0014-4754/95/020123-03$1.50 + 0.20

Expulsion of the placenta from the uterus is the principal initiator for collagen degradation in mouse uterus

K. Shimizu, T. Sato and J. Yamada

Department of Anatomy, Tokyo Medical College, Shinjuku, Shinjuku-ku, Tokyo 160 (Japan)
Received 28 July 1994; accepted 16 September 1994

Abstract. In unilaterally pregnant mice, collagen degradation in the non-pregnant uterine horn was not initiated by removal of the fetus only but by removal of both the fetus and placenta. The results indicate that expulsion of the placenta from the uterus is a principal factor in the initiation of the process of collagen degradation simultaneously in the whole uterus.
Key words. Placenta; collagen degradation; initiator; uterus; mouse.

The uterus grows during pregnancy to adapt to fetal growth. Following parturition, both the weight and protein content of the uterus show a rapid decrease. A conspicuous feature in this postparum involution is collagen degradation[1]. It is still obscure what factor(s) initiate the degradation process.

At parturition, the mechanical distension produced by the fetus is lost, and in addition the placenta is expelled. It is believed that the cessation of mechanical distension of the uterine wall is a factor in starting the degradation process[2,3], but it is unclear whether the expulsion of the placenta from the uterus also plays a role.

In the mouse, the postpartum collagen degradation occurs more in the endometrium than in the myometrium[4]. To investigate whether the expulsion of the placenta from the uterus is a factor in starting the process of collagen degradation simultaneously in the whole uterus, we observed the distribution of collagen bundles in the endometrium of the non-pregnant uterine horn of unilaterally pregnant mice under various conditions.

Materials and methods

The animals used were female mice of the IVCS strain. They were reared under a 12 h light and 12 h dark regime and given water and food ad libitum. At 7 weeks of age, they were anesthetized with ether, and the right oviduct was ligated with silk thread. After one week, they were mated. Successful mating was recognised by the presence of a vaginal plug (day 0 of pregnancy). Pregnancy lasted 19 days.

Group 1 (Control): 10 mice. Five animals were killed on day 16 of pregnancy, and 5 on the day of parturition.

Group 2 (Removal of fetus): 5 mice. On day 16 of pregnancy, the mice were anesthetized with ether. Fetuses were removed through small incisions in the adjacent uterine wall, care being taken to leave the placenta in situ. Three placentae in the uterine horn nearest to the ovary were sewn with silk thread to the uterine wall, to prevent the expulsion of the placenta from the uterus at parturition[5]. The mice were killed on postoperative day 3.

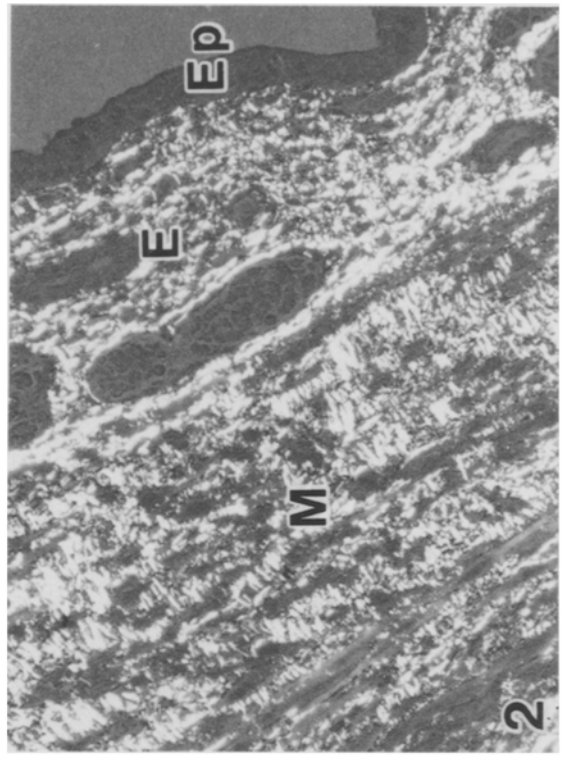
Group 3 (Removal of both fetus and placenta): 5 mice. On day 16 of pregnancy, mice were anesthetized with ether. A pregnant uterine horn was exposed and all fetal materials including the placentae were removed through a single incision. The mice were killed on postoperative day 3.

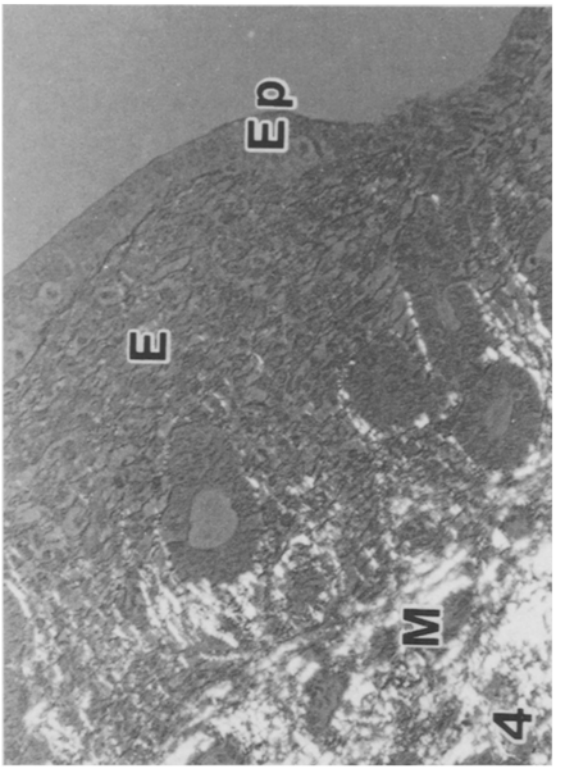
Group 4 (Sham operation): 5 mice. On day 16 of pregnancy, the mice were anesthetized with ether. Both the skin and abdominal wall were cut and then sewn. The animals were killed on postoperative day 3.

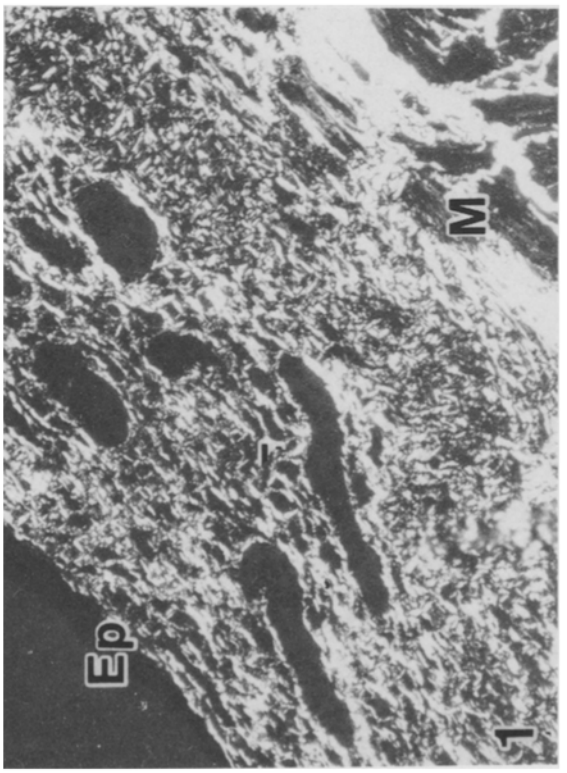
After the mice had been killed, the right uterine horn (non-pregnant horn, NPH) was removed and weighed. A difference of the organ weight postoperative day 0 (day 16 of pregnancy) and day 3 (day of parturition) was analyzed with one-way analysis of variance (ANOVA). One or two portions of the right uterine horn from each animal were fixed in 10% buffered formalin for one day. They were dehydrated in alcohol, passed through xylene, and embedded in paraffin. Sections 3 μm thick were deparaffined and stained for one hour in picrosirius red solution[6]. The stained sections were viewed with a light microscope with a polarizing filter (Olympus, BH-2, Tokyo). This picrosirius red polarizing method is useful for observing in detail the distribution of interstitial collagen bundles in tissue sections[7,8]. Several sections of the placenta were stained with hematoxylin and eosin.

Results

Changes of the organ weight of the NPH from day 16 of pregnancy to the day of parturition are summarized in the table. The organ weight of the NPH in both control and sham-operated mice increased. The NPH weight was not changed by removal of the fetus. After removal of both the fetus and the placenta, the NPH weight decreased significantly.


Ep
E
M
2


Ep
E
M
4


M
Ep
1

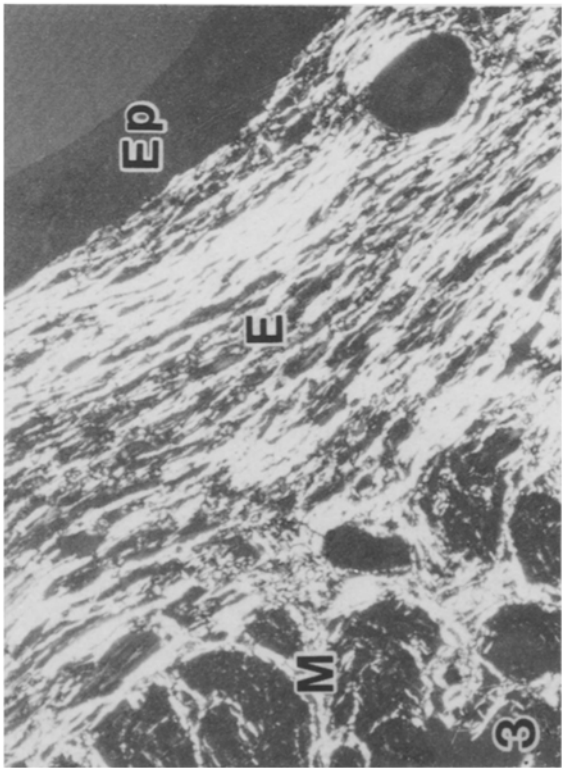

Ep
E
M
3

Table. Changes of organ weight of non-pregnant uterine horn of unilaterally pregnant mice from postoperative day 0 (day 16 of pregnancy) to postoperative day 3 (day of parturition) (mg, mean ± SE, n = 5).

	Day 0	Day 3
Control	64 ± 4	101 ± 8*
Sham		93 ± 6*
Placenta:		
Removed		45 ± 2*
Retained		65 ± 4

*$p < 0.01$ as compared with day 0 (ANOVA).

Collagen bundles were distributed throughout the whole of the endometrium from day 16 of pregnancy (fig. 1) to the day of parturition (fig. 2). On the day of parturition, the distribution of collagen bundles in the endometrium of the sham-operated animals was similar to that in the controls (not shown). The distribution of collagen bundles in the endometrium was not changed by removal of the fetus (fig. 3); it was similar in appearance to that of the control on the day of parturition (fig. 2). Collagen bundles in the immediate subluminal compartment of the endometrium disappeared after removal of both the placenta and fetus (fig. 4).

On postoperative day 3, the labyrinth zone of the placenta, which had no fetus, degenerated but its basal zone did not degenerate.

Discussion

When the mechanical distension produced by the fetus was removed by artificial removal of the fetus, the contralateral NPH did not involute, and the process of collagen degradation in it did not start. It has been reported previously that collagen degradation in the NPH of the unilaterally pregnant rodent which is not influenced by the loss of mechanical distension, occurs after parturition[9,10]. Therefore, it is possible that an initiating factor for the degradation process is not a factor which has a local influence such as the loss of mechanical distension, but one which functions systematically, such as a hormone.

The artificial removal of both placenta and fetus brought about the involution of the contralateral NPH, and also collagen degradation in it. Therefore, expelling the placenta from the uterus is probably a principal factor for initiating the simultaneous process of collagen degradation in the whole uterus.

The placenta can secrete many hormones[11]. Since collagenase in the uterus has never been detected during pregnancy[12], a hormonal factor secreted by the placenta might provide an inhibiting signal for collagenase synthesis in the whole uterus. Elimination of a hormone factor by the expulsion of the placenta at parturition, or by artificial removal of the placenta, might start the process of collagen degradation simultaneously in the whole of the uterus. Investigations are in process to identify this hormonal factor.

1 Harkness, R. D., Int. Rev. Connect. Tissue Res. *2* (1964) 155.
2 Wray, S., J. Physiol. *328* (1982) 1.
3 Morton, A. J., and Goldspink, D. F., Am. J. Physiol. *250* (1986) E114.
4 Shimizu, K., and Hokano, M., Anat. Rec. *220* (1988) 138.
5 Newton, W. H., J. Physiol. *84* (1935) 196.
6 Sweat, F., Puchtler, H., and Rosenthal, S. I., Archs Pathol. *78* (1964) 69.
7 Junqueria, L. C. U., Bingnolas, G., and Brantani, R. R., Histochem. J. *11* (1979) 447.
8 Piérard, G. E., Matrix *9* (1989) 68.
9 Adams, W. C., and Frieden, E. H., Biol. Reprod. *33* (1985) 1168.
10 Shimizu, K., Takahashi, T., and Hokano, M., Biomed. Res. India *2* (1991) 198.
11 Pragar, D., Weber, M. M., and Herman-Bonert, V., Semin. Reprod. Endocrin. *10* (1992) 83.
12 Jeffrey, J. J., Semin. Perinatol. *15* (1991) 118.

Distribution of collagen bundles in the endometrium. E, endometrium, Ep, luminal epithelium. M, myometrium. Picrosirius red polarization. ×250.

Figure 1. Control. Day 16 of pregnancy. Collagen bundles distributed throughout the whole of the endometrium.

Figure 2. Control. Day of parturition. Distribution of collagen bundles was similar to that on day 16 of pregnancy (fig. 1).

Figure 3. Removal of the fetus. Postoperative day 3 (day of parturition). Distribution of collagen bundles was similar to that of the control on the day of parturition (fig. 2).

Figure 4. Removal of both the fetus and placenta. Postoperative day 3 (day of parturition). Disappearance of collagen bundles in the immediate subluminal compartment of the endometrium was observed.