Imperforate Vagina in Mice: Per Cent Incidence and Surgical Repair

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An inbred strain maintained at Nagoya University, Laboratory of Animal Genetics, produced high incidence of imperforate vagina as 12.21% of occurrence in females, herein designated as Imv strain. Selected females with imperforate vagina in this strain were surgically repaired and subsequently mated with their littermates. These matings produced a 12.76% incidence of imperforate vagina. A detailed description of the surgical technique is herein reported.

The appearance of female mice with imperforate vagina occurring at a rather high incidence was first noticed by kondo among the dilute black strain kept in our laboratory. This finding was a fortuitous one because the purpose of producing this dilute black strain was for class demonstration of the dilute gene. The origin of this strain came from crosses among DBA/2, IXBL and AI (AI was an inbred strain but got extinct in 1963) [6]. The perineum of affected mouse had a noticeable swelling quite similar to the scrotum of male mice. However, upon careful examination it was noticed that affected mice were past their puberty age and had complete closure of the vagina, whereas the other apparently normal female littermates had their vaginas open without the perineal swelling. Because of this condition we decided to name this strain as Imv, hereafter referred to as such.

In the mouse, the presence of imperforate vagina has been reported by several researchers. In the black silver strain Marx [4] observed imperforate vagina in association with pituitary dwarfism. Gowen and Heidenthal [2] reported also the presence of imperforate vagina in the black silver strain. The same condition was reported by Chase [4] in the Aka strain. Strong and Hollander [7] reported the presence of imperforate vagina in association with loop-tail and nervous socking in the Strong A strain. In the rat this condition was reported by Plagge and Lamar [5] occurring in about one in every 150 females. Grüneberg [3] made an excellent review of imperforate vagina in mice. Review of literature for recent papers on imperforate vagina in mice yielded negative result. Because of this lack of report in the literature we feel it is timely to report our findings.

The purpose of this study is to find out the incidence of imperforate vagina in our colony of Imv mice. The authors also tried to repair the condition by surgery and attempted to mate the surgically-repaired female mice to see if they are capable of reproduction.
Materials and Methods

In our laboratory animal house, mice are kept under conventional conditions. It is only in winter when some form of heating is applied to keep the temperature above 15°C and in summer electric wall fans are installed to circulate the air and minimize increase in temperature. Feeding is once daily using commercial poultry feed (approximate analysis: crude protein 15.0%, crude fat 3.0%, crude fiber 6.0%, crude ash 13.0%, Calcium 2.5%, Phosphorus 0.30% and metabolizable energy in excess of 2,800 Kcal per 1 kg of feed). Water is given ad lib. Cages are conventional plastic cages for mice and/or manufactured wooden cages having the same dimensions as the plastic cages. Body weight of animals is taken once a week at which time the beddings are also changed if necessary.

The breeding method used for Imv was the usual sister-brother mating using normal females. Mice used in this experiment came from the 9th up to the 13th generation of sib inbreeding. Pairs were kept mated continuously until the production of the 7th litter. As opening of the vagina in mice generally occurs at about 35 days after birth, all litters under observation were kept for this length of time. All females were examined after the observation period for the presence or absence of imperforate vagina. Female mice with imperforate vagina were kept for surgical repair. The details of the surgical technique are explained in the section for results.

Results

Incidence of imperforate vagina in Imv strain: The incidence of imperforate vagina in some strains maintained in our laboratory is summarized in Tables 1 and 2. As data in these tables included only

<table>
<thead>
<tr>
<th>Strain</th>
<th>Males</th>
<th>Females</th>
<th>Imperforate females</th>
<th>Total</th>
<th>% in total</th>
<th>% in females</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS7BL/6</td>
<td>129</td>
<td>154</td>
<td>1</td>
<td>284</td>
<td>0.35</td>
<td>0.65</td>
</tr>
<tr>
<td>IXBL</td>
<td>377</td>
<td>438</td>
<td>0</td>
<td>815</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>AI</td>
<td>138</td>
<td>141</td>
<td>0</td>
<td>279</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>DBA/2</td>
<td>198</td>
<td>248</td>
<td>0</td>
<td>446</td>
<td>0.00</td>
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<tr>
<td>ITES</td>
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<td>76</td>
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<td>190</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>KR</td>
<td>61</td>
<td>64</td>
<td>4</td>
<td>125</td>
<td>3.20</td>
<td>6.25</td>
</tr>
<tr>
<td>CS</td>
<td>141</td>
<td>140</td>
<td>0</td>
<td>281</td>
<td>0.00</td>
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</tr>
</tbody>
</table>

Table 2. Incidence of imperforate vagina in the Imv strain

<table>
<thead>
<tr>
<th>Group</th>
<th>Nucleus line</th>
<th>No. of pairs mated</th>
<th>No. of offspring born</th>
<th>Total</th>
<th>% in total</th>
<th>% in females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Imperforate females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I*</td>
<td></td>
<td>10</td>
<td>127</td>
<td>131</td>
<td>16</td>
<td>258</td>
</tr>
<tr>
<td>Group II</td>
<td>SR-Imv**</td>
<td>10</td>
<td>74</td>
<td>47</td>
<td>6</td>
<td>121</td>
</tr>
</tbody>
</table>

*Normal females used
**Surgically-repaired imperforate females used
the record of the nucleus line and some of the experimental records, the per cent of occurrence is not so accurate. But it could be said that the incidence of occurrence of female with imperforate vagina in the Imv strain is extremely high.

Matings from the first group consisting of 10 pairs of apparently normal Imv mice (the nucleus line of Imv) produced a total of 131 female mice. Out of these 131 females, 16 were imperforate or 12.21%. All males in this strain were normal in morphological and breeding experiments. This high incidence of occurrence in Imv strain was supposed to be connected with some genetical basis, however the female mouse with imperforate vagina itself could not be used in mating experiment. In the second step, the surgical repairing of imperforate females was carried out and used in the mating experiment.

As can be seen in Fig. 1 the female Imv mouse with imperforate vagina is apparently normal externally except for the closed vaginal orifice which causes the characteristic perineal swelling. Incision of the swollen perineum revealed the same 3 layers of tissue as reported by Gowen and Heidenthal [1] i.e., from the outside: a layer of skin, a connective tissue membrane and the blindly ending vagina. Anatomical examination showed that the internal organs were all apparently normal except for the distended uterus (Fig. 2). Incision of the uterus or vagina causes the fluid inside to ooze out, and the color and consistency of the fluid vary with the age of the animal. The more aged the animal (2 years or more) the darker the color and the more viscous the consistency of the fluid was. Histological examination of the ovary in imperforate females approxi-
mately 3 months of age showed apparently normal stages of Graafian follicle.

**Surgical operation**: After weighing the animal it was anesthetized by injecting Nembutal Sodium solution (Abbott) I. P. at a dose of 0.06mg/10g body weight. Hair from the perineum and its vicinity were removed by a depilatory cream. The animal was then held down by tapes to the operating board and the perineum was washed with tap water and disinfected with 70% alcohol. To ease the problem of minute surgery, the operation was viewed under an adjustable illuminated magnifying lens. A straight incision was made through the skin at the site of the closed vaginal orifice using a pair of small pointed surgical scissors. After cutting the skin, the underlying connective tissue membrane was also cut exposing the blindly ending vagina. This was also cut and immediately fluid came out and had to be completely drained out before the next step of suturing was done. Black nylon U. S. P. No. 6-0 needled suture was used, the needle size was C-4. The cut edges of the vagina, connective tissue membrane and skin were sutured together using interrupted sutures. Three (3) sutures were applied on each side making a total of 6 sutures. To prevent the edges of the wound from coming in contact and closing again and to assure continuous drainage of fluid, a rubber tube (O. D. 11/3mm) approximately 8 mm long was inserted through the vagina and anchored to the vaginal wall by stay sutures. After surgery the wound was dressed with an antibiotic ointment (Terramycin, Pfizer). The animal was then observed for 7-10 days after which the sutures and tube were removed under ether anesthesia. By this time the wound has healed and the animal was immediately mated. Pairs were kept mated until the production of the 7th litter.

The above procedure was carried out in 10 female Imv mice with imperforate vagina, the ages of operated mice ranged from 35-40 days and they consisted the second group (surgically-repaired line). In this study we designated the surgically-repaired Imv mice as SR-Imv for short, and hereafter referred to as such. Likewise all litters born to SR-Imv were also kept for 35 days observation of imperforate vagina among the females. This group yielded a total of 47 female mice. Out of these 47 females, 6 were imperforate or 12.76%. The incidence of imperforates among the 2 groups (nucleus line and SR-Imv line) was almost the same. This result is shown in Table 2. However, it was noticed that the SR-Imv mice were not as productive as the nucleus line probably due to the influence of the surgical treatment or the former's inherent defect.

We would like also to mention that not all litters produced from SR-Imv were normal and some of the defects observed were: late opening of one or both eyes, partial to complete absence of some digits or limbs, tail defects such as short or amputated tails and crooked tails. One case of dwarfism was likewise observed. In contrast, the above mentioned findings were not observed in the nucleus line of Imv.

**Discussion**

The imperforate vagina appears occasionally in the mouse as reported by several researchers, but there exist some differences in the syndrome of imperforate vagina. In the report by Gowen and Heidehntal [2] they mentioned that in female mice with imperforate vagina the abdomen had a smooth swelling arising out of the pelvis and extending to the diaphragm. This swelling extended through the pelvis causing a marked swelling of the perineum. In the Imv no smooth swelling of the abdomen was noticed, but the perineum had a marked swelling. Anatomical observation showed that the uterus was distended. This observation was the same as reported by the above authors. However, the severity of the uterine swell-
ing could not be compared because severely affected animals seldom survive for more than a short time. In our case, we kept several female mice with imperforate vagina for more than 2 years and they remained alive in spite of the condition.

Similarly it was also shown that surgically-repaired imperforate mice may or may not produce litters with the same condition [2]. In the black silver strain [4] a 3.6% incidence of imperforate was reported while in the Aka strain [1] a 6.6% incidence was observed. In our case we observed 12.21% and 12.76% incidence of imperforate in the nucleus line of Imv and in the SR-Imv respectively. There is a big difference in the incidence between the results of the previous workers compared with the results of our present work. At present we still could not elucidate the reason for this big difference.

Using a straight incision instead of a circular incision produced better results. The tendency of the cut edges to pucker after suturing was prevented. The problem of adhesion of cut edges was likewise prevented by insertion of a rubber tubing, which also ensured continuous drainage of fluid.

The female mouse having imperforate vagina in the Imv strain is proved to be normal in its reproductive organs, except for the closed orifice of the vagina. In addition, the high incidence of occurrence of imperforate females in this strain may have some form of hereditary basis. The result, however, indicates a complicated base of inheritance, as the same per cent of occurrence could be observed in the nucleus line and SR-Imv line.

It could be supposed that some irregular maternal influence or some irregular situation in the uterus might occur and this particular condition may have disturbed the development of the vagina in the fetus, but this same condition causes little disturbance in the male fetus. The occurrence of malformed offsprings, like malformed digits, crooked tails, etc. came only from the SR-Imv and this might be a result of the severe condition in the surgically-repaired mother.

Chromosome observation is now in progress but analysis could not be done to determine whether there is a presence or absence of chromosomal aberration. A more detailed observation is necessary in chromosome and in mating experiment.

Acknowledgment

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References

マウスの臓閉塞：発生率と外科的治療

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名古屋大学農学部家畜育種学教室での学生実習用に維持・使用していた粒子の系統に臓閉塞の多発がみられた。この系統は、兄妹交配によって維持されていて、研究に用いたのは兄妹交配9〜13世代であり、Imv系と命名してある。Imv系の臓閉塞の出現率は、131例中16例（12.21%）で、他の系統と比較して著しく高い。形態学的観察によって臓開孔部のみの異常で他の器官に異常はないとわれたので、35〜40日齢の臓閉塞雄10例を手術によって開孔させ、治療後交配してみたところ、総べて正常に分娩した。しかし、生まれた子には臓閉塞雌が12.76%（47例中6例）生じImv系維持集団における発生率と同率であった。尚、この手術により治療した雌からは、臓閉塞雌以外に、指趾の奇形・尾曲り等が多発した。