+}-dependent deimination-induced disassembly of intermediate filaments involves specific modification of the amino-terminal head domain. J Biol Chem 264:18119–18127.

Jayatilak PG, Glaser LA, Basuray R, Kelly PA, Gibori G (1985): Identification and partial characterization of a prolactin-like hormone produced by rat decidual tissue. Proc Natl Acad Sci USA 82:217–221.

Kalra SP, Kalra PS (1974): Temporal interrelationships among circulating levels of estradiol, progesterone and LH during the rat estrous cycle: Effects of exogenous progesterone. Endocrinology 95:1711–1718.

Kohsaka T, Sasada H, Masaki J (1992): Subcellular localization of the antigenic sites of relaxin in the luteal cells of the pregnant rat using an improved immunocytochemical technique. Anim Reprod Sci 29:123–132.

Kubilus J, Baden HP (1983): Purification and properties of a brain enzyme which deiminates proteins. Biochim Biophys Acta 745:285–291.

Kubilus J, Baden HP (1985): The occurrence and immunology of peptidylarginine deiminase and its preparation from bovine epidermis by an improved method. J Invest Dermatol 85:232–234.

Kurokawa T, Hara S, Takahara H, Sugawara K, Ikenaka T (1987): Conversion of peanut trypsin-chymotrypsin inhibitor B-III to a chymotrypsin inhibitor by deimination of the P_1 arginine residues in two reactive sites. J Biochem (Tokyo) 101:1361–1367.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Lamensa JWE, Moscarello MA (1993): Deimination of human myelin basic protein by a peptidylarginine deiminase from bovine brain. J Neurochem 61:987–996.

Lin CW, Chapman CM, Delellis RA, Kirley S (1978): Immunofluorescent staining of histaminase (diamine oxidase) in human placnta. J Histochem Cytochem 26:1021–1025.

Manning JP, Steinetz BG, Giannina T (1969): Decidual alkaline phosphatase activity in the pregnant and pseudopregnant rat. Ann NY Acad Sci 166:482–509.

Markoff E, Zeitler P, Peleg S, Handwerger S (1983): Characterization of the synthesis and release of prolactin by an enriched fraction of human decidual cells. J Clin Endocrinol Metab 56:962–968.

McCormack JT, Greenwald GS (1974): Progesterone and oestradiol-17 β concentrations in the peripheral plasma during pregnancy in the mouse. J Endocr 62:101–107.

Morishige WK, Pere GJ, Rothchild I (1973): Serum luteinizing hormone, prolactin and progesterone levels during pregnancy in the rat. Endocrinology 92:1527–1530.

Read SM, Northcote DH (1981): Minimization of variation in the response to different proteins of the Coomassie blue G dye-binding assay for protein. Anal Biochem 116:53–64.

Rogers GE, Taylor LD (1977): The enzymic derivation of citrulline residues from arginine residues in situ during the biosynthesis of hair proteins that are cross-linked by isopeptide bonds. Adv Exp Med Biol 84A:283–294.

Senshu T, Sato T, Inoue T, Akiyama K, Asaga H (1992): Detection of citrulline residues in deiminated proteins on polyvinylidene difluoride membrane. Anal Biochem 203: 94–100.

Shaikh AA (1971): Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. Biol Reprod 5:297–307.

Takahara H, Oikawa Y, Sugawara K (1983): Purification and characterization of peptidylarginine deiminase from rabbit skeletal muscle. J Biochem (Tokyo) 94:1945–1953.

Takahara H, Okamoto H, Sugawara K (1985): Specific modification of the functional arginine residue in soybean trypsin inhibitor (Kunitz) by peptidylarginine deiminase. J Biol Chem 260:8378–8383.

Takahara H, Sueyoshi K, Sugawara K (1986): Activities and properties of peptidylarginine deiminases of several vertebrate brains. Agric Biol Chem 50:1303–1306.

Takahara H, Sugawara K (1987): Specific modification of an arginine residue in mouse contrapsin by peptidylarginine deiminase altered its inhibitory activities against trypsin and chymotrypsin. Agric Biol Chem 51:1657–1664.

Takahara H, Tsuchida M, Kusubata M, Akutsu K, Tagami S, Sugawara K (1989): Peptidylarginine deiminase of the mouse. Distribution, properties, and immunocytochemical localization. J Biol Chem 264:13361–13368.

Takahara H, Kusubata M, Tsuchida M, Kohsaka T, Tagami S, Sugawara K (1992): Expression of peptidylarginine deiminase in the uterine epithelial cells of mouse is dependent on estrogen. J Biol Chem 267:520–525.

Thordarson G, Southard JN, Talamantes F (1991): Purification and characterization of mouse decidual calcyclin: A novel stimulator of mouse placental lactogen-II secretion. Endocrinology 129:1257–1265.

Thrailkill KM, Golander A, Underwood LE, Handwerger S (1988): Insulin-like growth factor I stimulates the synthesis and release of prolactin from human decidual cells. Endocrinology 123:2930–2934.

Towbin H, Staehelin T, Gordon J (1979): Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354.

Tsuchida M, Takahara H, Minami N, Arai T, Kobayashi Y, Tsujimoto H, Fukazawa C, Sugawara K (1993): cDNA nucleotide sequence and primary structure of mouse uterine peptidylarginine deiminase. Detection of a 3′-untranslated nucleotide sequence common to the mRNA of transiently expressed genes and rapid turnover of this enzyme's mRNA in the estrous cycle. Eur J Biochem 215:677–685.

Watanabe K, Akiyama K, Hikichi K, Ohtsuka R, Okuyama A, Senshu T (1988): Combined biochemical and immunochemical comparison of peptidylarginine deiminases present in various tissues. Biochim Biophys Acta 966:375–383.

Watanabe K, Senshu T (1989): Isolation and characterization of cDNA clones encoding rat skeletal muscle peptidylarginine deiminase. J Biol Chem 264:15255–15260.

Watanabe K, Nomoto M, Nagata S, Itoh Y, Hikichi K, Maruyama N, Mita T, Senshu T (1992): The rat peptidylarginine deiminase-encoding gene: Structural analysis and the 5′-flanking sequence. Gene 114:261–265.

Weisburger WR, Mendelsohn G, Eggleston JC, Baylin SB (1978): Imunohistochemical localization of histaminase (diamine oxidase) in decidual cells of human placenta. Lab Invest 38:703–706.