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Surgical Correction of Rectal Prolapse in Laboratory Mice (*Mus musculus*)

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Rectal prolapse is a common clinical problem in laboratory mice. This condition may occur spontaneously, develop after genetic manipulations, result from infections with pathogens such as *Citrobacter* species, or arise secondary to experimental design such as colitis models. The current standard of care at our institution is limited to monitoring mice until tissue becomes ulcerated or necrotic; this strategy often leads to premature euthanasia of valuable animals prior to the study endpoint. Surgical correction of rectal prolapse is performed routinely and with minimal complications in larger species by using manual reduction with placement of a pursestring suture. In this report, we investigated whether the use of a pursestring suture was an effective treatment for mice with rectal prolapse. The procedure includes anesthetizing mice with isoflurane, manually reducing prolapsed tissue, and placing a pursestring suture of 4-0 polydioxanone. We have performed this procedure successfully in 12 mice. Complications included self-trauma, fecal impaction due to lack of defecation, and mutilation of the surgical site by cage mates. Singly housing mice for 7 d postoperatively, applying multimodal analgesia, and releasing the pursestring when indicated eliminated these complications. The surgical repair of rectal prolapses in mice is a minimally invasive procedure that resolves the clinical symptoms of affected animals and reduces the number of mice that are euthanized prematurely prior to the study endpoint.

Rectal prolapse is a common clinical condition in laboratory mouse colonies. Causes of rectal prolapse in mice include spontaneous occurrence, genetic manipulation,[3,14,15] and infections with pathogens such as *Helicobacter* and *Citrobacter* species,[6,13] or the condition may be a secondary complication of the experimental design.[2,3,11] Long-term complications of untreated rectal prolapse are associated with trauma to the prolapsed tissue and include ulceration and necrosis of the tissue,[14] secondary bacterial infections,[13] and systemic signs of illness.

Published information regarding treatment options for laboratory rodents with rectal prolapse is scarce. There is one case report on an FVB mouse with rectal prolapse.[8] This mouse was coinfected with pinworms and *Citrobacter freundii* and was treated medically for the pathogens in addition to manual reduction of the prolapsed tissue. However, details of the procedure and follow-up care were not available, and the success of the procedure may be attributed to a combination of eradication of the pathogens and manual reduction.

The current standard of care at our institution is limited to monitoring mice until tissue becomes ulcerated or necrotic or until affected animals display systemic signs of illness. This strategy often leads to premature euthanasia of valuable animals prior to the study endpoint. Standard operating procedures from other institutions indicate that the treatment for rectal prolapse is limited to empirical therapy, including applying ointment to reduce inflammation or desiccation and providing soft bedding. In large animals, rectal prolapse repairs are performed routinely by using manual reduction and the placement of a pursestring suture, particularly when the prolapse is large. Complications in large animals are minimal and include tenesmus, dyschezia, hematochezia, and recurrence.[5] However, to our knowledge, the surgical repair of rectal prolapse in mice has not been described.

In this report, we describe a surgical correction for rectal prolapse in mice that results in complete resolution of clinical signs, alleviates the need to monitor prolapsed tissue, and eliminates animal welfare concerns associated with exposed rectal tissue. Therefore, this treatment offers the potential to prevent the euthanasia of valuable animals with rectal prolapse, thus reducing the number of mice needed for studies and improving animal welfare.

Case Series

Animals. The mice described in this report were housed at an AAALAC-accredited animal facility at the University of Michigan. All procedures and housing were compliant with the *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Michigan's IACUC. Mice were SPF for mouse hepatitis virus, minute virus of mice, mouse parvovirus, enzootic diarrhea of infant mice virus, ectromelia virus, Sendai virus, pneumonia virus of mice, Theiler murine encephalomyelitis virus, reovirus 3, lymphocytic choriomeningitis virus, mouse adenovirus, polyomavirus, pinworms, and fur mites. *Helicobacter* and *Citrobacter* species are not routinely screened or excluded at the University of Michigan, unless specifically requested. Therefore, the pathogen status of mice included in this case series regarding these bacterial species is unknown.

A total of 22 mice with rectal prolapse (maintained by 9 different laboratories) are included in this report. These mice varied in age, sex, strain or stock, genetic background, and experimental manipulation. To our knowledge, rectal prolapse was not an expected phenotype in any of the mice included in this case series. Prior to each surgery, a physical examination was performed, and the prolapsed tissue was examined. Mice were considered to have the best prognosis when all of the following criteria were met: 1) the duration of the prolapse was less than 24 h; 2)

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the prolapsed tissue did not have any evidence of ulceration, necrosis, or desiccation; and 3) the mouse was bright, alert, and responsive on examination. Once mice were selected for surgery, the lesions were photographed and information regarding sex, approximate age of prolapse, and stock, strain, and genetic manipulations (when possible) was recorded (Table 1).

Procedure. Mice were anesthetized by using isoflurane and received a preoperative dose of analgesic (carprofen 5 mg/kg SC or buprenorphine 0.1 mg/kg SC). The perianal area was shaved and rinsed with sterile saline. Prior to the procedure, the padding of the swab was removed with a pair of hemostats until the tip fit smoothly into the rectum (Figure 1 A). For this procedure, cotton swabs for human use were selected because conventional swabs for animal use were determined to be abrasive to the mucosa. After mice reached a surgical plane of anesthesia, a cotton swab coated with a generous amount of lubricant was inserted gently into the rectum to replace the prolapsed tissue (Figure 1 B and C). As constant pressure was applied to the cotton swab, the surgeon placed an absorbable pursestring suture (4-0 polydioxanone) as close to the anus as possible without penetrating the mucosa (Figure 1 D). A total of 4 throws were placed, and the suture was tied off with 4 single knots (Figure 1 E and F). The surgeon removed the cotton swab with one hand while pinching the skin around the anus and applying pressure toward the body with the other (Figure 1 G). At the completion of the procedure, prolapsed tissue is replaced and maintained by the pursestring suture (Figure 1 H).

Postoperatively, a topical lidocaine–prilocaine cream (EMLA cream, AstraZeneca, Wilmington, DE) was applied to the surgical area. Mice were singly housed in clean cages so that fecal output could be assessed easily, and they each received a quarter of a carprofen chew tablet (2 mg/tablet, Bio-Serv, Flemington, NJ), shredded paper bedding, and a cotton nesting square. For our study, veterinarians or veterinary technicians assessed the mice once daily for 7 d to examine the surgical site and monitor fecal output. If no fecal output was noted 1 d after surgery, a physical examination was performed, the surgical site was inspected for evidence of self-mutilation or fistula, and injectable analgesia was provided. If there was still no evidence of defecation by 2 d after surgery, the suture was released, and injectable analgesia provided as long as the animal appeared healthy, and had no evidence of fistula or self-mutilation. Animals were euthanized when 1) there was evidence of a fistula or self-mutilation, 2) there were systemic signs of illness, or 3) no defecation had been noted by the third day after surgery.

Surgical outcomes. Follow-up was performed for 7 d; based on our observations in this study, reprolapse did not occur once mice had reached this point. Therefore, we considered the procedure successful when mice were bright, alert, and responsive; defecating normally; and had no evidence of reprolapse 7 d postoperatively (Figure 2). Mice continued to be monitored periodically until 3 mo after surgery, but most animals were euthanized prior to 3 mo when they reached their scientific study endpoint. Between February 2012 and June 2013, we performed 22 rectal prolapse repair surgeries. Most mice had defecated and were bright, alert, and responsive within 1 d after surgery. One mouse was found dead in the cage the morning after surgery, and the body was cannibalized by its cage mates. This mouse was excluded from the study because the cause of death appeared to be unrelated to the surgical procedure. Of the remaining 21 surgeries, one mouse was lost to follow up on day 6, and another mouse was euthanized on day 4 by the laboratory for unrelated reasons. The surgical sites for both of these mice appeared to be healing normally at the last examination; however they were not assessed on day 7 after surgery (and therefore could not be considered successful). One mouse had a small reprolapse (diameter, less than 2 mm; 1 mm protrusion) of the rectal tissue on day 5 that did not warrant an additional repair surgery (see Discussion). The degree of prolapse in this mouse did not progress nor did the tissue become ulcerated or necrotic before the animal reached the study-related endpoint. Of the remaining 18 mice, 12 had successful repairs, whereas 6 developed surgical complications.

Complications. Two types of complications—lack of defecation and mutilation of the surgical site—were associated with this procedure. Both complications primarily occurred in the beginning of the study. Mutilation of the surgical site by cage mates occurred in 2 mice, both of which developed fistulas in the perianal area and were euthanized. This complication was eliminated by singly housing mice for 7 d after surgery. We also gave each mouse a cotton nesting square and shredded paper bedding as a means of distraction to minimize self-induced trauma to the surgery site. Even with the enrichment, one mouse disrupted the knot 1 d after surgery. However, the pursestring suture was not disrupted, there was no evidence of reprolapse in this animal, and no intervention was necessary. This mouse continued to do well, no reprolapse was noted before the study endpoint, and the surgery was considered successful.

Laboratory A had an unexpectedly high incidence of rectal prolapse within their colony, and 9 of their animals were included in this case series. Rectal prolapse was not an anticipated phenotype of their genetic manipulation. All of the mice in this colony were bred inhouse and were on a C57BL/6 background. Both transgenic mice as well as their wild-type litter-mates had a high incidence of rectal prolapse. Specifically, 5 of 9 mice from laboratory A that had rectal prolapse repair were euthanized due to complications from self-trauma or fecal impaction. Neither self-trauma nor fecal impaction was seen in animals from any other colony or investigator. Although the mice were bright, alert, and responsive initially, they became lethargic with distended abdomens when no defecation was noted by 3 d after surgery. On necropsy, the rectum and colon appeared normal without evidence of tear or stricture, but fecal impaction with severe distension of the cecum was present (Figure 3). According to the laboratory staff, the genetic manipulation was not expected to cause any phenotype-related pathology in the large intestinal tract or rectum that would interfere with defecation. We initially approached this problem by providing subcutaneous fluids and multimodal analgesia (buprenorphine 0.1 mg/kg SC and carprofen 5 mg/kg SC) to address potential dehydration and pain, but this approach was ineffective. We subsequently released the pursestring by cutting the knot, leaving the rest of the suture in place. This treatment led to immediate defecation with no further complications.

Discussion

In this case series, we described the surgical repair and postoperative care of mice with rectal prolapse. The rectal prolapse was corrected by placing a pursestring suture. Although we observed complications including trauma (self-induced or from other cage mates) and lack of defecation during the initial stages of method development, they were eliminated by singly housing mice and providing enrichment postoperatively and by releasing the pursestring when clinically indicated.

Rectal prolapse is a common clinical problem in mice. This high incidence is thought to be due to the very short rectum in this species, which predisposes mice to this condition.[12,13] It may occur spontaneously from certain conditions such as aging,

Table 1. Mice included in this case series

Mouse	Lab	Sex	Approximate age of prolapse	Background strain	Genetic manipulation	Outcome after rectal prolapse repair
1	A	M	L, Pa	C57BL/6	RGS2 KO	Euthanized due to no defecation
2	A	M	S, R.	C57BL/6	RGS2 KO	Success
3	1	F	L, R B.	C57BL/6	Unknown	Euthanized due to fistula
4	1	M	Med, Pa, B	C57BL/6	HIF2 KO	Success
5	2	M	Med, Pa, B	B6.129	RASA mutant	Excluded from the study
6	3	M	Med, R	C57BL/6	WT	Lost to follow-up (day 4)
7	1	M	Med, P	129	VHL, HIF2α, Apc KO	Lost to follow-up (day 6)
8	1	M	Med, R	129	VHL, HIF2α, Apc KO	Relapse (minor)
9	4	F	L, Pa	Unknown (albino)	Unknown (donated to the training core)	Success
10	A	M	Med, R/Pi,	C57BL/6	WT	Success
11	5	F	Med, Pa, D	BALB/c	Unknown (donated to the training core)	Success
12	6	M	S, Pa, D	C57BL/6	RIP–Cre	Success
13	7	M	L, Pi	C57BL/6	WT	Success (chewed knot)
14	A	M	L, R/Pi	C57BL/6	RSG2 KO	Euthanized due to fistula and no defecation
15	A	M	Med, Pa	C57BL/6	RSG2 KO	Euthanized due to fistula
16	A	M	Med, R, B	C57BL/6	Unknown	Success
17	A	M	S, Pa	C57BL/6	WT	Euthanized due to no defecation
18	A	M	S, Pa	C57BL/6	Unknown	Euthanized due to no defecation
19	8	M	Med, Pa	B6.129	Sff–GFP	Success
20	A	M	Med, Pi	C57BL/6	Gai2 conditional	Success
21	A	M	L, Pi	C57BL/6	WT	Success (with suture release)
22	9	M	Med, Pa	C57BL/6	PGDFRα KO conditional in astrocytes[b]	Success

F, female; KO, knockout; M, male; WT, wild type; L, large (>4mm diameter, >3mm protrusion); Med, medium (>3mm diameter, >2mm protrusion); S, small (>2mm diameter, >1mm protrusion); Pa, pale; Pi, pink; R, red; B, bleeding; D, dry.
[a]For mice nos. 1 through 8, optimized postoperative care (single housing and extra enrichment) had not yet been initiated.
[b]From references 16 and 17.

pregnancy, abdominal masses, or diarrhea.[13] It can also be seen in some transgenic mice,[14,15] or in mice infected with pathogens such as *Helicobacter hepaticus*, *Citrobacter rodenticum*, or heavy burden of pinworms.[6,13] Rectal prolapse can also be a complication in colitis models using DSS[2] and in colorectal cancer models.[3,13]

The rectal prolapse repair procedure described in this case series is a straightforward procedure that can be performed by veterinary technicians with basic suturing and tissue-handling skills. Each procedure typically lasted less than 10 min, and mice recovered from anesthesia very quickly. It is helpful to have 2 operators when performing this procedure: a surgeon to place a purse string suture, and an assistant to apply constant gentle pressure on the cotton swab to keep the prolapsed tissue replaced. Postoperative care was minimally labor-intensive, given that most cases required only the confirmation of fecal output and examination of the surgical site. Even cases that required suture release were minimally labor-intensive, because no additional anesthesia was needed. In cases with no complications, postoperative care required approximately 15 min of veterinary technician (or veterinarian) time over the course of the 7-d postoperative period.

Although the surgical procedure itself was simple and straightforward, several interventions were investigated to address complications and establish an effective postoperative management plan. Most of the complications occurred in the first 8 cases, and with the exception of laboratory A, the success rate of this procedure rose to 100% after instituting individual housing and providing cotton nesting squares and shredded-paper bedding. Because all of the mice in laboratory A were on a C57BL/6 background and because both wild-type and transgenic mice were affected, we initially hypothesized that mice on a C57BL/6 background were more sensitive to the pain associated with the surgical procedure and thus refused to defecate postoperatively. When compared with other inbred strains, C57BL/6 mice have been shown to be one of the most sensitive strains in terms of thermal nociception, but they demonstrate a variable pain response to chemical and mechanical stimuli.[9,10] In addition, C57BL/6 mice are one of the least sensitive strains to opioids.[10] This characteristic suggests that our choice of buprenorphine as a secondary analgesic agent was not an optimal choice to address pain in these mice, and we still cannot rule out unalleviated postoperative pain in these mice. However, we performed the same procedure by using carprofen and EMLA cream as the only analgesic agents on 4 C57BL/6 mice from different laboratories and saw no complications. This outcome may be explained by the different pain sensitivities displayed by various C57BL/6 substrains.[1] We concluded that the complications seen in mice from the laboratory A likely were associated with either the substrain maintained by laboratory A or their specific genetic manipulation.

In addition, 2 of the mice belonging to laboratory 1 had genetic manipulations involving HIF2α and the *VHL* and *Apc* genes. The manipulation of *Apc* genes in mice has been associated with a high incidence of rectal prolapse.[4,7] Although rectal prolapse was not listed as an expected phenotype, it is possible

Figure 1. Description of the rectal prolapse repair procedure. (A) Padding from the cotton swab was removed with hemostats until the tip fit smoothly into the rectum without damaging mucosa. (B). Lubricant was applied to the tip of the cotton swab. (C) The mouse was anesthetized and received a preoperative analgesic. After the perianal area was flushed with saline, the cotton swab was inserted gently. (D) A pursestring suture was placed as close to the rectum as possible without penetrating the mucosa. (E) Placement of the first throw. (F) Four throws were placed, and the suture was tied by using 4 single square knots. (G) The cotton swab was removed gently as the skin around the anus was pinched closed and pressure was applied toward the body. (H) Immediate postoperative photograph. After recovering from anesthesia, each mouse was singly housed in a clean cage so that fecal output could be monitored.

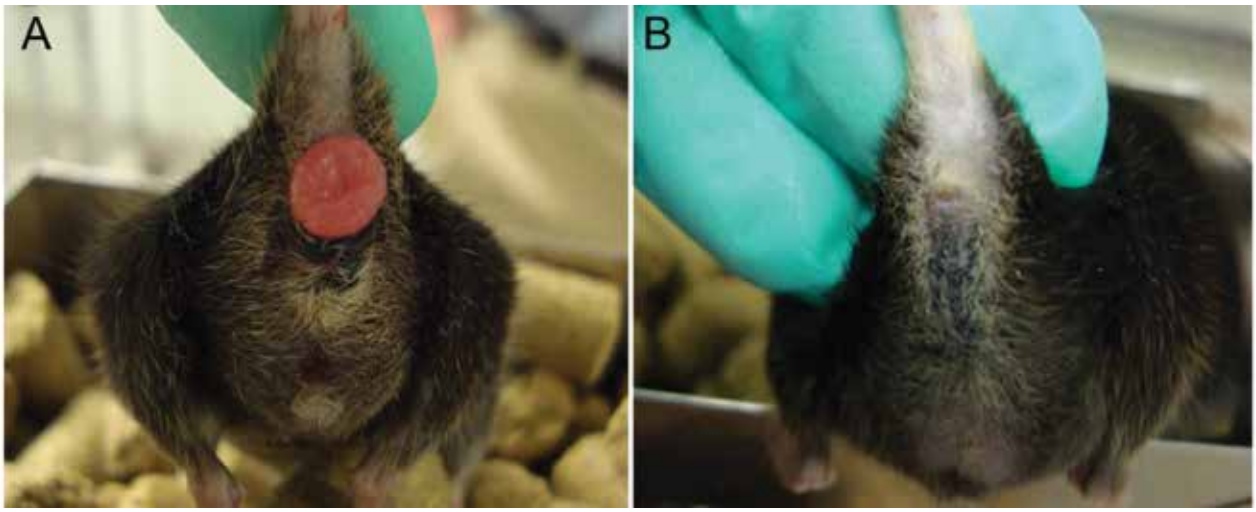

Figure 2. (A) Preoperative photograph of a mouse with a large, pink prolapse. (B) The same mouse 16 d after surgery, with no evidence of reprolapse.

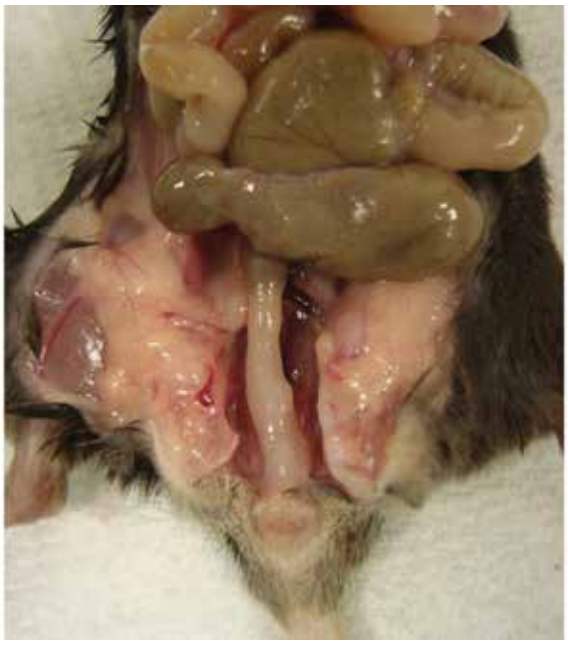

Figure 3. An example of a surgical complication. No defecation was noted, and the mouse was euthanized 3 d after surgery, due to lethargy and distended abdomen. Necropsy showed a distended cecum with a large amount of feces. The rectum appeared normal, with no evidence of stricture or fistula formation.

that this pathology was an experimentally related condition. The efficacy of the procedure for these particular mice, or mice with *Apc* mutation in general, is inconclusive given that these mice presented at the very early stages of this study (when the surgical procedure and postoperative care measures were still being refined) and the fact that they did not all complete the 7-d postoperative evaluation (1 of the 2 mice was lost to follow up). However, with the refinements of the postoperative care, surgical repair may be beneficial for mice with clinical rectal prolapses due to infections or experimental manipulations.

In addition to substrain-specific caveats regarding outcome, there are other considerations when performing rectal prolapse repair in mice. In our study, mice with acute prolapses had the best prognosis, given that prolapses older than 24 h were difficult to reduce (unless the prolapse occurred intermittently). We attempted to reduce a very small prolapse (diameter, less than 2 mm; protrusion, 1 mm protrusion) in a number of mice, and felt that this was very damaging to the rectal mucosa. However we found that such prolapses typically did not ulcerate or become necrotic in the absence of additional treatment. Therefore, surgery on small prolapses likely causes more damage to the mucosa than does simply monitoring the site. In addition, in our experience, manual reduction alone was insufficient to correct rectal prolapse. Most of the mice had prolapses of significant size (diameter, 4 to 6 mm; protrusion, 2 to 3 mm), and reprolapse occurred almost immediately after initial replacement of the prolapsed tissue as soon as the pressure on the tip of the cotton swab was released. Therefore, we did not formally compare the 2 techniques (manual reduction alone compared with manual reduction and pursestring). In addition, the efficacy of a prophylactic pursestring suture placement is unknown. One laboratory requested prophylactic placement of pursestring sutures because of a high incidence of rectal prolapse during their study, which involved *Helicobacter* infection. We placed pursestrings prophylactically in 2 mice, but both sutures were removed the day after placement because of the lack of defecation. Those 2 mice defecated immediately after suture removal, and no additional prophylactic procedures were performed.

This case report describes the defining criteria, surgical method, and appropriate postoperative care for mice with rectal prolapse. After successful procedures, mice were bright, alert, and responsive with no evidence of dyschezia or reprolapse. The procedure is suitable for mice that are systemically healthy and in which prolapsed rectal tissue is viable. Surgical repair is a viable treatment option that can refine the current standard of care for mice with rectal prolapse and minimize the loss of otherwise healthy animals.

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