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Effects of Vaginal Septa on the Reproductive Performance of BALB/cByJNarl Mice

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From March through December 2010, the incidence of vaginal septa in our SPF breeding colony of BALB/cByJNarl mice was 14.2%. In general, septa obstructed half of the vaginal orifice. Here we sought to determine the effect of this defect by comparing the reproductive performance of affected (septate) mice with that of unaffected (nonseptate) mice. Our results showed that the rates of both copulatory plugs and pregnancy were significantly lower in septate mice than in nonseptate mice. Specifically, 23 of 45 bred septate female mice (51%) had vaginal plugs compared with 49 of 68 bred nonseptate females (72%). Only 12 septate female mice (27%) had successful pregnancies, compared with 37 nonseptate females (54%). Septate mice had a 1-logfold fewer intrauterine sperm after mating than did nonseptate mice. Three cases of dystocia were noted among septate mice whereas none occurred in nonseptate mice. Septate dams had a higher percentage of septate pups (15.5%) than did nonseptate dams (6.1%). Our findings indicate that vaginal septa affect the reproductive performance of laboratory mice and that such a defect should be considered as an exclusion criterion for the selection of future breeders in a mouse colony.

Vaginal septa in mice vary from a thin dorsoventral band of tissue that bisects the vaginal orifice to a thick band that divides the vaginal canal longitudinally (Figure 1). Microscopically, the septum consists of fibrous connective tissue covered by normal vaginal mucosa.[1,3] Vaginal septa occur in many species, including humans, multiple strains of laboratory mice, rats, and dogs.[1,2,6,9,10] Previous reports have indicated that genetic background is important for the expression of this polygenic anomaly in mice.[1,11]

While evaluating mice for copulatory plugs, animal caretakers noticed a high incidence of vaginal septa in our SPF BALB/cByJNarl colony. The BALB/cByJNarl subline was derived from BALB/cByJ mice after more than 20 generations of brother–sister mating in our facility. The C57BL/6J strain was reported to have an increased incidence of vaginal septa after several decades of inbreeding.[3] The incidence in BALB/cJ mice had been reported to be as high as 38%, but that in BALB/cBy mice was only 1.3% to 3.2%.[1,11] Here we sought to determine the effects of vaginal septa on the reproduction performance of BALB/cByJNarl mice. In this strain, the presence of vaginal septa correlated with decreased intrauterine sperm count and decreased reproductive performance.

Materials and Methods

Animals. This study was conducted in a BALB/cByJNarl breeding colony in the animal facility of the National Laboratory Animal Center (Tainan, Taiwan), an AAALAC-accredited program. The BALB/cByJNarl substrain was derived in 1995 from the BALB/cByJ founder line (The Jackson Laboratory, Bar Harbor, ME). A total of 1480 virgin female mice were examined during the study period (March through December 2010). The mice were housed in open, wire-top cages in the facility's barrier production facility, which has a stringent quality assurance program to ensure the health and genetic integrity of the animals produced.[7] Caging and bedding (Aspen, Tapvei, Kortteinen, Finland) were autoclaved. Mice received autoclaved rodent diet (diet 5010, PMI, St Louis, MO) and autoclaved (121 °C, 20 min) reverse-osmosis water ad libitum. Environmental temperature was maintained at 23 ± 2 °C and humidity at 50% ± 10%, with a 12:12-h light:dark cycle.[5] All husbandry and sentinel surveillance practices strictly followed the institutional guidelines to ensure that mice were free of pneumonia virus of mice, Theiler encephalomyelitis virus, minute virus of mice, mouse hepatitis virus, mouse adenovirus, Sendai virus, lymphocytic choriomeningitis virus, ectromelia (mousepox) virus, Hantaan virus, mouse parvovirus, mouse norovirus, reovirus type 3, *Mycoplasma pulmonis, Bordetella bronchiseptica, Corynebacterium kutscheri*, *Salmonella* spp., *Citrobacter rodentium*, *Pseudomonas aeruginosa*, *Clostridium piliforme*, *Helicobacter* spp., and endo- and ectoparasites.[7] Quarterly genetic monitoring (11 microsatellite markers: Mit446, Mit309, Mit5, Mit136, Mit78, Mit14, Mit226, Mit184, Mit64, Mit19, and Mit210) confirmed a well-defined genetic profile consistent with the BALB/cByJ strain. All procedures were reviewed and approved by the facility's IACUC (approval number, NLAC[TN]-100-R-007).

Examination for vaginal septa. Female mice were examined after weaning for the presence of a vaginal septum by using a technique similar to that for checking for copulatory plugs. Briefly, one set of forceps was used to lift mice by the tail, while a second set of forceps was used to apply gentle external pressure on the vagina to open the vaginal orifice. When present, a vaginal septum was easily visualized as a longitudinal, transverse, or oblique dorsal-to-ventral band of tissue bisecting the vagina. To quantify obstruction of the vaginal orifice by the vaginal septum, age- and weight-matched virgin female mice (n = 16 septate, n = 16 nonseptate) were selected, and photographs of their perineums were taken by using a digital camera (Figure 1). The area (in mm^2) of the vaginal orifice was determined by using ImageJ software (National Institutes of Health, Bethesda, MD). The percentage obstruction was calculated as:

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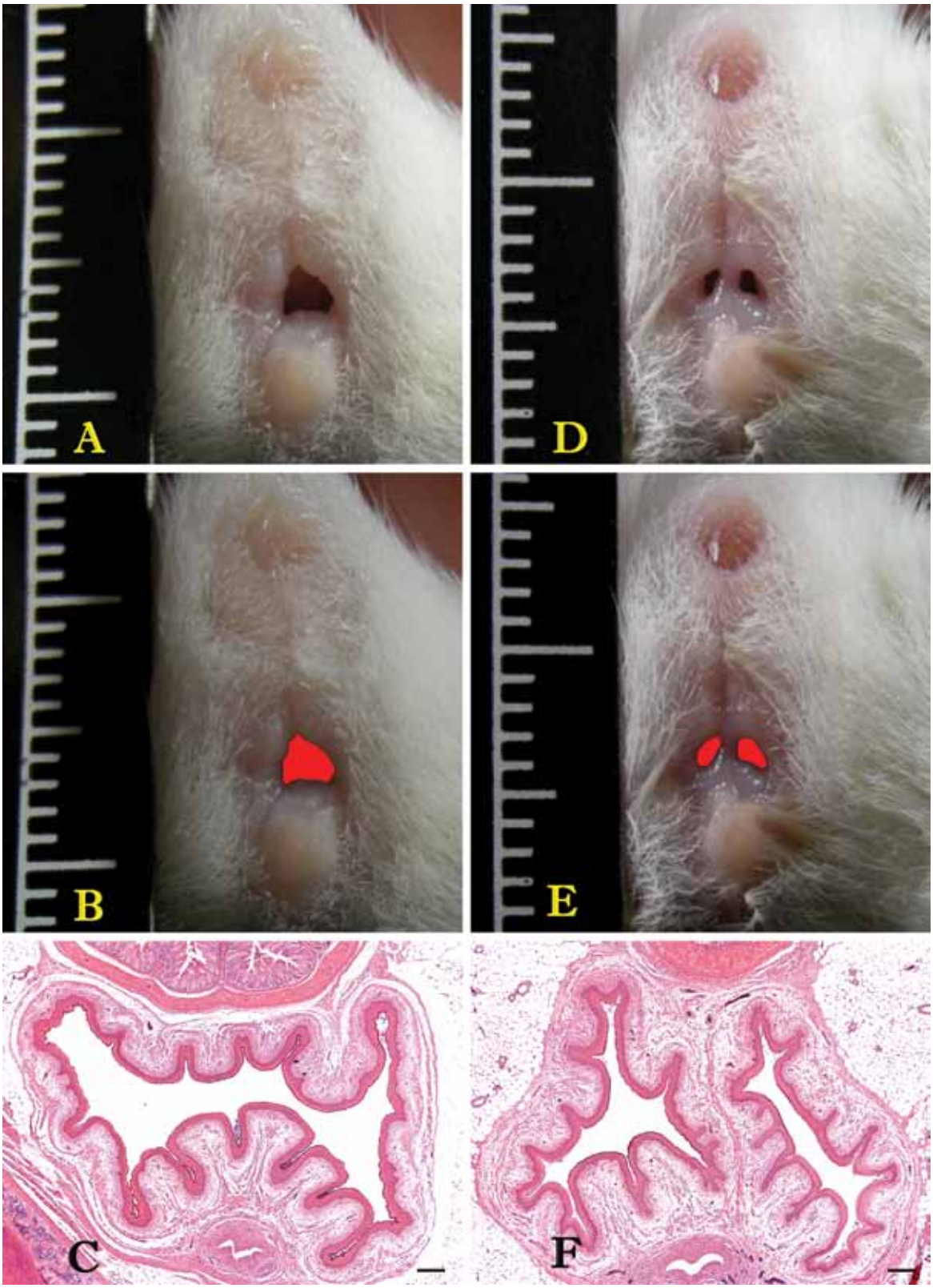

Figure 1. The gross and histopathologic manifestations of vaginal septa. A primiparous female mouse has (A) a nonseptate vaginal orifice with (B) a fully open vaginal space and (C) normal histologic appearance. A septate female mouse exhibits (D) a longitudinal vaginal septum with (E) a smaller vaginal orifice. The septum is a dorsoventral band of connective tissue that extends longitudinally in the vagina canal. It is covered by mucosa of stratified squamous keratinizing epithelium. Scale bar, 200 µm.

(Average area of nonseptate vaginal orifice – Average area of obstructed vaginal orifice) ÷ Average area of nonseptate vaginal orifice × 100%.

Breeding performance. To examine the effect of vaginal septa on breeding performance, a total of 68 nonseptate and 45 septate virgin female mice (age, 9 to 12 wk) each was mated with 1 of 20

individually caged proven male breeder mice. The female mice were introduced into the cage of the sire at 1600 and examined for copulatory plugs at 0900 the following morning by using the same technique as for vaginal septum. Mice with plugs then were housed individually; those without plugs were group-housed. All mated mice were examined by palpation at 14 d after mating to assess for pregnancy. Pregnant mice were monitored for delivery or dystocia, which was defined as the delivery of dead, full-term pups. When dystocia occurred, the dam and surviving pups were euthanized to remove them from the production colony. At the end of the study, nonseptate successful dams remained in the breeding colony, and septate dams were euthanized. The rate of copulatory plugs was defined as:

No. of female mice with plugs ÷ the total no. of female mice mated × 100%.

Pregnancy rate was defined as:

No. of female mice that delivered ÷ the total no. of female mice mated × 100%.

The rate of dystocia rate was defined as:

No. of mice that experienced dystocia ÷ the total no. of female mice mated × 100%.

The rate of plugged and pregnant mice was defined as:

No. of female mice with plugs that delivered ÷ the total no. of female mice with plugs after mating × 100%.

Sperm count in the uterus. Six adult male mice that had at least one successful mating record were housed individually. Each male was pair-housed with a nonseptate female ($n = 6$) and a female with a vaginal septum ($n = 6$) for periods of 1 wk each; the order of mating with nonseptate or septate mice was randomized for each male mouse. Female mice were examined every 30 min after pairing for the presence of copulatory plugs; those with plugs were euthanized immediately. The uterine horns were removed by cutting at the cervical and ovarian ends. Each uterine horn was flushed with 1 mL PBS.[14] The number of spermatozoa in 10 µL of the wash solution was counted immediately on a Makler Counting Chamber (New York Microscope Company, New York, NY).[8]

Heritability. Nonseptate ($n = 57$) and septate ($n = 64$) virgin female mice at 9 wk of age were individually randomly mated with 1 of 15 proven sires. Pregnant mice were housed individually in a cage with nesting materials. The female offspring of these mice were examined for the presence of a vaginal septum at 4 wk of age. At the end of the study, nonseptate dams and pups remained in the breeding colony, whereas those with a vaginal septum were euthanized.

Euthanasia method. Mice were euthanized by carbon dioxide inhalation with or without cervical dislocation.

Histopathology. Two nonseptate and 6 septate female mice were euthanized with carbon dioxide, and their vagina and uteri were removed and fixed in 10% neutral buffered formalin. Tissue samples were processed by routine methods and were imbedded in paraffin wax. Sections (6 µm) were stained with hematoxylin and eosin.

Statistical analyses. Frequency data were analyzed by using the χ^2 test (copulatory plug rate, pregnancy rate, and heredity) or Fisher exact test (dystocia), and quantitative data (vaginal orifice and sperm count) were analyzed by using an unpaired Student t test. All statistics were analyzed by using SigmaPlot software (Systat Software, San Jose, CA). Results are expressed as mean ± 1 SD. A P value of less than 0.05 was considered statistically significant.

Results

Incidence of vaginal septum in BALB/cByJNarl mice. During the 10-mo study period, 14.2% (210 of 1480) of BALB/cByJNarl virgin female mice born in our facility were found to have a vaginal septum. All but 2 of these septa were longitudinal in orientation.

Obstruction of vaginal orifice by vaginal septum. The vaginal orifice of septate female mice was obstructed on visual inspection (Figure 1). Measurement from digital images showed that a vaginal septum approximately obstructed 49% of the vaginal orifice of septate mice. Consequently, the vaginal orifice of septate mice ($n = 16$; mean ± 1 SD, 1.36 ± 0.28 mm^2) was significantly ($P < 0.0001$) smaller than that of nonseptate mice ($n = 16$; 2.67 ± 0.88 mm^2).

Effect of vaginal septa on reproductive performance. In a timed mating trail, in which female mice were mated at 1600 and checked for copulatory plugs at 0900 the next morning, nonseptate mice had a significantly (χ^2, $P < 0.05$) higher copulatory plug rate than did septate mice. Specifically, 49 of 68 (72%) nonseptate mice had copulatory plugs, compared with 23 of 45 (51%) septate female mice. The female mice were further monitored for successful pregnancy after mating. The pregnancy rate was significantly (χ^2, $P < 0.01$) higher in nonseptate mice than septate mice: 37 of 68 (54%) mice delivered litters compared with 12 of 45 (27%) septate mice. In addition, no dystocia occurred in the nonseptate group, whereas 3 cases were confirmed in septate mice (Fisher exact test, $P < 0.05$). Among mice with copulatory plugs, 37 of 49 (75.5%) nonseptate mice had successful pregnancies, compared with 12 of 23 (52.2%) septate mice (χ^2, $P < 0.05$).

Rupture of vaginal septa during breeding or parturition. Among the 23 septate mice with plugs after mating, 13 (56.5%) experienced ruptured septa. These mice were fertile, and a small-scale breeding study showed that they had a 50% copulatory rate (4 of 8 mice) and a 25% pregnancy rate (2 of 8 mice) after mating.

Uterine sperm counts. To determine whether a vaginal septum interferes with the mating of septate mice, we counted the number of sperm in the uteri shortly after mating. The average number of spermatozoa in the vagina of nonseptate mice ($1.2 \pm 0.29 \times 10^7$/mL) was significantly (unpaired t test, $P < 0.001$) higher than that of mice with vaginal septa ($1.45 \pm 1.17 \times 10^6$/mL).

Heritability of vaginal septa. Among the 131 female pups born to 57 nonseptate dams, 8 (6.1%) had a vaginal septum, whereas 24 (15.5%) of the 155 female pups born to 64 septate dams carried the defect (χ^2, $P < 0.05$).

Histologic examination. Microscopic examination was consistent with earlier findings.[1] The vaginal septum of our mice was a longitudinal band of tissue covered by a mucosa of stratified squamous keratinizing epithelium (Figure 1 C and F), consistent with normal vaginal mucosa.

Discussion

BALB/cByJ breeder mice were introduced to our facility in 1995 and maintained under SPF conditions. A new subline, designated as BALB/cByJNarl, was established in 1998 after 10 generations of inbreeding, and there have been 32 additional inbreeding generations since then. We demonstrated that the incidence of vaginal septa in the BALB/cByJNarl subline

was much higher than that of the parental strain BALB/cBy reported previously (14.2% versus1.3%),[1] suggesting that the trait became fixed during the establishment of the subline. Breeding with nonseptate female mice reduced but did not eliminate the trait in subsequent generations, consistent with previous reports[12] of the polygenic recessive nature of this trait and the fact that male mice can carry relevant alleles also.

Our results show significant association between vaginal septa and poor reproductive performance in mice. The effects of vaginal septa on reproduction were clear. The 14.2% incidence of vaginal septa reflects that 468 female mice with this defect had been selected as breeders (among the total of 3300 female breeders at our facility), corresponding to a considerable waste of both time and money.

The presence of copulatory plugs in the vagina and sperm in the uterus strongly indicated that septate mice could mate with male mice. However, the decreases in plug rate, sperm count, and pregnancy rate of septate mice imply decreased successful intercourse. The 1.33-fold increase in time for a success mating (data not shown) supported this assumption. In addition, 3 septate mice delivered dead pups (interpreted as dystocia), compared with no dead pups delivered in the nonseptate group. Therefore, although septate mice can successfully mate and carry fetuses to full-term, they seem to be more susceptible to dystocia than are nonseptate mice.

Grossly, more than 99% of the vaginal septa among our mice were longitudinal. Imperforate vagina has not been seen in BALB/cByJNarl mice, although a high incidence of this defect has been noted in other strains of inbred mice.[4,13] Importantly, approximately half of the septa ruptured after the first mating, and the mice with ruptured septa were fertile but had a poorer reproductive performance than that of nonseptate female mice.

Collectively, our results indicate that genetic drift of sublines can occur over time even with a strict brother–sister mating scheme[12] and that vaginal septa affect the reproductive performance of laboratory mice. Removing septate female mice from a breeding colony may reduce the incidence of vaginal septa in population, improve breeding performance, and improve animal welfare by facilitating intercourse and parturition.

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