Comparative Medicine
Copyright 2004
by the American Association for Laboratory Animal Science

Vol 54, No 4
August 2004
Pages 418-421

Increased Incidence of Vaginal Septum in C57BL/6J Mice Since 1976

Sonya Gearhart, DVM,[1,*] Jennifer Kalishman, DVM,[1] Hranush Melikyan,[2] Carol Mason, PhD,[2] and Dennis F. Kohn, DVM, PhD[1]

Decreased fertility was observed in a breeding colony of C57BL/6J mice. On examination, a dorsoventral vaginal septum was detected in many females. This defect was identified in 1976, with incidence of 4.0% in this strain. Our objective was to determine whether incidence of this condition has increased and whether this defect was associated with the observed infertility. We report incidence of 11.3%, nearly triple the original reported incidence. For comparison, incidence of vaginal septum in C57BL/6N females was determined and was found to be 1%. We performed a breeding study using normal and affected C57BL/6J females to evaluate fertility in affected females. Our data were consistent with those of the 1976 report; fertility was decreased in females with an intact vaginal septum. In 50% of affected females, the septum remained intact after breeding. The fertility for this subgroup of vaginal septum-retained females was 14.3%, compared with 85.7% in females whose septum ruptured and 75.0% in normal females (statistically significant, $P = 0.02$). On the basis of our results, we provide animal and financial loss data due to the defect. Lastly, we provide suggestions on how to minimize animal losses and be in accordance with the principles of the 3Rs (replacement, refinement, reduction).

Presence of a vaginal septum is a cause of reduced fertility in mouse breeding colonies and results in increased use of animals. A vaginal septum is a band of tissue that bisects the vagina and can be longitudinal, transverse, or oblique (Fig. 1). This defect has been documented in multiple species, including various strains of mice, rats, dogs, and humans (3-6, 8). A longitudinal septum was documented in multiple strains of mice at the Jackson Laboratories in 1976. The septum was characterized in the BALB/cJ strain; reproductive efficiency studies were conducted, and incidence was recorded at 38.1% (the highest recorded incidence in the strains reported). At that time, incidence in C57BL/6J mice was 4.0% (3). On the basis of results of genetic transmission studies, vaginal septum was determined to be a polygenic trait, and susceptibility to the trait was found not to be maternally determined (1-3, 9).

During 2002, a researcher consulted one of the authors (JK) at the Institute of Comparative Medicine with concern over reduced fertility in her breeding colony of C57BL/6J mice. While performing routine examinations for copulatory plugs, the research technician noted a vaginal defect in many of the female mice. On physical examination by a veterinarian, the affected mice were found to have a longitudinal vaginal septum bisecting the vaginal canal.

The goals of our investigation were to determine whether the incidence of vaginal septum in C57BL/6J mice had increased since the original report in 1976, whether the defect was found at the same frequency in another vendor's line of C57Bl/6 female mice, and whether this defect was the cause of reduced fertility in this colony. Further, an animal use analysis was performed to determine the potential animal and financial losses directly associated with the vaginal defect.

Received: 3/31/04. Revision requested: 4/13/04. Accepted: 4/27/04.
[1]Institute of Comparative Medicine and [2]Department of Pathology, College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York, New York 10032.
[*]Corresponding author.

Materials and Methods

Animals. All animals were acquired through, and all procedures were performed in accordance with, an IACUC-approved protocol at Columbia University Medical Center, an AAALAC-International-approved institution. Virgin female C57BL/6J (Jackson Laboratories, Bar Harbor, Maine) mice at 6 weeks of age were routinely ordered for a small breeding colony. The mice were housed under barrier conditions in static polycarbonate microisolation shoebox cages with autoclaved corncob bedding. Room temperature ranged from 18 to 26°C. A 12:12-h light:dark cycle was in effect. They were fed Purina 5058 PicoLab irradiated mouse diet 20 (Purina Mills, St. Louis, Mo.) and were offered autoclaved tap water ad libitum. On the basis of results of quarterly testing of sentinels exposed to soiled bedding, animals were considered free of cilia-associated respiratory bacillus, epizootic diarrhea of infant mice, Theiler's mouse encephalomyelitis virus, lymphocytic choriomeningitis virus, mouse hepatitis virus, *Mycoplasma pulmonis*, mouse parvovirus, pneumonia virus of mice, Sendai virus, endoparasites, and ectoparasites. Cage bedding was changed weekly, and all manipulations were performed in a laminar flow transfer station (Lab Products, Inc., Maywood, N.J.).

On arrival at the facility, all females were examined by the research technician for the presence of a vaginal septum and were separated accordingly. To examine for the presence of a septum, the tail was lifted and a blunt probe was used to apply gentle pressure to the ventral aspect of the vulva to slightly open the vagina (the same technique used to examine for a copulatory plug). The septum was easily visualized as a longitudinal band of tissue bisecting the vagina.

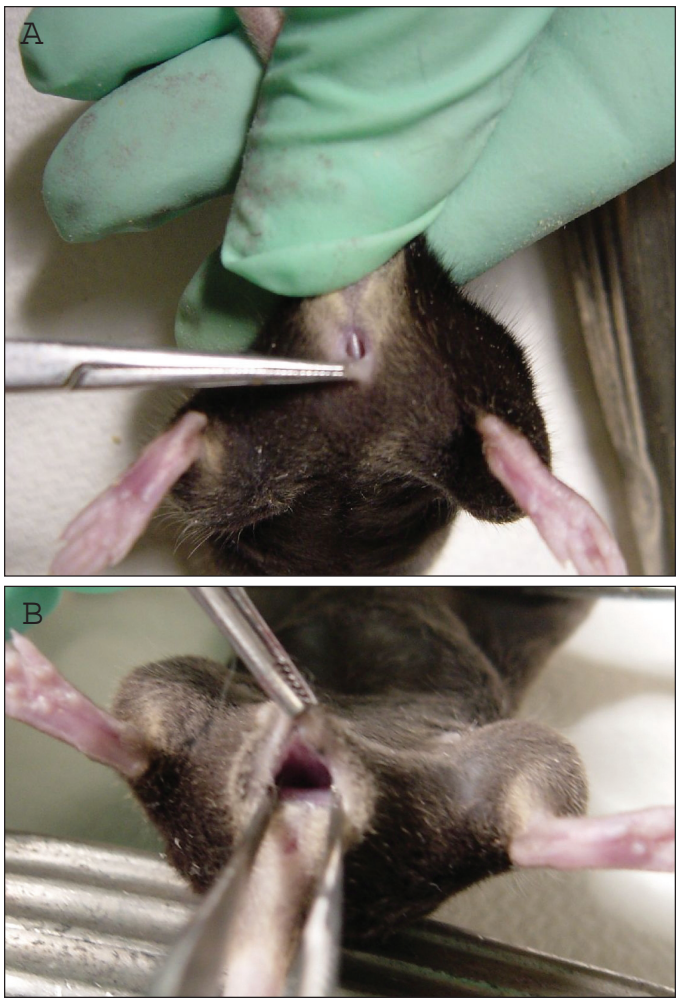

Figure 1. Photographs at necropsy of the vaginal opening of (A) a C57BL/6J female mouse with a longitudinal vaginal septum and (B) a C57BL/6J normal primiparous female for comparison.

Evaluated mouse strains. Over the course of a year, 337 C57BL/6J female mice in a total of 6 shipments were obtained for use in the breeding colony. Each female was examined on arrival for the presence of a vaginal septum as described previously. The number of affected females was recorded, and these females were euthanized without being incorporated into the breeding colony. For study purposes, however, 14 of the affected females from 2 shipments were maintained for the breeding trial (power analysis indicated the need for 12 animals/group to detect a difference in fertility).

For comparison, 100 C57Bl/6N (Taconic, Germantown, N.Y.) mice were examined for the presence of a vaginal septum shortly after arrival at another facility (Merck Pharmaceuticals, Rahway, N.J.). For accurate comparison, these virgin mice were 5- to 6-week-old females to be used in a breeding colony.

Breeding trial. Twelve normal virgin female C57BL/6J mice (without a vaginal septum) and 14 affected virgin female C57BL/6J mice (with a vaginal septum) were available for the breeding trial. This group was composed of individuals from 2 shipments received within 3 months of each other, and ranged in age from 9 to 15 weeks at the start of the trial. Each female was reexamined to verify presence of the septum, and was tattooed using an Aramis laboratory animal microtattooing system (Ketchum Manufacturing, Inc., Ottawa, Canada) for individual identification. Affected females were numbered 1-14, and normal females were numbered 15-26. A harem breeding scheme was developed, and as the animals were reexamined and tattooed, they were randomly assigned to 1 of 9 breeding cages. Eight cages contained 3 females, and one cage contained 2 females. Each cage contained at least one normal female and at least one affected female.

Table 1. Incidence of vaginal septum in C57BL/6J female mice in 6 shipments for a 1-year period

Shipment	Total no. of females received	No. with vaginal septum	Incidence
1	50	5	10.0%
2	20	2	10.0%
3	25	2	8.0%
4	95	14	14.7%
5	97	12	12.4%
6	50	3	6.0%
Total	337	38	11.3%

After a 5-day acclimation period to allow the females to adjust to their new cagemate(s), one C57BL/6J male mouse was placed in each cage. The males were from the breeding colony and were proven breeders as previously documented by their siring of litters. The harems remained together for 16 nights to allow multiple breeding opportunities (assuming a 4-day estrous cycle, each female would have had the opportunity to breed 3 to 4 times). After introduction of the males, females were examined each day, Monday through Friday, at the same time each morning, for the presence of a copulatory plug. At the same time, females 1-14 were reexamined to document whether the vaginal septum had been ruptured.

At the end of the 16-day breeding trial, the males were returned the regular breeding colony and each female was examined for pregnancy by use of abdominal palpation. Each pregnant female was placed in its own cage and was provided nesting material. Females that did not appear pregnant on the basis of results of abdominal palpation were group housed 4 or 5 to a cage, were monitored for pregnancy, and were separated as indicated. The pregnant females were monitored daily for successful pregnancy or dystocia. A successful pregnancy was defined as the delivery of live young without development of dystocia. The experimental design did not include counting or rearing of pups. If dystocia developed, the female and any delivered pups were euthanized by use of carbon dioxide inhalation.

After successful delivery, the affected females were once again examined for the continued presence of the vaginal septum. At the end of the study, normal females and females with ruptured vaginal septum were placed in the breeding colony. The remaining females were euthanized by use of carbon dioxide inhalation.

Light microscopy. Necropsy was performed on four of the affected mice to characterize the vaginal septum. Sections were treated with H&E and trichrome stains, then were examined.

Results

Incidence of vaginal septum in C57BL/6J mice. During 2002, a total of 337 C57BL/6J female mice in a total of 6 shipments were ordered for the breeding colony (Table 1). Incidence of females with a vaginal septum ranged from 6.0 to 14.7% per shipment. The incidence of the defect in C57BL/6J mice for the year was 11.3%.

Table 2. Comparison of successful pregnancies, dystocias, and infertile females in the three C57BL/6J female subgroups of vaginal septum

	Total	No. of successful pregnancies	Dystocia	Not pregnant
Normal	12	9	1	2
Intact	7	1	0	6
Ruptured	7	6	1	0

Incidence of vaginal septum in C57Bl/6N mice. Only 1 of the 100 females examined was found to have a vaginal septum.

Rate of vaginal septum rupture. At the start of the study, 14 female mice were identified as having a vaginal septum. The septum in 7 of these females ruptured during the breeding trial and was detected while examining for the presence of a copulatory plug. Thus, there was 50% rupture rate. The rupture likely occurred during copulation.

Copulatory plug rates. Throughout the breeding period, individuals were monitored Monday through Friday for copulatory plugs. Of the 14 affected females, 11 (79%) had a copulatory plug, and 8 of the 12 normal females (67%) had a copulatory plug. On the basis of results of the Fisher's exact test, the rates were not significantly different ($P = 0.67$).

In the affected females whose septum remained intact after mating, the copulatory plug was typically located on one side of the intact septum. Furthermore, in four of these females, the copulatory plug was observed over subsequent days. In one of the females with a septum, a different copulatory plug was recorded on two separate breeding occasions 10 days apart, indicating that the first mating was infertile. This female never became pregnant despite evidence of mating on two separate occasions.

Fertility. Females were monitored for successful pregnancy. As previously mentioned, a successful pregnancy was defined as the ability to give birth to live, viable young without development of dystocia. There were a total of 16 successful pregnancies and 2 dystocias (Table 2). One dystocia was in a normal female, and the other was in a female with a ruptured vaginal septum. Of 12 normal females, 9 had a successful pregnancy (75%) and 7 (50%) of 14 affected females had a successful pregnancy. Comparing just these 2 rates using the Fisher's exact test, the rates were not significantly different ($P = 0.25$). However, when data for the affected female group were subdivided into 2 groups, females with intact septum after breeding and those with ruptured septum after breeding, the following was found: 6 of the 7 females with postbreeding ruptured vaginal septum had a successful pregnancy (86%), whereas only 1 of the 7 females with postbreeding intact septum had a successful pregnancy (14%). Comparing the 3 groups, the normal females, the females with intact vaginal septum, and the females with ruptured vaginal septum, there was a significant difference in fertility using the Fisher's exact test ($P = 0.02$).

Light microscopy. Results of light microscopy were consistent with earlier findings in the BALB/cJ mice (3). The septum was found to be a longitudinal band of tissue covered by a mucosa of stratified squamous keratinizing epithelium. It was supported by a fibrous connective tissue stroma. The band contained few blood vessels.

Discussion

Our findings suggest that the incidence of vaginal septum in C57BL/6J female mice has increased. We found the defect in 11.3% of the females ordered for the year, almost triple the 4.0% expected incidence on the basis of that of the original report (3). In contrast, we report only 1% incidence in C57Bl/6N female mice.

In defining fertility for the purposes of this study, we considered only the ability of a female to have a successful pregnancy. A successful pregnancy was defined as the ability to deliver live young without development of dystocia. Results of a previous study (3) completed in the BALB/cJ strain indicated that fecundity was not altered due to the presence of the septum. The vaginal septum was, therefore, considered to be merely a physical barrier to breeding. It was not expected to have any effect on the number of pups delivered should a female become pregnant, nor was it expected to have any effect on the female's ability to nurse, rear, or wean the young. As such, neither the number of pups born was recorded, nor were the litters followed until weaning.

When the normal female group was compared with the vaginal septum group (those identified with vaginal septum at the start of the trial), there was no significant difference in fertility. This outcome was unexpected and likely was the result of the number of females enrolled in the study. Our original analysis indicated that only 12 animals were needed in each of the 2 groups (the normal group and the vaginal septum group) to determine a difference in fertility. This analysis did not account for the unexpected rupture of 50% of the septa in the vaginal septum group. These septa ruptured during the course of the breeding trial and were noted while examining for a copulatory plug. In a new power analysis taking into account the 50% rupture rate, 60 animals would be required in each group to detect a difference.

When data for the affected female group were subdivided into those for females with postbreeding intact septum and females with postbreeding ruptured septum, we noted two outcomes. There was not a significant difference in fertility between normal females and females with ruptured vaginal septum. Therefore, if the vaginal septum ruptures, the female can be considered as fertile as a normal female. Also, when the postbreeding intact vaginal septum group was compared with the postbreeding ruptured vaginal septum group and the normal group, there was a significant decrease in fertility in the intact vaginal septum group. Because copulatory plugs were identified in all groups, this decreased fertility cannot be attributed to lack of physical breeding. Only one of the females with postbreeding intact septum was fertile. This female unexpectedly delivered a litter without development of dystocia, as the septum was expected to serve as a physical barrier. The septum was noted to be stretched to the right side of the vagina after parturition, thus it no longer created a barrier to delivery. We expect that, in a trial with a larger number of females with intact septum, fewer than the 14% reported here would be fertile. We also expect that, in those that become pregnant, dystocia may be a problem, as the septum would serve as a physical barrier to delivery.

As we have reported, there has been a significant increase in the incidence of vaginal septum in C57BL/6J mice compared with that of the 1976 report. We have also documented, as was previously reported, a decrease in fertility in affected females with an intact vaginal septum (3). As a result, this defect can lead to large numbers of female C57BL/6J mice being culled, and to large financial burdens on the laboratory. This is particu-

larly true when one considers the fact that this strain is one of the most common strains used in research, and it has become one of the favorites in transgenic colonies. The small breeding colony of this study suffered a loss of 38 mice solely due to this defect, as the laboratory culls all affected mice at arrival. The laboratory suffered a financial loss of at least $558.60 for the year, not accounting for the loss of valuable research time. As one can imagine, the larger the colony, the greater the losses.

One of the major contemporary goals of our profession is to reduce the number of animals used in research. In terms of this defect, there are many ways this could be accomplished to be in accordance with the principles of the 3Rs (replacement, refinement, and reduction of animal use) of Russel and Burch (7). One of the most obvious recommendations is to breed the trait out of the line. This method, however, does not appear feasible, as the trait is polygenetic (1-3, 9). The most feasible method that results in the greatest reduction of animal use is training technicians at the vendor facility to examine for the defect as shipping containers are filled, and separate accordingly. This defect is easily identified by examination of the vaginal region by use of a blunt probe. Only females without the vaginal septum should be sent to investigators with breeding protocols. This route is the most effective way to reduce animal use and decrease financial losses suffered by laboratories such as this one. The researcher in this instance orders breeding animals from the vendor and uses the fetuses or pups in the research protocol. However, in laboratories that order only an occasional animal and mainly use the offspring to maintain the colony, the septum phenotype may appear in later generations in the colony due to the polygenetic nature of the trait.

A less effective alternative to reduce the number of animals is to incorporate all affected females received into the breeding colony. As our results indicate, the vaginal septum in approximately half of the females will rupture and these animals will be as fertile as normal females. The drawback to this approach for the laboratory is cost (cost of mice plus per diems of the infertile mice) and lost research time as a result of the infertility. This option, however, results in fewer animals used and less cost than does culling all the affected females on arrival at the laboratory.

Another consideration is to attempt to rupture the septum before incorporating an affected female into the breeding colony. Some of the septa appeared to be a thin band of tissue, whereas others appeared to be thicker and extended deep into the vagina (data not recorded, but previously observed [3]). Because there were no overt signs of trauma or distress in the females whose septum ruptured, an attempt to rupture the septum could be made prior to incorporating the affected female into the breeding colony. If the septum is thin, it will break easily in response to gentle pressure. One technique that was performed during necropsy was to place the tip of a blunt probe gently into the caudal portion of the vagina along one side of the septum. The septum was then 'hooked' with the tip of the probe. If the septum was successfully 'hooked,' gentle caudal retraction was used to break the septum. If the septum could not be 'hooked,' or the septum did not break in response to gentle caudal pressure, it is likely that the septum was too thick or deep, and this individual should be culled. This method does not eliminate animal loss due to the defect; however, it is equivalent to incorporating all females with a vaginal septum into the colony. This method enables the researcher to cull females with a septum that would likely not have broken during copulation and, thus, decreases the lost research time and per diem costs associated with the infertile females due to the septum.

One final way to reduce animal use due to this defect is to change vendors. Our results indicated significantly lower incidence of vaginal septum in C57Bl/6N mice, the only other strain evaluated in this study. However, due to genetic drift, changing vendors should proceed with caution in ongoing research protocols. "Even though each vendor strain can trace its origin back to a common pair of founder animals, each independently maintained line slowly drifts genetically from its ancestors over time" (10). Therefore, changing strains once a research project has been started may yield variable results.

In conclusion, on the basis of our findings, the incidence of vaginal septum has almost tripled since the original report in 1976 in C57BL/6J mice (3). Because this defect is linked to reduced fertility in affected females, the increased incidence has led to increased use of animals and increased costs to investigators. Since we have been charged with reducing animal use in research as part of the 3Rs philosophy, it is our responsibility to take measures to decrease the animal loss in breeding colonies due to this defect.

Acknowledgments

We would like to extend our gratitude to the following for their assistance in the preparation of this manuscript: Ana Rivas for cutting and staining the histologic slides, Judy Fenyk-Melody for her assistance in the assessment of incidence of the defect in C57Bl/6N female mice, Shing Lee of the Biostatistics department at Columbia University for her assistance in the statistical analysis, and Heike Heuer for pointing out the increased infertility in the breeding colony. This work was supported by grants from the National Institutes of Health (NS16951 and EY012736 to CAM).

References

1. **Bonner, J. J.** 1981. Vaginal septa frequency influenced by major histocompatibility complex, H-2. J. Immunogenet. **8:**455-458.
2. **Bonner, J. J. and M. L. Tyan.** 1983. Map of major histocompatibility complex subregions influencing vaginal septa frequency. J. Immunogenet. **10:**413-416.
3. **Cunliffe-Beamer, T. L. and D. B. Feldman.** 1976. Vaginal septa in mice: incidence, inheritance, and effect on reproductive performance. Lab. Anim. Sci. **26:**895-898.
4. **Fritz, E. B., S. J. Carlan, and L. Greenbaum.** 2002. Pregnancy and transvaginal septation. J. Matern. Fetal Neonatal Med. **11:**414-416.
5. **Rock, J. A., H. A. Zacur, A. M. Dlugi, H. W. Jones, and R. W. TeLinde.** 1982. Pregnancy success following surgical correction of imperforate hymen and complete transverse vaginal septum. Obstet. Gynecol. **59:**448-451.
6. **Root, M. V., S. D. Johnston, and G. R. Johnston.** 1995. Vaginal septa in dogs: 15 cases (1983-1992). J. Am. Vet. Med. Assoc. **206:**56-58.
7. **Russell, W. M. S. and R. L. Burch.** 1959. The principles of human experimental technique. Methuen Press, London.
8. **Schaepdrijver, L. M., J. L. Fransen, E. S. Van der Eycken, and W. C. Coussemnet.** 1995. Transverse vaginal septum in the specific-pathogen-free Wistar rat. Lab. Anim. Sci**. 45:**181-183.
9. **Shire, J. G. M.** 1984. Studies on the inheritance of vaginal septa in mice, a trait with low penetrance. J. Reprod. Fertil. **79:**333-339.
10. **Silver, L.** 1995. Mouse genetics, concepts and applications. Oxford Press University, Inc., New York.