Effect of Surgically Induced Endometriosis on Pregnancy and Effect of Pregnancy and Lactation on Endometriosis in Mice[1] (44022)

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Abstract. **Endometriosis, a disease of women and nonhuman primates in which endometrial tissue grows outside the uterus, can be mimicked surgically in rats and mice. The disease is related to infertility in women, and surgical induction of endometriotic lesions reduces fertility in rats according to some studies. Conversely, pregnancy appears to have a beneficial effect on endometriosis in women and some rat studies. Our objective was to evaluate a new mouse model of surgically induced endometriosis with respect to the effects of pregnancy on endometriosis and the effects of endometriosis on pregnancy. Female B6C3F1 mice were divided into four groups. Those in Group A and B underwent induction surgery for endometriosis and hemi-ovariectomy, those in Group C underwent sham surgery and hemi-ovariectomy, and animals in Group D received no surgery at all. Three weeks later, Group A, C, and D were bred. Eighteen days later one half of the dams in Groups A, B, and C only were sacrificed, and evaluations included endometriotic lesion diameter, number of pups, fetal weight, and various organ weights. The remaining dams delivered, and, 18 days after parturition, dams and pups were sacrificed. Evaluations included gestation length and those listed above. Endometriotic lesion diameter was significantly reduced in pregnant animals when compared with nonpregnant controls, but the reduction was not a full regression. Lactation returned the mean lesion diameter to prepregnancy dimensions. When effects of endometriosis on pregnancy were evaluated, no effects on the litter size, pup weight, or gestation length were found, but trends toward increased resorptions and malformations were evident. Thus, in the mouse model of induced endometriosis, pregnancy produced a significant reduction in endometriotic lesion diameter while fertility was largely unaffected by the surgically induced endometriosis. The mouse model of endometriosis thus appears more resistant than the rat model to effects of endometriosis on fertility.** [P.S.E.B.M. 1996, Vol 212]

Endometriosis, a disease of women in which endometrial tissue is found outside the uterus, is also found in primates (1, 2) and can be mimicked by surgical techniques in rodents (3, 4). The disease appears to be related to infertility, with 30% to 40% of endometriosis patients estimated by one study to be infertile (5). Studies in rats where endometriosis was surgically induced have demonstrated a reduction of the number of pups at term (3). In other studies, where the method for the induction of endometriosis differed, a reduction in pregnancy rate with no differences in gestation length or number of pups (6), or no effect on fertility at all (7) were found.

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Conversely, pregnancy has been reported to have an ameliorative effect on endometriosis in women (8). Other reports provide evidence in animals of a beneficial effect of pregnancy. According to Vernon and Wilson (3), surgically implanted endometriotic lesions completely regressed in pregnant rats but increased in size in nonpregnant rats. Barragan *et al.* (6) reported that lactation promoted the regression of surgically induced endometriotic lesions in rats. Rajkumar *et al.* (7) described the regression of implants in rats during pregnancy and their later recurrence.

Recently, a mouse model of surgically induced endometriosis was developed (4). This study described the importance of the novel technique and the stimulation of the growth of the endometriotic lesions by estrogen. Further work on the interaction of pregnancy and endometriosis was necessary to extend this model. The current study had two objectives. The first was to evaluate the effect of pregnancy and lactation on surgically induced endometriotic lesion diameter in mice. The second objective was to evaluate the effect of the endometriotic lesions on parameters relating to pregnancy including litter size, gestation length, pup weight, and other relevant measures. These data are necessary to compare the physiology of endometriosis of rats and mice, and to evaluate the mouse as a model system for studying human disease.

Materials and Methods

Animals. Male and female B6C3F1 mice were obtained from Charles River Animal Supply Company (Raleigh, NC) at 70 days of age and allowed to acclimate for 1 week. Female mice were housed in groups of five in clear plastic cages containing heat-treated pine shavings (Northeastern Products Corp., Warrensburg, NY). Males were housed individually. All animals received Prolab rat, mouse hamster 3000 (Agway, Syracuse, NY), and water *ad libitum*. A 12:12-hr light:dark cycle was maintained. Temperature and humidity in the animal room was 20°–24°C and 40%–50%, respectively.

Experimental Design. Initially, mice were divided into four groups. Three weeks prior to breeding (Day −21), Groups A (n = 20) and B (n = 20) underwent surgery for the induction of endometriosis (4) as briefly described below. During this surgery, all animals except those in Group D (n = 14) were hemiovariectomized in order to maximize the number of ovulations from the contralateral ovary attached to the patent uterine horn. Group C (n = 20) received sham surgery in which the left uterine horn and ovary were removed and sutures alone were tied in the mesenteric vessels. Animals in Group D served as controls and received no surgery. Three weeks after the surgeries, Groups A, C, and D were bred with proven fertile males. The presence of a vaginal plug was considered Day 0. Animals in Group B were not bred and were maintained for the three weeks. On Day 18 of pregnancy, one-half of the mice in Groups A and C were sacrificed by severing the carotid artery; one-half of the mice in Group B (not pregnant) were sacrificed 18 days after Groups A and C were bred. None of the animals in Group D were sacrificed on day 18 of pregnancy. The remaining animals in Groups A and C, and all mice in Group D, were allowed to undergo parturition and then lactation for 18 days, and gestation length was determined. Mice in Group B were kept for an additional 18 days as a period of time equivalent to lactation.

Surgery. The surgical method for the induction of endometriosis in mice (4) is described briefly as follows. The animal was placed under general anesthesia, and, with aseptic technique, a midventral incision was made to expose the uterus and intestines. One uterine horn was ligated at both ends and removed to warm media (Ham's F-12 supplemented with antibiotics). The uterine horn was split longitudinally then cut into three square pieces measuring approximately 2–2.5 mm on a side. Each piece of uterus was sutured to a vessel of the intestinal mesentery. The muscle and skin were then closed with suture and wound clips following lavage of the peritoneal cavity with warm media.

Necropsy. Parameters measured at necropsy on Day 18 of pregnancy included the diameter of all three endometriotic lesions (measured to the nearest 0.05 mm with fine calipers), dam body weight, uterine and ovarian weights, number of fetuses, fetal weights, number of implantation sites in the uterus, and gross exterior evaluation of the pups for malformations. Blood was collected when the carotid artery was severed, and serum progesterone was measured by RIA (Diagnostic Products, Los Angeles, CA) following the storage of the serum frozen at −60°C for less than 30 days. Use of the Diagnostic Products kit for RIA of progesterone in the mouse has been previously validated (9). On Day 18 of lactation, all remaining dams and pups were sacrificed. Parameters assessed included endometriotic lesion diameter, the body, uterine, and ovarian weights of each dam, number of pups and their weights, gestation length, and the number of implantation sites in the uterus.

Statistics. The measures of the three lesion diameters for each animal were averaged and taken as the animal mean, on which further analyses were done (no significant variation among within-animal lesion diameters was found). Effects of pregnancy and lactation on mean endometriotic site diameter and the effects of endometriosis on the measured parameters of pregnancy except resorptions were analyzed by two-way analysis of variance (ANOVA) (GLM) (10).

Resorptions were calculated by the following formula:

$$\frac{\#\text{ Implantation Sites} - \#\text{ Live Pups}}{\#\text{ Implantation Sites}} \times 100 = \%\text{ Resorptions.}$$

Following an arcsine-square root transformation of the percentages, data were analyzed by ANOVA as above. When significant effects on the overall ANOVA were detected ($P < 0.05$), post hoc comparisons among treatments were made with t tests (LSM) (10).

Results

Two animals in the Day 18 lactation group, one with endometriosis and one sham, failed to deliver any pups (were not pregnant), and one mouse in the Day 18 lactation group with endometriosis had only one pup, which died; all three of these dams were removed from the study. Also, the breeding date (and thus delivery date) of one mouse was unknown, and thus the animal was removed. Four mice scheduled to be sacrificed on day 18 of pregnancy and three mice scheduled for sacrifice on day 18 of lactation died due to anesthesia or complications of surgery or did not breed (as is expected for a proportion of animals). These losses led to the n values listed in Table I, II, and III.

As shown in Figure 1, endometriotic lesion diameter was significantly smaller in pregnant mice on Day 18 of pregnancy when compared with lesions measured in nonpregnant mice on the equivalent day (Group A mean = 5.15 ± 0.129 vs Group B mean = 6.01 ± 0.292). On Day 18 of lactation, however, there was no difference in lesion diameter between animals that were lactating and nonpregnant controls (Group A mean = 6.12 ± 0.199 vs Group B mean = 5.92 ± 0.299).

When the effects of the induction of endometriosis were evaluated (Table I), no significant effect on gestation length, number of pups delivered or surviving, pup weight on Day 18 of pregnancy or lactation, or the number of resorptions was detected. Also, there was no effect of endometriosis on serum progesterone levels (Table II). Litter size in mice that received induction surgery ranged from 5 to 9, whereas the range in sham-operated controls was 7–10 pups. In addition, two cases of exencephaly were detected in the surgically induced group, while no malformations were found in the sham control group.

Evaluation of serum progesterone, organ weights, and dam weights (Table II and III) revealed significant differences between pregnant and lactating animals but no differences resulting from the surgical induction of endometriosis. Ovarian weight was unchanged between Day 18 of pregnancy and Day 18 of lactation; a lower weight was seen in nonpregnant animals at these times and in the no-surgery control animals on which hemi-ovariectomies were not performed. Uterine weights were smaller on Day 18 of lactation than on Day 18 of pregnancy. Also, as expected, mean uterine weights in nonpregnant mice were smaller than those found in pregnant or lactating mice.

Discussion

We have previously reported the development of an endometriosis model in the mouse. Following the surgical procedure as described here, the endometriotic lesions that developed were fluid-filled, translucent, ovoid structures (4). Histological evaluations of these structures revealed an outer layer of myometrium, a middle layer of endometrial stroma containing glands, and an inner layer of epithelial cells; the lumen contained amorphous eosinophilic material and the endometriotic site was surrounded by serosa and blood vessels (4).

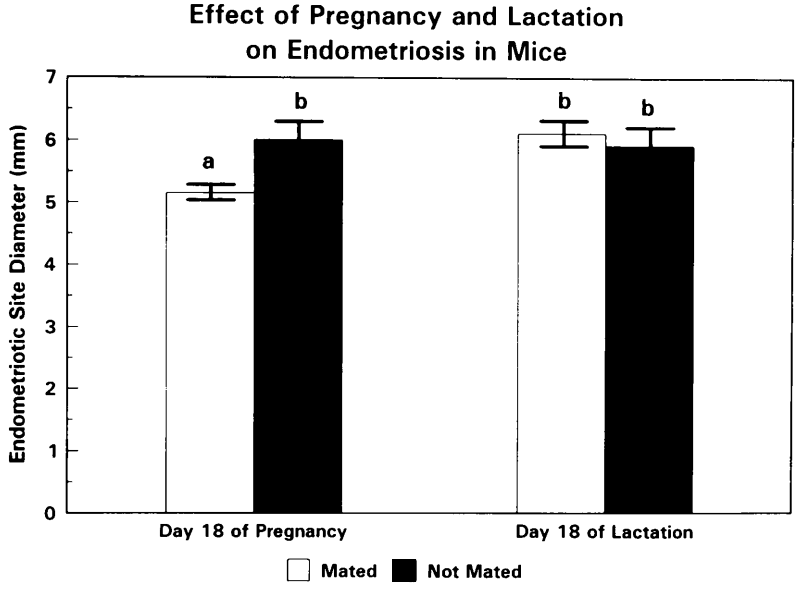

Figure 1. Effect of pregnancy and lactation on endometriosis in mice. Mice were bred 3 weeks after surgery for the induction of endometriosis; some animals were sacrificed on Day 18 of pregnancy, and the remainder were sacrificed on day 18 of lactation. Pregnancy produced a significant reduction in endometriotic site diameter. Lactation returned site diameter to nonpregnant dimensions. Data are expressed as mean ± SEM. Lowercase letters represent statistical differences between groups; $P < 0.05$.

Table I. Effects of Endometriosis on Pregnancy

	n	Parameter			
		Gestation length (days)	# pups	Pup weight (g)	Resorptions (%)
Day 18 of pregnancy					
(C) S/P[a]	8	N/A	8.5 ± 0.4*	1.2 ± 0.02*	2.9*
(A) E/P[b]	9	N/A	8.4 ± 0.2*	1.2 ± 0.02*	9.1*
Day 18 of lactation					
(C) S/P[a]	7	18.0 ± 0.0*	8.0 ± 0.4*	10.3 ± 0.3†	6.3*
(A) E/P[b]	6	18.0 ± 0.3*	7.5 ± 0.8*	10.6 ± 0.3†	9.2*
No surgery[c]					
(D)	14	18.3 ± 0.2*	9.1 ± 0.5*	10.9 ± 0.3†	6.8*

Note. Mice were bred 3 weeks after surgery for the induction of endometriosis; some animals were sacrificed on Day 18 of pregnancy, and the remainder were sacrificed on Day 18 of lactation. Data are presented as mean ± SEM except for percentage resorptions. Values with different superscripts (* and †) represent data that are different from each other, analyzed across all groups including pregnancy and lactation, $P < 0.05$.

[a] S/P, sham/pregnant.

[b] E/P, endometriosis/pregnant.

[c] No surgery, pregnant, no surgery control.

Table II. Serum Progesterone Levels: Effects of Surgically Induced Endometriosis during Pregnancy and Lactation

	n	Serum progesterone (ng/ml)
Day 18 of pregnancy		
Endometriosis/pregnant (A)	9	5.11 ± 0.74
Sham/pregnant (C)	8	4.36 ± 0.69
Endometriosis/not pregnant (B)	9	6.26 ± 1.18
Day 18 of lactation		
Endometriosis/pregnant (A)	6	1.22 ± 0.17
Sham/pregnant (C)	7	1.33 ± 0.25
Endometriosis/not pregnant (B)	10	8.27 ± 2.22[a]

Note. Hormone measures were made using an RIA kit (Diagnostic Products) after treatment of animals, as described in Materials and Methods. No effect of endometriosis on serum progesterone was evident on Day 18 of pregnancy or on Day 18 of lactation.

[a] Significantly different from mated groups on Day 18 of lactation; $P < 0.001$.

The data shown here demonstrate that in the mouse pregnancy produces a significant reduction in endometriotic lesion diameter. When pregnancy is followed by lactation, a return to the control or nonpregnant size of the endometriotic lesion diameter was observed. The reduction in lesion diameter produced by pregnancy, while significant, was minor when compared with the complete regression in endometriotic sites seen in the rat following or during pregnancy (3, 7). The pregnancy-related regression of endometriosis in the rat may involve hormone-dependent mechanisms. For example, the size of the surgically induced endometriotic lesions was maintained in ovariectomized rats treated with estrogen, while rats receiving progesterone had endometriotic lesions that were fully regressed, as were those in ovariectomized, untreated rats (11). Our data on serum progesterone levels show no difference between pregnant mice with lesions (5.11 ± 0.74 ng/ml) and nonpregnant mice with endometriotic lesions (6.26 ± 1.18 ng/ml), perhaps because the difference in the lesion diameter between the two groups is also small. Our data indicating that lesion diameter returns to pre-pregnancy diameter during lactation in the mouse are also in contrast to a report that surgically induced endometriotic lesions undergo regression during lactation in the rat (6). In mated animals, serum progesterone is considerably lower on Day 18 of lactation (1.22 ± 0.17 ng/ml) than on Day 18 of pregnancy (5.11 ± 0.74 ng/ml). This lower level of the hormone that appears to inhibit endometriotic lesion growth in the rat (11) may be responsible for the return to control diameter of the lesions during lactation in our study. A comparison of the progesterone levels in nonpregnant mice is difficult because the animals are cycling and the average serum progesterone level is relatively meaningless. The mouse, therefore, differs from the rat in the magnitude of effect on endometriotic lesion size following pregnancy and on the effect of lactation on endometriosis.

Mechanisms responsible for the differences between rats and mice may include the fact that the surgical procedures used to induce endometriosis in two of the rat studies (6, 7) placed the implants on the peritoneal wall and not in the mesentery as in the report by Vernon and Wilson (3), and in the mouse technique (4). Also, there are three implants in each mouse in contrast to six in each rat, and the lesions reach approximately the same diameter in both species over time even though the rat is a much larger animal. As a result of the greater size of the lesions relative to the size of the animal and her organs, there may be a limitation in the mouse of the degree of vascularization necessary to bring ovarian steroids to the lesions during pregnancy and lactation and thus produce regres-

Table III. Effect of Induction Surgery on Parameters Other Than Endometriotic Site Diameter

		Parameter		
	n	Ovarian weight (mg)	Uterine weight (mg)	Dam weight (g)
Day 18 of Pregnancy				
(C) S/P[a]	8	12.5 ± 0.86*	529 ± 41.4*	39.9 ± 1.1*
(A) E/P[b]	9	12.2 ± 0.23*	526 ± 9.7*	42.0 ± 0.7*
(B) E/NP[c]	9	8.5 ± 0.5†	39.0 ± 3.0‡	25.4 ± 0.5‡
Day 18 of lactation				
(C) S/P[a]	7	11.3 ± 0.6*	69.5 ± 11.2†	36.0 ± 0.8†
(A) E/P[b]	6	11.9 ± 0.7*	76.7 ± 11.5†	34.8 ± 2.0†
(B) E/NP	10	10.2 ± 0.7*	32.6 ± 3.3‡	27.9 ± 0.8‡
No surgery[d]				
(D)	14	6.9 ± 0.3‡	60.4 ± 6.9†	36.9 ± 0.8†

Note. Data are expressed as mean ± SEM. Numbers bearing different superscripts (*, †, and ‡) represent data that are significantly different from each other ($P < 0.05$); data analysis included all groups within each parameter including Day 18 of pregnancy and Day 18 of lactation.

[a] S/P, pregnancy/sham.

[b] E/P, pregnant/endometriosis.

[c] E/NP, not pregnant/endometriosis.

[d] No surgery, pregnant, no surgery control.

sion. Likewise, such a limitation would reduce the ability of chemical products of the endometriotic lesions to travel to the uterus and affect pregnancy.

The remainder of the data show no significant effect of endometriosis on parameters related to pregnancy such as gestation length, number of pups per litter, and pup weight. However, trends toward increases in resorptions and malformations were evident. Also, while there was no significant effect on litter size for either Day 18 of pregnancy or Day 18 of lactation, the groups of mice in which endometriosis was induced had a range of five to nine pups, while groups of mice receiving sham surgery has a range of seven to ten. Effects on organ weights correspond to either mated versus not mated or pregnancy versus lactation. One exception is the ovarian weight in the no surgery Day 18 of lactation group. Ovarian weight in the no surgery group was smaller than that found in the endometriosis/pregnant, Day 18 lactation group because the animals in the former group were not hemi-ovariectomized. Ovaries in the latter group perhaps underwent compensatory hypertrophy as a result of the hemi-ovariectomy performed during the induction surgery. Overall, the effect of endometriosis on pregnancy was negligible in the mouse. In the rat, surgically induced endometriosis reduced litter size (3). Thus, endometriosis appears to have a greater deleterious effect on pregnancy in rats than in mice.

According to Halme and Surrey (12), a number of mechanisms may contribute to the infertility observed in women with endometriosis. These include anatomic factors, ovulatory/hormonal dysfunction, abnormal fertilization, early pregnancy loss, altered systemic immunity, and altered peritoneal fluid/local immune response (12). The observed trends toward increased resorptions and malformations and a decrease in the range of the number of pups per litter in mice having endometriotic lesions may be attributable to similar mechanisms. For the most part, however, mice in which endometriosis is surgically induced do not exhibit the severe reduction in fertility seen in women with endometriosis.

In summary, the mouse differs from the rat and human in the effect of pregnancy on endometriosis and the effect of endometriosis on pregnancy. Whereas pregnancy may have a beneficial effect on endometriosis in rats and humans, it produces only a small but significant reduction in lesion diameter, not full regression, in mice. This may be due to the technique, amount of tissue produced, and vascularization of the endometriotic lesions. Conversely, the finding of no effect of endometriosis on fertility in mice is in contrast to at least some other reports for rats. There are a number of potential mechanisms by which endometriosis may affect fertility in women. It is apparent that the rat and mouse differ as models for the study of endometriosis and in their relationship to the human with respect to endometriosis and pregnancy.

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