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The disappearance of CD34-positive and alpha-smooth muscle actin-positive stromal cells associated with human intra-uterine and tubal pregnancies

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Summary. In order to elucidate the change in alpha-smooth muscle actin (ASMA)-positive and CD34-positive stromal cells associated with pregnancy, we examined endometrial and Fallopian tube tissues from 40 patients including normal endometrium (n=10), intra-uterine pregnancy (n=10), normal Fallopian tube (n=10), and tubal pregnancy (n=10), using immunohisto-chemistry. In normal endometrium, only a few ASMA-positive cells were focally observed. Additionally, a wide range of CD34-positive stromal cell abundance was observed. In normal Fallopian tube mucosa, a small to moderate number of both ASMA-positive and CD34-positive stromal cells was observed. Neither ASMA-positive nor CD34-positive stromal cells were observed anywhere in the decidual stroma during both intra-uterine and tubal pregnancies. Likewise, a varying abundance of ASMA-positive cells but no CD34-positive stromal cells were observed at the fetal side during both intra-uterine and tubal pregnancies. In conclusion, the disappearance of CD34-positive and ASMA-positive stromal cells may be an indicator of decidualisation induced change in the stroma during both intra-uterine and tubal pregnancies. ASMA-positive stromal cells at the fetal side associated with pregnancy may play a role in the production of villous extracellular matrix or regulation of blood flow.

Key words: Pregnancy, CD34, ASMA, Stromal cells

Introduction

CD34 has been discovered as a marker of human hematopoietic cells. and this molecule is a 110kDa transmembrane cell surface glycoprotein. CD34-positive neoplastic cells were detected in various tumors, including leukemia, vascular tumors, dermato-fibrosarcoma protuberans, solitary fibrous tumors and gastrointestinal stromal tumors (van de Rijn and Rouse, 1994). CD34-positive stromal cells and myofibroblasts are distributed widely under various normal organ or pathological conditions (Yamazaki and Eyden, 1995, 1996a,b, 1997; Nakayama et al., 2000; Papadas et al., 2001; Barth et al., 2002a-c). Although a relationship between CD34-positive stromal cells and myofibroblasts is not evident, some researchers suggest that there is a population of stromal cells expressing both CD34 and alpha-smooth muscle actin (ASMA) (Yamazaki and Eyden, 1996a; Barth et al., 2002c). Myofibroblasts express ASMA, vimentin and/or desmin. On the other hand, high molecular weight caldesmon (h-CD) is a novel smooth muscle actin-specific antibody and is not expressed in myofibroblasts (Ueki et al., 1987; Ceballos et al., 2000; Watanabe et al., 2000; Rush et al., 2001). In an attempt to identify a stromal change associated with pregnancy, we examined the expression of CD34 and ASMA in stromal cells of human normal endometrium, normal Fallopian tube, and intra-uterine and tubal pregnancies, using immunohistochemistry.

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Materials and methods

Tissue specimens

In the present study we selected 40 cases including normal endometrium (n=10), intra-uterine pregnancy (n=10), normal Fallopian tube (n=10), and tubal pregnancy (n=10). We selected those normal endometrium cases which underwent endometrial curettage for suspected endometrial hyperplasia or cancer. Likewise, we selected normal tube cases which underwent a salpingectomy for uterine leiomyoma or ovarian tumor. The 40 patients ranged from 21 to 49 years old (mean, 37.0 years old) and all women were pre-menopausal. For light microscopy, all specimens were immediately fixed in 10% neutral formaldehyde solution and embedded in paraffin.

Immunohistochemistry and its interpretation.

Using a streptoavidin-biotin immunoperoxidase technique, 3-μm sections of each specimen were evaluated for the detection for antigens of ASMA (1:50 dilution, monoclonal, clone:1A4, Dako Cytomation, Glostrup, Denmark), h-CD (1:50 dilution, monoclonal, clone:h-CD, Dako Cytomation, CA, USA), CD34 (1:20 dilution, monoclonal, clone:MY10, Becton-Dickinson, San Jose, CA, USA), and CD31 (1:20 dilution, monoclonal, clone:JC/70A, Dako Cytomation, Glostrup, Denmark). The sections for h-CD were microwaved for five minutes three times in 10 mmol/L citrate buffer, pH 6.0. The sections for CD31 were pretreated before immunostaining with 0.1% pronase E for 20 minutes at 37 ºC. Vascular smooth muscle cells and endothelial cells were used as the internal positive controls for ASMA and h-CD, and CD34 and CD31, respectively. Appropriate negative controls for all antibodies were also performed.

We considered stromal cells that were positive for both ASMA and h-CD as smooth muscle cells, and ASMA-positive and h-CD-negative cells as ASMA-positive stromal cells. Furthermore, CD34-positive and CD31-negative stromal cells were considered as CD34-positive stromal cells. Thus, we evaluated the distribution of CD34-and/or ASMA-positive stromal cells in normal mucosal tissues and during each pregnancy.

Double immunostaining

A double immunostaining for CD34 and ASMA was performed for all specimens from the 40 patients in order to identify the relationship between ASMA-positive stromal cells and CD34-positive stromal cells. Sections were treated with 0.3% hydrogen peroxide/methanol for 10 min at room temperature (RT) and incubated overnight with anti-CD34 antibody. Then the sections were incubated with peroxidase-conjugated mouse IgG and rabbit IgG (Simple stain PO-MAX (multi), Nichirei, Tokyo, Japan) for 1h at RT and immersed in 0.2% DAB and 0.1% hydrogen peroxide in 0.05M Tris buffer. After washing with PBS, the sections were incubated for 2 hr at RT with anti-ASMA antibody. They were incubated with biotinylated rabbit anti-mouse IgG $F(ab')_2$ fragment (Dako Cytomation, Glostrup, Denmark) for 1h at RT and alkaline phophatase-conjugated streptoavidin (Nichirei, Tokyo, Japan) for 30 min at RT. To visualize this reaction the sections were stained with Fast blue.

Results

Distribution of stromal cells in normal endometrium and fallopian tube

Stromal cell distribution is summarized in Tables 1 and 2. In normal endometrium, ASMA-positive stromal cells were generally absent, and only a few ASMA-positive cells were focally observed (Fig. 1a). However, a small to large number of CD34-positive stromal cells was distributed in a reticular network (Fig. 1b). In normal Fallopian tube, ASMA-positive stromal cells were distributed in the lamina propria and submucosal layers (Fig. 1c, d). A small to moderate number of CD34-positive stromal cells was observed in normal mucosa of Fallopian tubes (Fig. 1e). Additionally, a dense reticular network containing CD34 was observed in or around the smooth muscle layer.

Double immunostaining

A number of ASMA and CD34 positive cells was observed in the normal mucosa of Fallopian tubes (Fig. 1f) and at the peridecidual mucosa of both intra-uterine and tubal pregnancies. Cells positive for both antigens seemed to be more abundant in normal mucosa of

Table 1. Distribution of stromal cells in normal endometrium and intra-uterine pregnancy.

	NORMAL ENDOMETRIUM	INTRA-UTERINE PREGNANCY (n=10)		
	(n=10)	Decidual stroma	Endometrium adjacent to decidua	Chorionic villi
ASMA-positive stromal cells	+-	-	+++	+~+++
CD34-positive stromal cells	+~+++	-	+-	-

Table 2. Distribution of stromal cells in normal mucosa of fallopian tube and tubal pregnancy.

	NORMAL MUCOSA OF FALLOPIAN TUBE	TUBAL PREGNANCY (n=10)		
	(n=10)	Decidual stroma	Mucosa adjacent to decidua	Chorionic villi
ASMA-positive stromal cells	+~++	-	+++	+~+++
CD34-positive stromal cells	+~++	-	+-~+	-

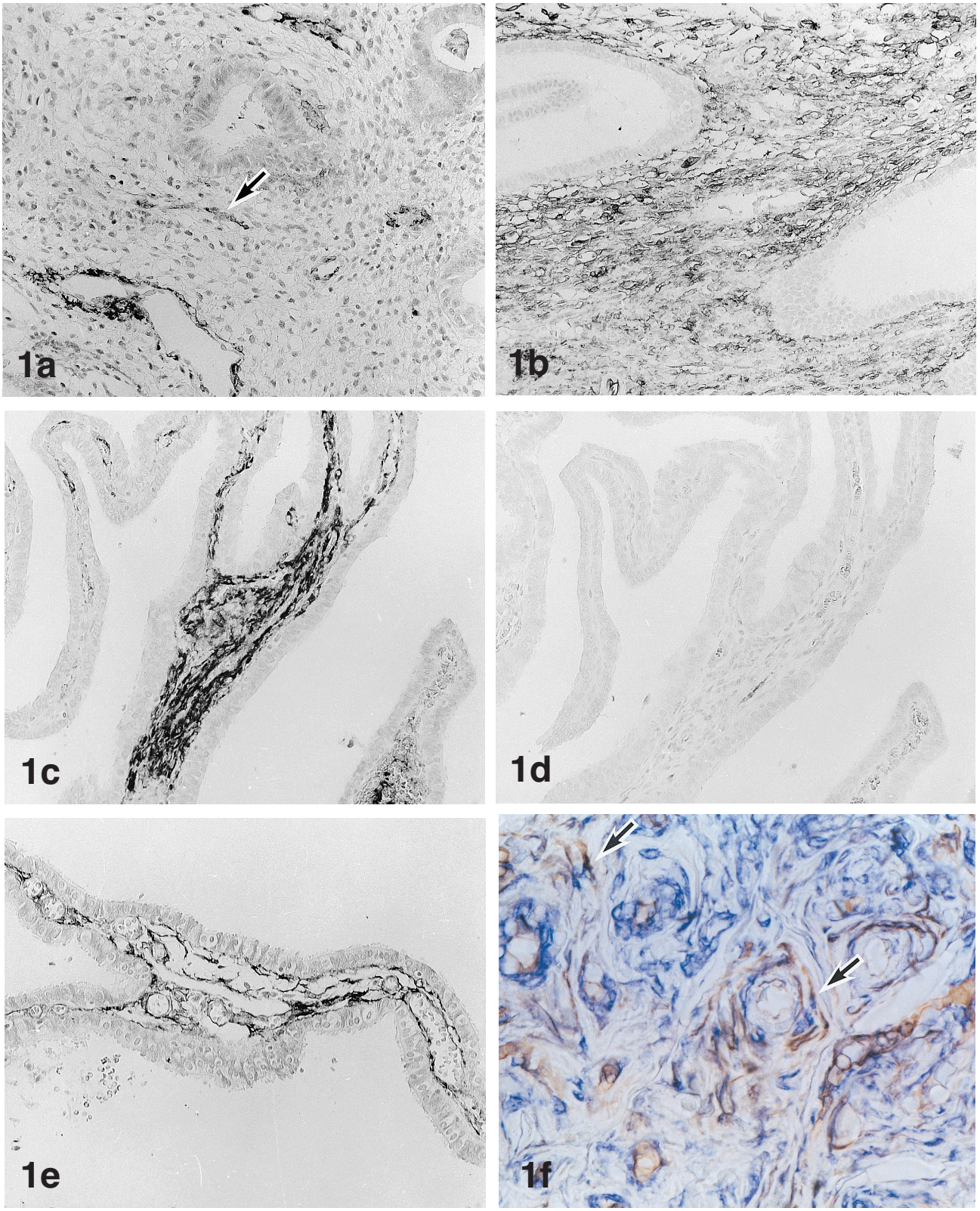

Fig. 1. a. Normal endometrium. Only a few ASMA-positive stromal cellls (arrow) are focally observed. x 50. **b.** Normal endometrium. A dense reticular network of CD34 cells is observed x 50. **c,d.** Normal mucosa of Fallopian tube. Some ASMA-positive stromal cells are present. c) ASMA x 50, **d.** h-CD (x 50). **e)** Normal mucosa of Fallopian tube. A small to moderate number of CD34-positive stromal cells is observed (x 50). **f)** Double immunohistochemistry of ASMA (blue) and CD34 (red) in the mucosa of normal Fallopian tube. Some cells (arrows) positive for both ASMA and CD34 are observed. x 100

Fallopian tubes than at the peridecidual mucosa of intra-uterine and tubal pregnancies.

Distribution of stromal cells in the decidua and adjacent mucosa of intra-uterine and tubal pregnancies

Neither ASMA-positive nor CD34-positive stromal cells were observed anywhere in the decidual stroma of intra-uterine and tubal pregnancies (Fig. 2a-d). In contrast, many ASMA-positive and h-CD-negative stromal cells were observed at the peridecidual endometrium of intra-uterine pregnancy (Fig. 3a, b). However, few CD34-positive stromal cells were present at this site (Fig. 3c). The distribution of stromal cells at the peridecidual Fallopian tube mucosa of tubal pregnancy was identical to that of intra-uterine pregnancy. Namely, many ASMA-positive stromal cells were observed, whereas the number of CD34-positive stromal cells was scarce or small.

Distribution of stromal cells at the fetal side of intra-uterine and tubal pregnancy

A small to large number of ASMA-positive cells was observed at the fetal side from both intra-uterine and tubal pregnancies, which focally formed a reticular network (Fig. 4a). However, no CD34-positive stromal cells were identified in these stroma (Fig. 4b).

Discussion

Yamazaki and Eyden (1996a) found for the first time

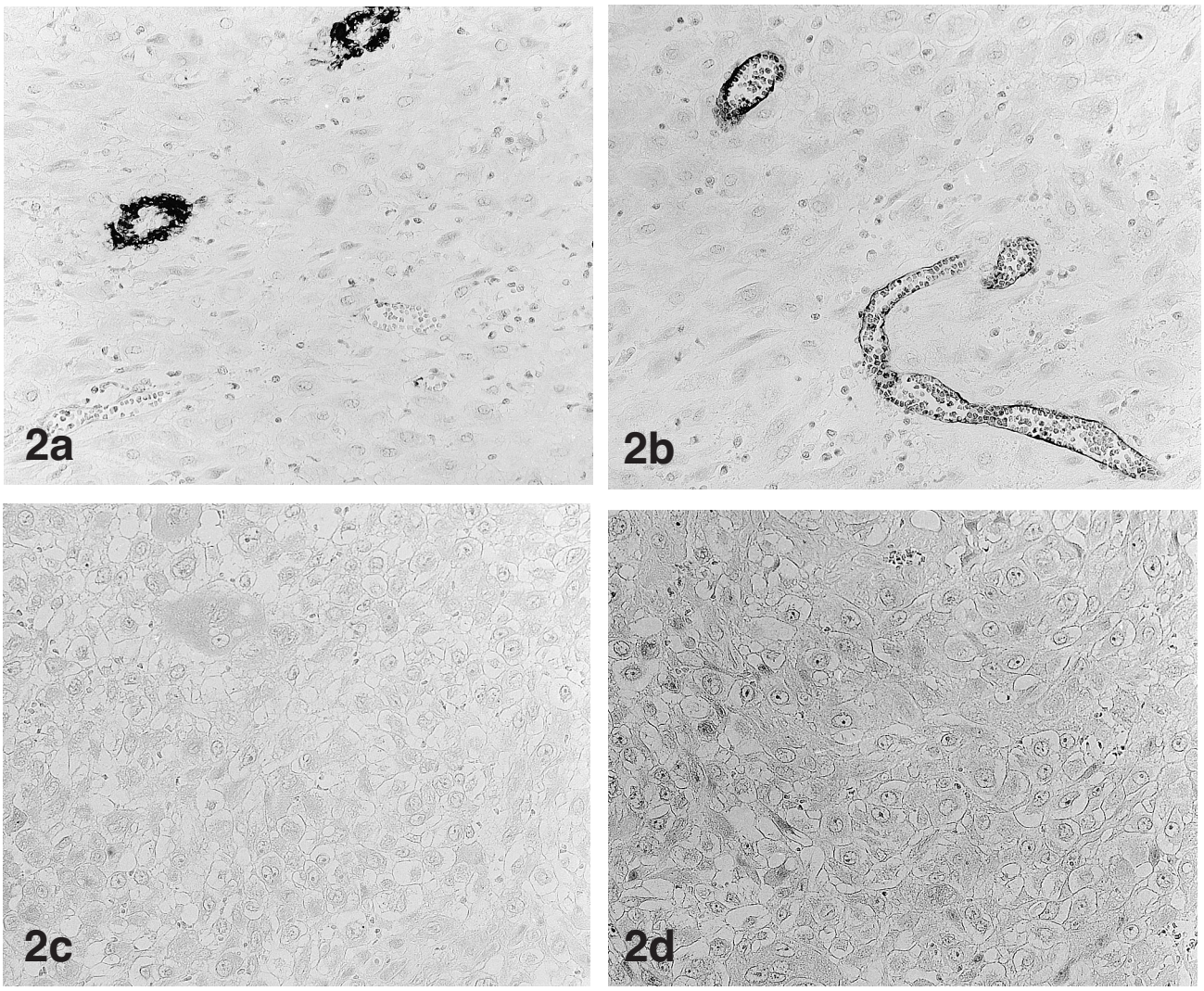

Fig. 2. Immunohistochemistry of ASMA **(a, c)** and CD34 **(b, d)** in intra-uterine **(a, b)** and tubal **(c, d)** pregnancies. Vascular smooth muscle cells and endothelial cells are positive for ASMA and CD34, respectively. However, ASMA-positive and CD34-positive stromal cells are completely absent from the decidual stroma. x 50

the presence of CD34-positive stromal cells of Fallopian tube in female genital organs. In the present study, we elucidated the presence of a CD34 reticular network in normal endometrial stroma. To the best of our knowledge, CD34-positive stromal cells have not been previously reported in normal endometrium. In the present study, ASMA-positive stromal cells were observed in the lamina propria and submucosal layer of normal Fallopian tubes. The double immunostaining for CD34 and ASMA identified a population of cells expressing both CD34 and ASMA in the lamina propria and submucosal layer of normal Fallopian tubes. This suggests that CD34-positive stromal cells and ASMA-positive stromal cells may share a common origin, as was suggested by Yamazaki and Eyden (1996) for stromal cells in Fallopian tubes and by Barth et al. (2002c) for stromal cells of the uterine cervix. Our data suggest that cells positive for CD34 and negative for ASMA and h-CD may include undifferentiated fibroblastic cells including fibroblasts, and cells positive for ASMA and negative for h-CD, irrespective of CD34 expression, may differentiate into myofibroblasts as suggested by Eyden (2001).

CD34- and ASMA-positive stromal cells were not observed in the decidual stroma of intra-uterine and tubal pregnancies. This finding suggests that the disappearance of CD34-positive and ASMA-positive stromal cells may be associated with decidualisation induced change in both intra-uterine and tubal pregnancies. Kimatrai et al. (2003) have reported that decidual stroma cells in human endometrium exhibit contractile activity and resemble the phenotype of myofibroblasts, whereas the present date showed that ASMA-positive stromal cells, namely myofibroblasts, were completely absent from the stroma of intra-uterine and tubal pregnancies.

However, at the peridecidual mucosa in both intra-uterine and tubal pregnancies, we elucidated that compared with normal mucosa, the abundance of CD34-positive stromal cells decreased and ASMA-positive stromal cells increased The possible common origin of ASMA-positive and CD34-positive stromal cells suggests a phenotypic change in stromal cells through down-regulation of CD34 expression and up-regulation of ASMA expression. In other words, we suggest that the decidualisation induced change may induce the differentiation of undifferentiated fibroblastic cells existing in the neighbouring mucosa into myofibroblasts. A small to moderate number of ASMA-positive stromal cells was also observed at the fetal side of both intra-uterine and tubal pregnancies. The presence of

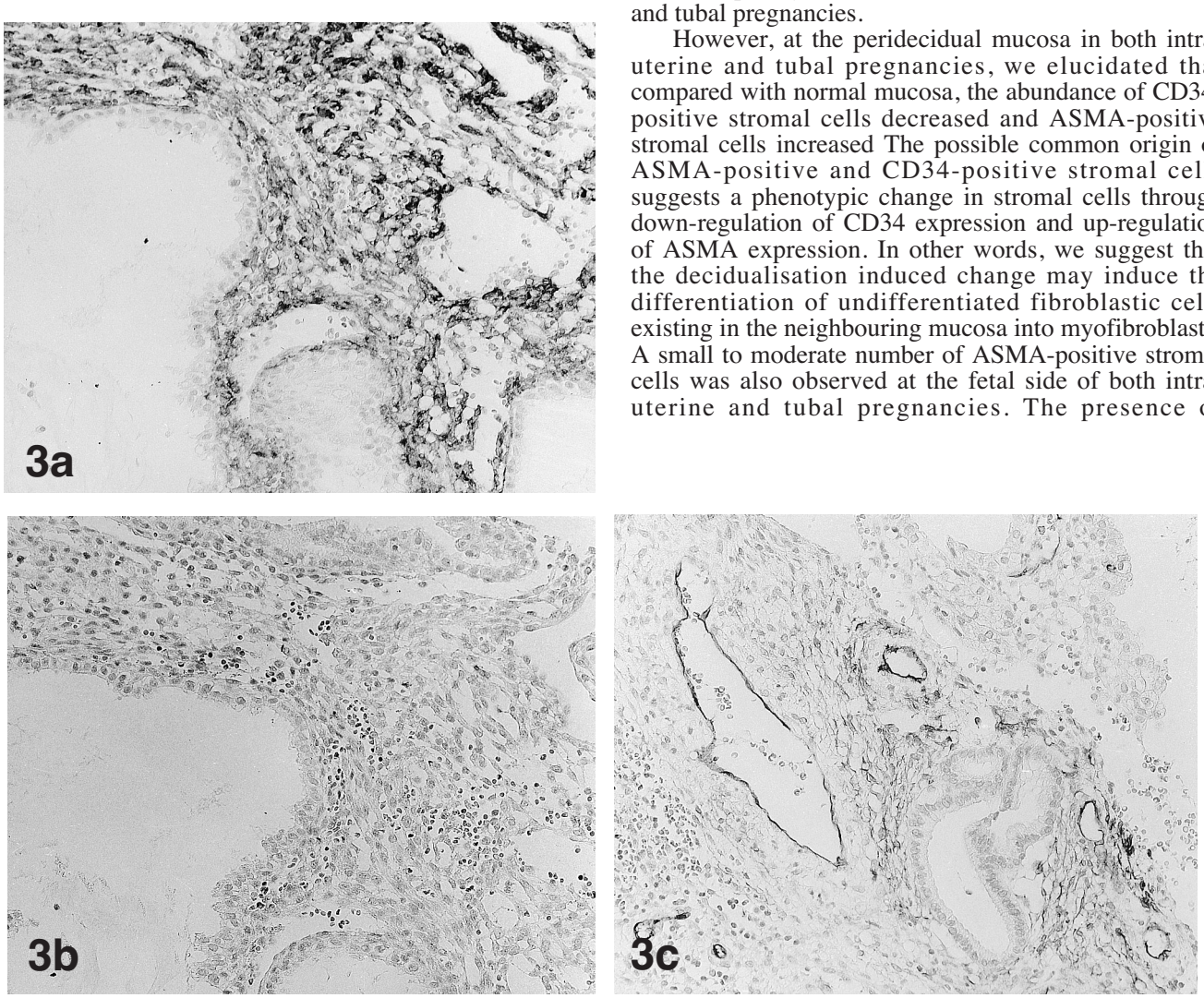

Fig. 3. Immunohistochemistry of ASMA **(a),** h-CD **(b)** and CD34 **(c)** at the peridecidual endometrium during intra-uterine pregnancy. Many ASMA-positive and h-CD-negative stromal cells are observed, but only a few CD34-positive stromal cells are focally identified. x 50

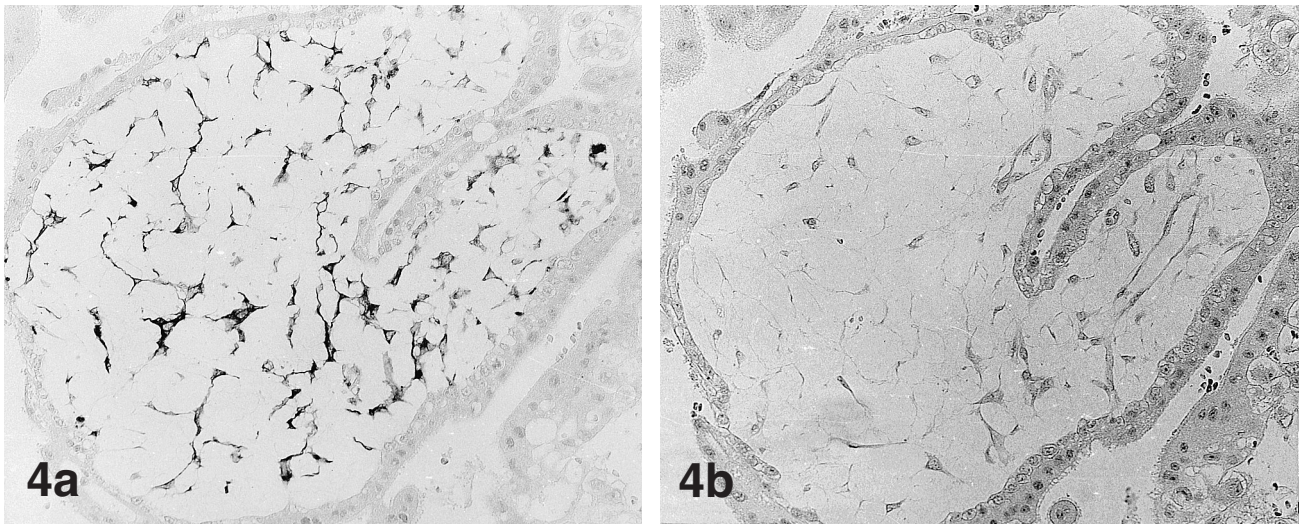

Fig. 4. Immunohistochemistry of ASMA and CD34 at the fetal side of tubal pregnancy. **a.** Many ASMA-positive cells are observed. x 50. **b.** No CD34-positive stromal cells are observed in the stroma. x 50

myofibroblastic cells at the fetal side has previously been demonstrated in intra-uterine pregnancy (Haigh et al., 1999; Trovato et al., 2002). The present study suggests that the cellular composition at the fetal side during tubal pregnancy is essentially identical to that during intra-uterine pregnancy. ASMA-positive stromal cells at the fetal side may play a role in the production of villous extracellular matrix (Kohen et al., 1996) or regulation of blood flow during tubal pregnancy.

Finally, CD34- and ASMA-positive stromal cells were completely absent from the decidual stroma during both intra-uterine and tubal pregnancies. The disappearance of CD34- and ASMA-positive stromal cells during both intra-uterine and tubal pregnancies may be an indicator of decidualisation induced change in stroma whereas it in the peridecidual tissues gives an up-regulation for fibroblasts to differentiate in myofibroblasts. Further examinations will be required to clarify the significance of CD34- and ASMA-positive stromal cells in the endometrium and Fallopian tubes.

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