Research Articles

Effects of ovariectomy on removal of collagen bundles in the postpartum uterus of the mouse

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Abstract. Ovariectomy before and after parturition did not influence the rate of postpartum involution and did not affect the removal of collagen bundles in the endometrium during the first three postpartum days.
Key words. Ovariectomy; postpartum; collagen; uterus; mouse.

Following parturition, the uterine weight shows a rapid decrease, which is known as postpartum involution. A conspicuous feature in this involution is collagen degradation, and the rate of degradation is one of the fastest known rates for this process in any connective tissue [1]. In vivo injection of estradiol retards postpartum involution [2–4] and reduces the amount of collagenase in the postpartum uterus [3]. Progesterone prevents the production of collagenase in cultured postpartum uterus [5, 6]. Thus, it is believed that ovarian hormones play a critical role in the regulation of collagenase synthesis. However, it is unclear whether the ovary actually affects the removal of collagen bundles in the postpartum uterus.

In the mouse, the postpartum collagen degradation occurs more in the endometrium than in the myometrium and most of it is finished by postpartum day 3 [7]. To determine whether the ovary actually regulates the removal of collagen bundles in the postpartum uterus during the first three postpartum days, we observed the distribution of collagen bundles in the endometrium of uteri of mice which had been ovariectomized before and after parturition, using the picrosirius red polarization (Picro–SR POL) method. This Picro–SR POL method is useful for observing the complete and detailed distribution of collagen bundles in tissue sections [8, 9].

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 8 weeks of age, the mice were mated. A successful mating was validated by the presence of a vaginal plug (day 0 of pregnancy).

Group I (Control). 24 mice. They were normal parturient mice. All pups were removed on the day of parturition. Animals were killed on the day of parturition or one, two or three days postpartum. Six animals were killed on each day.

Group II (POVX). 18 mice. On the day of parturition, mice were anesthetized with ether and both ovaries taken out. All pups were removed on the day of parturition. Animals were killed one, two or three days postpartum. Six animals were killed on each day. The organ weight on the day of parturition was cited from that of Group I.

Group III (18 OVX). 24 mice. On day 18 of pregnancy (the last day of pregnancy), mice were ovariectomized as in Group II. They proceeded to a normal delivery. All pups were removed on the day of parturition. Animals were killed on the day of parturition or one, two or three days postpartum. Six animals were killed on each day.

After the mice had been killed, the uterine horns were dissected free and weighed. Data for the organ weight were analyzed by the one-way analysis of variance (ANOVA). The half time of the decrease for the organ weight was calculated with a regression line (postpartum day, x, versus organ weight, y).

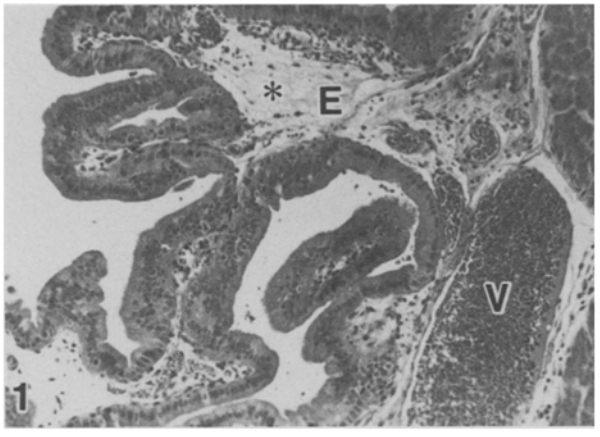
Two portions of uterine horn from each animal were fixed in 10% buffered formalin for one day. They were dehydrated in alcohol, passed through xylene, and embedded in paraffin. Sections 3 μm thick were deparaffined and stained for one hour in picrosirius red solution [10] (0.1% sirius red FB 200, Polyscience, USA). The stained sections were viewed with a light microscope with a polarizing filter (Olympus, BH-2, Tokyo). A section near the one stained with the picrosirius red was stained with hematoxylin and eosin (H.E.).

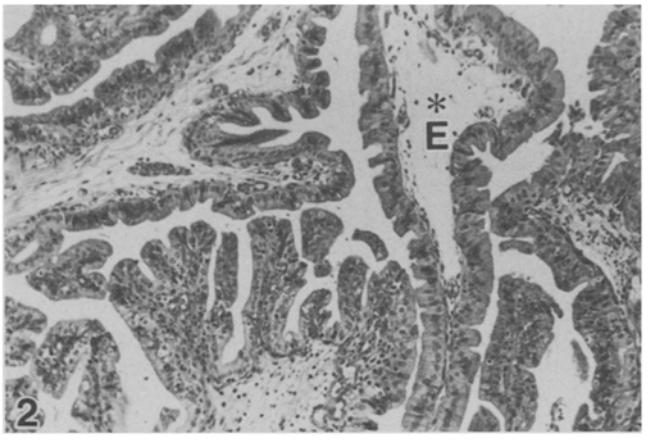
Results

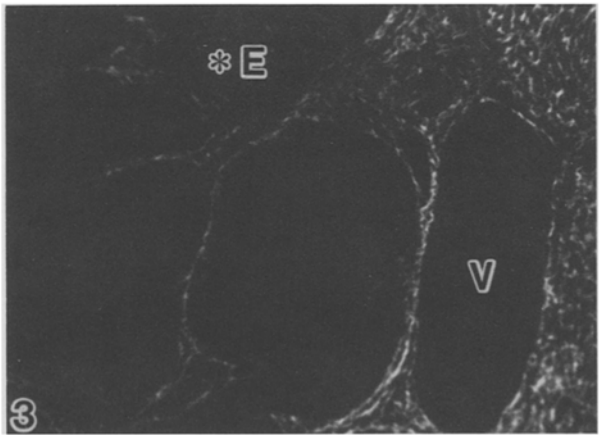
Changes of the organ weight are summarized in the table. Overall variance for the organ weight was significant in control ($Fs(3,20) = 792{,}9$, $p < 0.01$), in POVX ($Fs(3,20) = 293.2$, $p < 0.01$) and 18 OVX ($Fs(3,20) = 81.1$, $p < 0.01$), respectively. There were a good correlation between the organ weight and the postpartum day in control ($r = -0.94$, $y = -197.7x + 939.2$), in POVX ($r = -0.95$, $y = -213.1x + 968.9$) and in 18 OVX

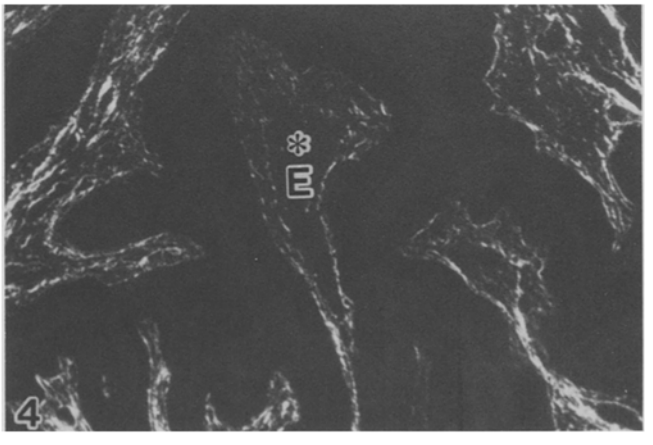
Changes in organ weight (mg, mean ± SE) during the first three postpartum days (N = 6)

	Parturition	Day 1	Day 2	Day 3	Half time of the decrease days
Control	810 ± 29	470 ± 23	382 ± 15	215 ± 5	1.70
Ovariectomy (POVX)	810 ± 29	488 ± 35	270 ± 4	174 ± 7	1.64
Ovariectomy (18 OVX)	741 ± 34	385 ± 19	256 ± 11	209 ± 16	1.66









Figures 1 and 2. On the day of parturition. Edema (asterisk) was observed in the endometrial plicae in control (fig. 1) and in 18 OVX (fig. 2), respectively. E, endometrium. V, blood vessel. H.E. × 100.

Figures 3 and 4. On the day of parturition. Collagen birefringence was decreased in the edematous area (asterisk) in the endometrium of control (fig. 3) and of 18 OVX (fig. 4), respectively. Asterisks in fig. 3 and fig. 4 correspond to those in fig. 1 and fig. 2, respectively. Picrosirius red polarization. × 100.

($r = -0.89$, $y = -172.5x + 892.5$), respectively. A half time for the decrease in weight was 1.70 days in controls, 1.64 days in POVX and 1.66 days in 18 OVX, respectively.

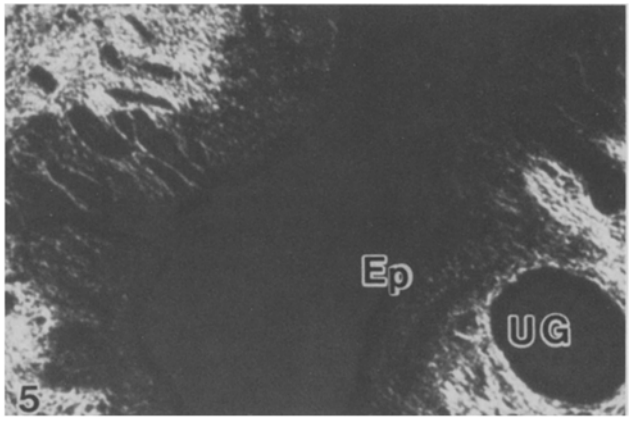
On the day of parturition, many plicae of the endometrium of controls (fig. 1) and of 18 OVX (fig. 2), respectively, projected into the uterine lumen. Edema was observed in some plicae. Collagen birefringence decreased in the edematous area of controls (fig. 3) and of 18 OVX (fig. 4), respectively. Collagen birefringence was distributed wholly in the non-edematous plicae.

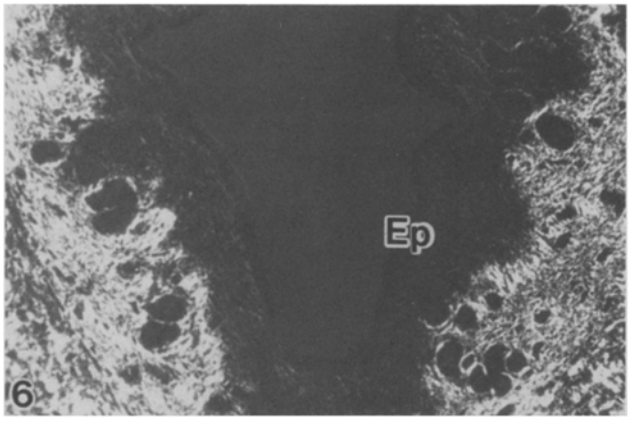
On postpartum day 3, collagen birefringence in the immediate subluminal compartment of the endometrium disappeared in controls (fig. 5), in POVX (fig. 6) and in 18 OVX (fig. 7). Removal of collagen bundles was more apparent in the immediate subluminal compartment than in the deep compartment of the endometrium. The extent of the disappearance of collagen birefringence in the immediate subluminal compartment of the endometrium of POVX and 18 OVX groups was similar to that in the controls.

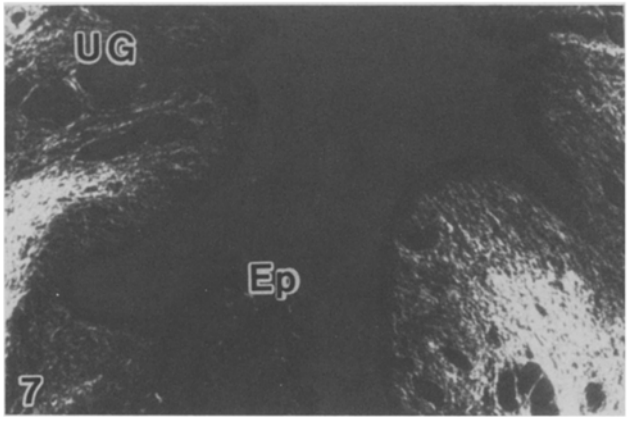
Discussion

The plasma estradiol levels in rodents rise slowly during the last week of pregnancy and increase suddenly on the day of parturition to induce the postpartum ovulation[11–13]. On postpartum day 1, the estradiol levels decrease rapidly and then continue low by at least postpartum day 3[11]. On the other hand, the plasma progesterone levels decrease slowly during the last week of pregnancy[11] and show a nadir on the day of parturition[12, 14, 15]. The plasma progesterone levels remain very low during the first three postpartum days[11]. If ovarian hormones actually play a role in vivo in the regulation of the removal of collagen bundles in the endometrium, estradiol will regulate the degrading process more than progesterone.

At parturition, the uterus in control animals was exposed to a high plasma estradiol concentration, similar to that on the proestrous day in the cycling mouse[12]. In the POVX group, a small fraction of this estradiol was present, and the 18 OVX group had virtually none. However, the present results showed that ovariectomy either







Figures 5–7. Postpartum day 3. Collagen birefringence disappeared in the immediate subluminal compartment of the endometrium in control (fig. 5), in POVX (fig. 6) and in 18 OVX (fig. 7). Ep, luminal epithelium. UG, uterine gland. Picrosirius red polarization. × 105.

before or after parturition did not affect the rate of postpartum involution or of the disappearance of collagen bundles in the endometrium during the first three postpartum days.

The rate of the postpartum involution of POVX was similar to that described in previous reports[4, 16]. Furthermore, the results of a biochemical study[16] were in agreement with the present histological results; they also showed that the removal of collagen bundles in the endometrium of POVX was similar to that in controls. Therefore, the present results indicate that the ovary does not play a critical role in the regulation of the removal of collagen bundles in the endometrium during the first three postpartum days.

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