

The Use of Porcine Small Intestinal Submucosa as a Biomaterial for Perineal Herniorrhaphy in the Dog

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Objectives—To develop an in vivo perineal hernia model, to develop a technique for using small intestinal submucosa (SIS) in perineal hernia repair, to further elucidate the biological behavior of SIS, and to compare SIS herniorrhaphy with the internal obturator muscle transposition (IOT) technique.

Study Design—Prospective evaluation comparing SIS herniorrhaphy with IOT.

Animals—Twelve adult castrated male, large-breed dogs.

Methods—All dogs had bilateral pelvic diaphragm defects created by complete excision of the levator ani muscle. Each dog had one side repaired using SIS and the other by IOT. Pain and inflammation were subjectively scored. Dogs were killed 2 weeks ($n = 4$), 12 weeks ($n = 4$), or 16 weeks ($n = 4$) after surgery. Each pelvic diaphragm was biomechanically tested to failure. The pelvic diaphragms from 2 normal dogs ($n = 4$ sides) were also biomechanically tested. Failure site, maximum pressure, displacement at failure, and initial linear stiffness values were determined. Histologic assessment was performed. Statistical analysis was performed with significance set at $P < .05$.

Results—No significant postoperative complications were noted. There were no significant differences in maximum pressure to failure, displacement, or stiffness when comparing normal, SIS, and IOT at any time point. The SIS group had significantly less displacement ($P = .004$) at 2 weeks than at weeks 12 or 16. For all herniorrhaphy techniques, the failure site was central ($n = 22$) or at the suture line ($n = 2$). At 2 weeks, histologic evaluation of tissues from the IOT group showed inflammation, mineralization, and necrosis, which were not present in tissues from the SIS group. Histologic examination at 12 and 16 weeks showed no microscopic differences in cell population or tissue characteristics between the IOT and SIS groups.

Conclusions—SIS herniorrhaphy was successfully performed in this in vivo model of perineal hernia in the dog.

Clinical Relevance—This study suggests that SIS can be used as a primary means of repair, as augmentation when the internal obturator muscle is thin and friable, or as a salvage procedure in cases of recurrence in dogs with perineal hernia.

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PERINEAL HERNIA has been described as a failure of the supporting structures of the pelvic outlet that results in an inability of the pelvic diaphragm to contain the pelvic organs.¹ The defect in the

pelvic diaphragm is thought to develop as a result of weakening of the muscles of the pelvic diaphragm, with the levator ani muscle most commonly affected.^{2,3} The cause of perineal hernia has yet to be

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defined. However, several theories have been proposed including tenesmus secondary to prostatomegaly or chronic constipation, imbalances of gonadal hormone concentrations, and neurogenic atrophy of the levator ani muscle.⁴⁻¹¹ Several techniques for the surgical treatment of perineal hernia have been described, including direct apposition of surrounding tissue with nonabsorbable sutures, internal obturator transposition, superficial gluteal muscle transposition, semitendinosus muscle flap, synthetic mesh implants, and porcine dermal collagen implants.^{3,5,9,12-18} The internal obturator transposition (IOT) is the most commonly recommended technique. Technique complications include sciatic nerve injury, rectal prolapse, fecal incontinence, wound infection, wound abscess, urinary incontinence, and failure with recurrence of the hernia.^{3, 9,12,18-21}

An ideal material for hernia repair has been described as one that is inexpensive, is technically easy to use, promotes host tissue ingrowth, results in a healed repair with strength equal to normal tissue, provides resistance to infection, elicits no enhanced inflammatory response, and inhibits adhesion and fistula formation.²² Porcine small intestinal submucosa (SIS) has been reported to be biocompatible and resistant to infection, to possess predictable mechanical properties before implantation, and to induce a response that results in regeneration of site specific tissues.²³⁻²⁶ Recent studies have evaluated the use of SIS for arterial or venous grafts, urinary bladder augmentation, and repair of abdominal wall hernias.^{22,23,27,28} The SIS graft permits vascular and tissue ingrowth that is morphologically similar to that of the host tissues.^{22,23,27,28} The surgical alternative that we investigated was the use of a new biomaterial with known tissue-inductive and conductive properties.²²⁻²⁸

The objectives of this study were to develop an *in vivo* model for the study of perineal hernia in dogs, to develop a technique for the use of SIS in perineal hernia repair, to further elucidate the biological behavior of SIS, and to compare SIS herniorrhaphy to the IOT technique. The IOT was chosen because it is generally regarded as the standard of care technique for the treatment of perineal hernia in the dog.

METHODS

Animals

The study protocol was approved by the University of Missouri Animal Care and Use Committee. Twelve healthy,

conditioned, castrated male research mongrel dogs weighing 19 to 28.4 kg were studied. Before inclusion in the study, complete physical examinations, complete blood counts, serum biochemical profiles, and urinalyses were performed. Two weight-matched, healthy research mongrel dogs killed for reasons unrelated to this study were used as control subjects.

Surgical Procedure

Dogs were anesthetized in random order by premedication with xylazine (0.5 mg/kg) and morphine (0.5 mg/kg) intramuscularly and then induced with 10 to 20 mg/kg intravenous (IV) thiopental administered to effect. Dogs were intubated orotracheally, and anesthesia was maintained with isoflurane in oxygen. Esophageal temperature, pulse rate, and blood pressure were monitored during anesthesia. The perineum was clipped bilaterally and was aseptically prepared. The tail was positioned over the back, and the dogs were positioned in sternal recumbency with the pelvis elevated. All surgical procedures were performed by the same investigator (M.R.S.).

A curvilinear skin incision was made 1 to 2 cm lateral to the anus, beginning at the base of the tail and extending 1 to 2 cm ventral to the ischium. The perineal fascia was incised. Once the pelvic diaphragm was observed, the levator ani muscle was identified and completely excised to create the pelvic diaphragm defect.

The side and order in which each herniorrhaphy technique (SIS or IOT) was performed were randomized for each dog. Each dog had one side repaired by IOT and the other side repaired by the SIS technique. The randomization of technique was such that each technique was performed in each dog with equal distribution between right and left sides.

SIS Herniorrhaphy

A 4-ply SIS sheet (Cook Biotech Inc., West Lafayette, IN) was trimmed to dimensions slightly larger than the pelvic diaphragm defect and then rehydrated in sterile saline (0.9% NaCl) solution. Sutures were pre-placed in a horizontal mattress pattern using 2-0 polydioxanone through the SIS and into the pelvic diaphragm muscles as described below, leaving a 5- to 10-mm edge on the SIS. Three to four sutures were placed from the SIS to the coccygeus muscle, 3 to 4 from the SIS to the internal obturator muscle, and 3 to 4 from the SIS to the external anal sphincter muscle. After all sutures were placed, they were tied, securing the SIS to the pelvic diaphragm muscles (Fig 1). If a defect was present dorsally, an additional interrupted suture was placed from the coccygeus muscle to the external anal sphincter muscle.

The surgical site was lavaged with sterile saline solution,

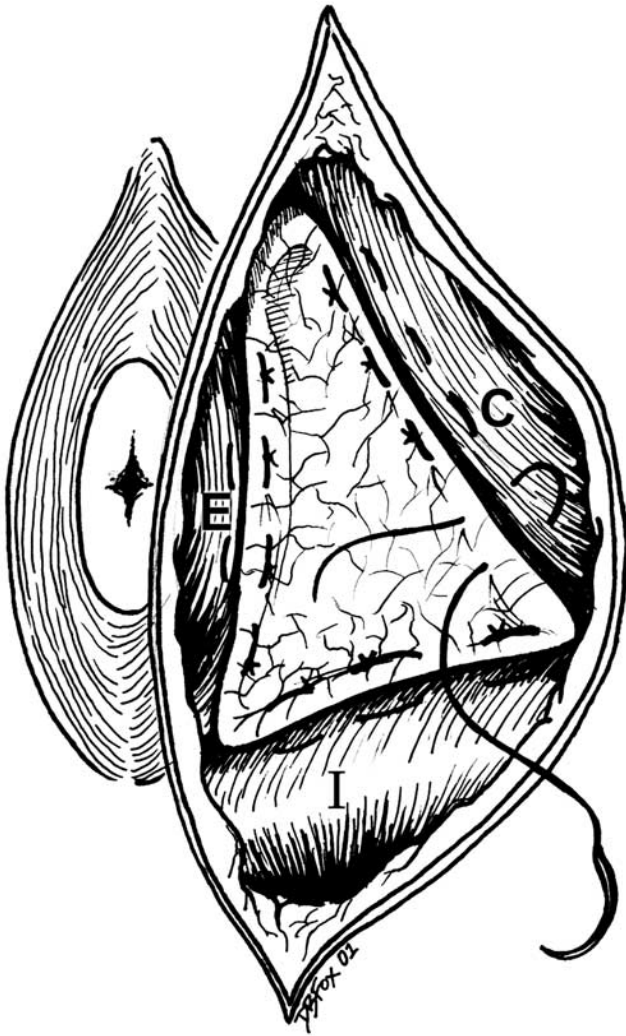


Fig 1. The SIS herniorrhaphy technique. E, external anal sphincter; C, coccygeus muscle; I, internal obturator muscle. SIS sutured into the defect using multiple horizontal mattress sutures.

and the deep subcutaneous tissue was apposed using 3-0 polydioxanone in a simple continuous pattern. The superficial subcutaneous tissue (perineal fascia) was closed in a simple continuous pattern with 3-0 polydioxanone. The skin was apposed using 3-0 nylon in a simple interrupted pattern. Total surgical time including creation of the pelvic diaphragm defect and herniorrhaphy was recorded for each side of each dog beginning with the initial skin incision until the last skin suture was placed.

Internal Obturator Transposition

The internal obturator muscle transposition was performed as described by Orsher,¹⁷ using 2-0 polydioxanone suture. Closure of the surgical wound was the same as that described for the SIS technique.

Postoperative Care

All dogs were fed a maintenance diet, and no stool softeners were added. Morphine (0.25 mg/kg IV and 0.25 mg/kg subcutaneously) was administered every 6 hours for 24 hours postoperatively. Each dog was confined to a kennel with minimal activity for 10 days after surgery. Each dog was assessed daily for general pain, inflammation at each surgical site, and any postoperative complications for the first 14 days after surgery. Skin sutures were removed at 14 days.

Pain Assessment

Pain assessment was based on a subjective scoring system established by the investigators before initiation of the study. Scores were assigned by one observer based on the dogs' willingness to ambulate and sit, vocalization, and physiological variables (aural temperature, pulse rate, and respiratory rate). Scoring was based on a 0 to 3 scale, with 0 = no pain, 1 = mild pain, 2 = moderate pain, and 3 = severe pain.

Inflammation Score

Assessment of inflammation was based on a similar subjective scoring system that was determined by the investigators before the onset of the study. Each incision site on each dog was assessed by one observer for redness and swelling and given a score of 0 to 3 of the perineum. A score of 0 was assigned for no redness or swelling; 1 for mild redness or swelling; 2 for moderate redness or swelling; and 3 for severe redness or swelling. The observer was unaware as to which repair technique was used.

Specimen Preparation

The dogs were randomly selected to be euthanatized using IV pentobarbital (Beuthanasia-D; Schering-Plough Animal Health, Union, NJ) at one of 3 time points: 2 weeks ($n = 4$), 12 weeks ($n = 4$), or 16 weeks ($n = 4$) after surgery. After euthanasia, the cadavers were transected cranial to the ilium, and the caudal portion of the cadaver was frozen at -80°C for subsequent biomechanical testing and histologic evaluation.

Biomechanical Testing

A group of 3 to 4 specimens were thawed to room temperature and biomechanically tested during a 12-hour period. A curvilinear incision was made in the skin over both sides of the perineum, creating a skin flap, to expose the pelvic diaphragm so that the plunger tip with a 1-inch diameter suction cup of the linear variable differential transformer (LVDT) could be directly placed on the pelvic

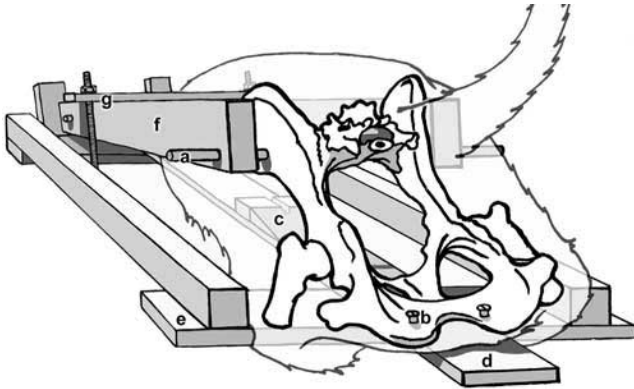


Fig 2. Drawing of the holding apparatus used to perform biomechanical testing. (A) Metal rod placed transileally, incorporating the sacrum. (B) Deck screws in the ischial plateau are securing the pelvis to the holding apparatus. (C) Rostral restraining wedge with a groove in the wood to restrain the air hose. Foam triangle used to conform the pelvis, constraining the pelvic cavity. (D) Base board. (E) Holding board. (F) Holding apparatus. (G) Securing clamp.

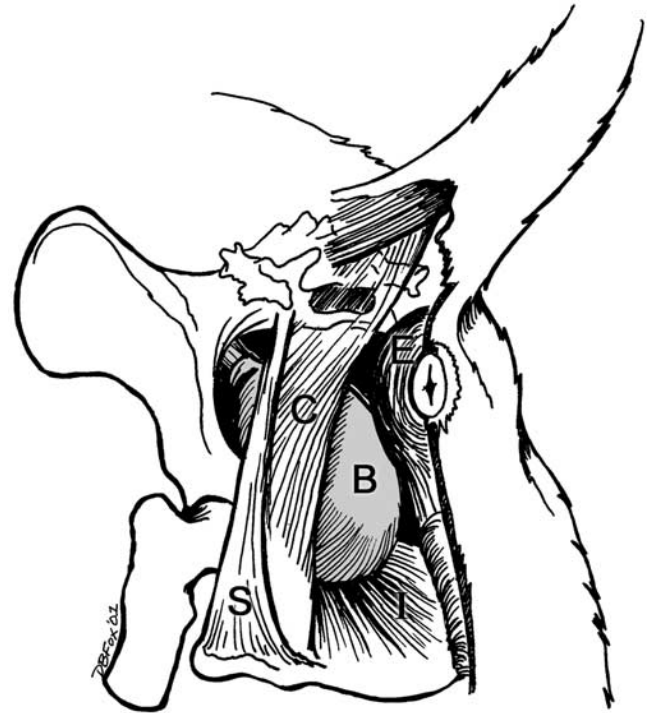


Fig 3. Illustration of the balloon placed within the pelvic diaphragm. The levator ani is absent to allow identification of the balloon within the pelvic diaphragm. E, external anal sphincter; C, coccygeus muscle; I, internal obturator muscle; S, sacrotuberous ligament.

diaphragm. The LVDT allowed for direct measurement of the lateral displacement of each diaphragm. A 5/16-inch (7.94 mm) diameter hole was drilled transileally with a power drill bit, incorporating the sacrum, through which a pointed 5/16-inch steel rod was driven. The holding apparatus (Fig 2) was mounted onto the 5/16-inch rod. A 16-inch latex balloon secured to the end of an air supply hose was pre-placed into the pelvic canal (Fig 3). The pelvis was then centered over the base board and aligned with the proximal one third of the ilium over the restraining wedge (Fig 2). The sides of the holding apparatus were then clamped to the base board to squeeze the cranial end of the specimen against the cranial restraining wedge. Deck screws (3.75 to 5 inches long as required by dog size) were power driven, one through each side of the ischial plateau, and into the 2 × 6-inch base board to confine the pelvic cavity and secure the caudal end of the specimen (Fig 2). The rectal tissue was everted, tied with 1/4-inch umbilical tape and clamped with Kelly forceps to prevent the balloon from protruding through the anus. An LVDT was positioned on each side of the pelvic diaphragm, perpendicular to the tissue to be tested, to measure lateral displacement of the tissue (Fig 4). As the tissue displaced, the LVDTs could potentially move away from the test area. To prevent cranial and caudal movement, but still allow lateral movement, 2 loops of 1/4-inch umbilical tape were placed around each LVDT. The umbilical tape was placed around the holding board and perpendicular to the LVDTs, with one placed cranial to caudal and the second caudal to cranial so that they did not restrain plunger movement.

Figure 5 is a schematic representation of the balloon pressurization system. Applied pressure and right and left

perineal tissue displacement data were obtained at 100 Hz. To set the air flow rate, the flow control valve was adjusted during initial tests on trial specimens so that pressure

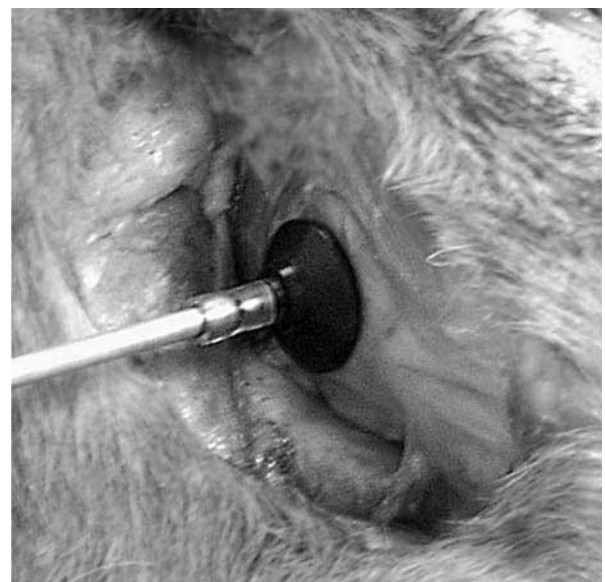


Fig 4. LVDT in place on the pelvic diaphragm to measure its lateral displacement.

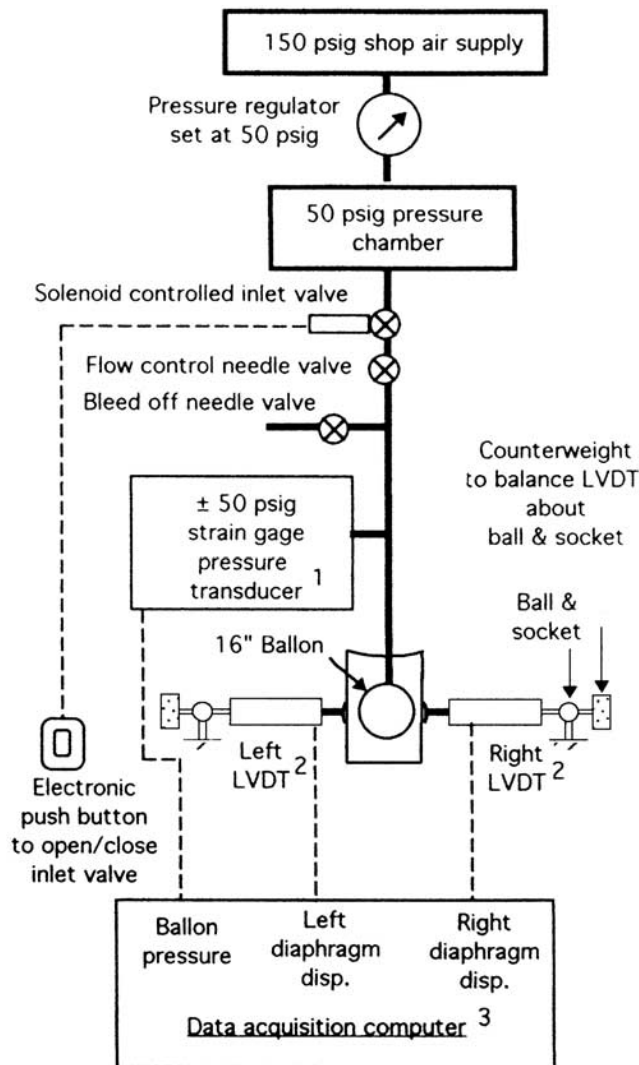


Fig 5. Schematic of the overall experimental setup. Equipment used: (1) pressure transducer: PM280TC-350; Statham Strain Gage Conditioner 3270, Dayton, OH. (2) Displacement LVDT; 3002 XS-D, Schaevitz Signal Conditioner ATA-101, Schaevitz, Hampton, VA. (3) DAQ board; AT-MIO-16XE-50, National Instruments DAQ software, Lab-View, National Instruments, Austin, TX.

increases from 0 to 10 psi (0 to 69 kPa) occurred in 24.6 ± 3.4 seconds. The flow control valve was not changed thereafter.

After mounting the specimen, the video recording and data acquisition systems were started, and shortly thereafter the inlet pressure valve solenoid was manually actuated. Pressurization was continued until ultimate failure of the diaphragm occurred on one side. Failure was defined as a visual defect in the pelvic diaphragm (Fig 6), which was accompanied by a sudden reduction in pressure (Fig 7) as the balloon rapidly expanded in a semi-constrained fashion through the failure site. The site of failure was visually

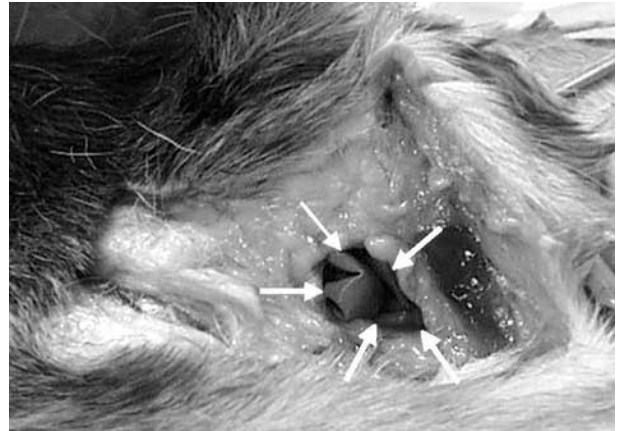


Fig 6. Failure of the repair, centrally located in the SIS herniorrhaphy. Note the balloon within the failure site (arrows).

evaluated and recorded as either central, dorsal, ventral, or at the suture line. To complete data acquisition on the side that remained intact, the rectal restraint was removed, the air hose with the old balloon was carefully exteriorized through the rectum to allow a new balloon to be secured to the end of the air hose, and the air hose was pulled cranially to reposition the new balloon within the pelvic canal. The rectum was again secured as previously described. Surgical gauze (4 × 4 inch) was placed in the failure site, and the skin flap was closed over the site using 2 polypropylene in a Ford-interlocking pattern. A 2 × 2-inch board was used as a lever arm to force a laterally protruding 3/4-inch diameter dowel on which a 3-inch diameter hard rubber squeeze bulb was mounted against the ruptured side to contain it as the new balloon was pressurized to test the second side (Fig 8). During some tests, the balloon protruded through areas other than the pelvic diaphragm. When this occurred, a new balloon was installed as previously described, the area was secured, and testing was repeated.

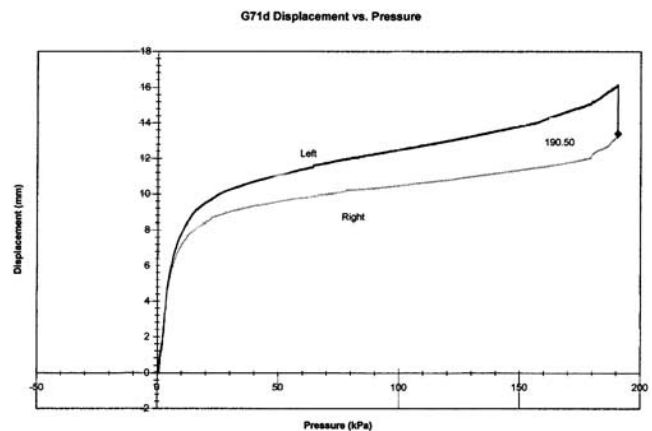


Fig 7. Representative displacement-pressure curve.



Fig 8. Counter pressure applied to the left side of the perineal region, enabling biomechanical testing of the contralateral side.

Two balloons were tested when not constrained by the pelvic cavity to determine their relative resistance to pressurization. The maximum pressure reached by both was 5.5 kPa followed by continued expansion at a slowly decreasing pressure.

A spreadsheet program was used to plot lateral displacement data as a function of applied pressure for each test. Maximum applied pressure and corresponding lateral displacement of the side that failed were recorded as ultimate failure pressure and displacement for that side (Fig 7). Typically, there was an initial rapid rate of displacement followed by relatively little displacement as pressure increased until abrupt failure. In general, the initial portion had a linear segment, the slope of which was calculated and its reciprocal taken as the initial stiffness in kPa/mm. The initial stiffness for each side during the first pressurization of a specimen was used to characterize and compare the initial resistance of the pelvic diaphragms. This was done to eliminate the influence of stretching that may have taken place during previous tests.

Histologic Evaluation

Once biomechanical testing was complete, the pelvic diaphragm was removed and the tissue was placed in 10% buffered formalin. Tissue samples were taken from the margin of the failure site, embedded in paraffin, sectioned at 5 μ m and stained with hematoxylin and eosin (H & E), and examined by a pathologist (J.M.K.) who was unaware of the dog number or treatment group. Histologic sections were

subjectively assessed for the cell and matrix composition and morphology of tissues comprising the herniorrhaphy site, the number and nature of inflammatory cells present within the tissues, and the amount and morphology of skeletal muscle within the tissues. Subjective assessments of each of these parameters were provided as written descriptions by the pathologist. Subjective comparisons between groups and among time points were then made by investigators (M.R.S., J.L.C.) based on the histologic descriptions provided.

Statistical Analysis

All statistical analyses were performed using a computer software program (SigmaStat; SPSS Inc., Chicago, IL). Data were recorded as mean (\pm SEM) and range. Surgical times for the SIS and IOT groups and for the first 12 procedures and last 12 procedures were compared using a paired *t* test. Pain and inflammation scores were compared at days 1, 3, and 7 using a one-way analysis of variance (ANOVA) and Dunn's method. Maximum pressure to failure, lateral displacement, and stiffness of all pelvic diaphragms on the left and right side were compared using a *t* test. Maximum pressure to failure, displacement, and stiffness of all measurements for SIS repair sides were then compared with those for IOT using a *t* test. Differences between SIS and IOT for each of these variables at individual time points (2, 12, 16 weeks) were also compared with the *t* test. Changes in maximal pressure to failure, displacement, and stiffness within each group at 2 weeks, 12 weeks, and 16 weeks were analyzed with a one-way ANOVA. Maximum pressure to failure, displacement, and stiffness of normal dogs were compared with operated dogs (includes all IOT and SIS) using a *t* test. Significance for all tests was set at $P < .05$.

RESULTS

General

All dogs survived the initial surgical procedure and the intended study period. No abnormalities were found on preoperative physical examinations, and the results of complete blood counts, urinalyses, and serum biochemical profiles were within reference ranges. Weights of the dogs ranged from 19 to 28.4 kg with a median of 24.7 kg and a mean of 24.7 (\pm 0.96).

Surgical Time

Mean (\pm SEM) surgical times were 36.3 (\pm 2.2) minutes (range, 24 to 55 minutes) for the SIS group and 44 (\pm 3.7) minutes (range, 23 to 65 minutes) for

the IOT group. There were no statistically significant differences in surgical times between the two surgical techniques. However, the surgical time for the SIS technique was less than the time for the IOT technique in 10 of 12 dogs. Surgical time for the first 12 (1 to 12) procedures ranged from 24 to 65 minutes with a mean $45.1 (\pm 3.7)$ minutes. Surgical time for procedures 13 to 24 ranged from 23 to 47 minutes with a mean $35.3 (\pm 1.9)$ minutes. When these two groups were compared, a decrease in surgical time with experience was demonstrated ($P = .027$).

Pain and Inflammation Scores

The mean pain scores for dogs was 1.17 ± 0.17 (day 1), 0.25 ± 0.13 (day 3), and 0 ± 0 (day 7). Pain scores were significantly greater on days 1 than on days 3 and 7 ($P < .001$).

The mean inflammation scores for the SIS side were 1.42 ± 0.15 (day 1), 0.83 ± 0.17 (day 3), and 0.17 ± 0.11 (day 7). The mean inflammation scores for the IOT side were 1.08 ± 0.15 (day 1), 0.83 ± 0.11 (day 3), and 0.17 ± 0.11 (day 7). There were no significant differences between groups at any time point. However, there were significant differences in inflammation scores within the SIS and IOT groups when comparing day 1 to day 7 ($P < .001$).

Postoperative Complications

Skin sutures were prematurely removed in 3 dogs (2 IOT and 1 SIS), and Elizabethan collars were subsequently placed on these dogs. No adverse effects to the surgical sites were noted. Skin and superficial subcutaneous dehiscence occurred in 1 dog from the SIS group on day 3 postoperatively because of self-mutilation. An Elizabethan collar was placed, and the wound was flushed with sterile saline solution twice daily and allowed to heal by second intention. No other complications were noted.

Biomechanical Testing

Twenty-three sides from 12 study dogs and 4 from 2 normal dogs were tested to ultimate failure. Complete failure was not achieved on one SIS repair where a thin membrane of tissue persisted. This specimen was assigned an ultimate failure pressure of 134.8 kPa, which was the maximum pressure reached during testing. Failure mode was established for all sites and occurred in the central portion of the repair by tissue

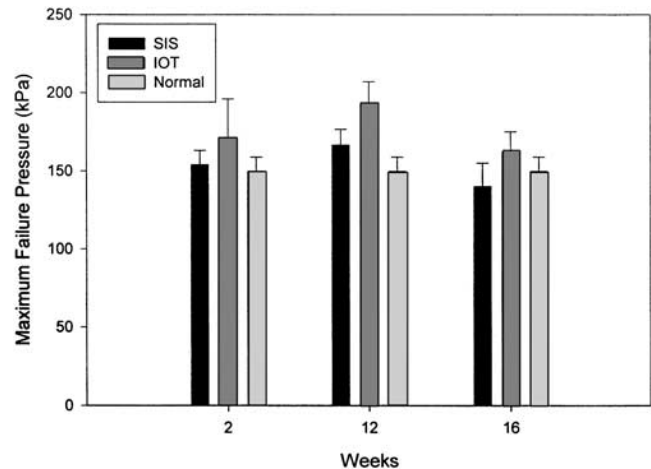


Fig 9. Mean (\pm SEM) of the maximum failure to pressure comparing normal pelvic diaphragms to that of the SIS herniorrhaphy and IOT herniorrhaphy.

disruption in 22 sites and at the suture line by suture failure in 2 sites, 1 SIS and 1 IOT. Failure occurred by central disruption of the levator ani muscle in all the normal pelvic diaphragms tested.

For MPF_{max} , there were no significant differences between SIS, IOT, and normal specimens (Fig 9, Table 1), nor was there a significant difference between pooled MPF_{max} data and the MPF_{max} for normal specimens. Likewise there were no significant temporal differences in MPF_{max} within groups. MPF_{max} of the right side of the pelvic diaphragm, including operated and normal dogs, was 169.61 ± 9.52 kPa (range, 107.37 to 190.50 kPa) and for the left side was $155.10 (\pm 6.51)$ kPa (range 99.19 to 192.19 kPa): These differences were not significant.

For mean displacement (maximum displacement at failure), there were no significant differences between SIS, IOT, and normal specimens. (Fig 10, Table 1). Pooled mean displacement data from operated dogs had significantly less displacement ($P = .027$) than that of the normal group. There were no significant temporal differences in mean displacement between groups or within the IOT group. However, mean displacement of SIS at 2 weeks was significantly ($P = .004$) less than that at weeks 12 and 16.

For initial mean stiffness, there were no significance differences between SIS, IOT, and normal specimens (Fig 11, Table 1), nor was there a significant difference between pooled initial mean stiffness data and the initial mean stiffness for normal dog specimens. Likewise, there were no significant temporal differences in initial mean stiffness within groups.

Table 1. Mean \pm SEM (range) Biomechanical Variables Measure on Canine Pelvic Diaphragm Specimens After Perineal Herniorrhaphy by Porcine Small Intestine Submucosa or Internal Obturator Muscle Transposition

Weeks After Surgery	Maximal Pressure to Failure (MPFmax), kPa			Maximum Displacement at Failure, mm			Initial Stiffness, kPa/mm		
	Normal n = 2	SIS n = 4	IOT n = 4	Normal n = 2	SIS n = 4	IOT n = 4	Normal n = 2	SIS n = 4	IOT n = 4
0	149.78 \pm 9.05 (124.80-168.77)			18.76 \pm 2.67 (15.10-21.50)			1.47 \pm 0.39 (0.74-2.55)		
2		154.03 \pm 9.25 (143.92-181.75)	171.97 \pm 25.82 (99.19-219.29)		7.81 \pm 1.25 (12.95-20.68)	7.24 \pm 2.53 (0.69-11.79)		5.07 \pm 1.31 (1.84-7.50)	5.30 \pm 1.62 (2.14-9.18)
12		166.56 \pm 10.67 (140.70-190.50)	193.04 \pm 14.64 (152.15-217.65)		13.86 \pm 1.27 (10.62-16.66)	14.36 \pm 4.69 (3.60-25.63)		2.43 \pm 0.66 (0.92-4.06)	1.18 \pm 0.18 (0.69-1.53)
16		139.46 \pm 15.77 (107.37-178.11)	162.96 \pm 12.85 (137.94-198.56)		16.74 \pm 1.59 (12.95-20.68)	10.07 \pm 2.60 (4.50-14.78)		2.88 \pm 0.49 (2.05-3.96)	3.58 \pm 0.70 (1.52-4.63)
Pooled data*		153.36 \pm 7.20 (107.37-190.50)	175.99 \pm 10.47 (99.19-219.29)		12.80 \pm 1.33 (4.93-20.68)	10.55 \pm 1.99 (0.69-25.63)		3.46 \pm 0.58 (0.92-7.50)	3.35 \pm 0.74 (0.69-9.18)

* Pooled data, confirmation of data from weeks 2, 12, and 16.

Histologic Findings

On evaluation of histologically prepared sections from SIS repairs 2 weeks after surgery, no SIS material could be identified. In both SIS and IOT groups, sections from the pelvic diaphragm contained a band of fibrous connective tissue at one margin with adjacent skeletal muscle. The fibrous band was subjectively wider on average in the IOT repairs. Histologic sections from the IOT repairs (Fig 12) consistently contained multifocal, randomly scattered aggregates of lymphocytes within the fibrous band, whereas the SIS sections (Fig 13) did not. Small clusters or individual myofibers were occasionally shrunken and eosinophilic in the IOT sections. Small foci of mineralization and necrosis were also present at

the fibrous tissue-muscle interface of the IOT group. Minimal or no inflammation was noted in the SIS group, and necrosis was not a feature. Some sections from the SIS repair samples contained areas with vascular invasion. One sample from each group contained several suture granulomas within the fibrous band.

At weeks 12 and 16, it was not possible to differentiate the two groups histologically. There was a narrow, dense band of fibrous connective tissue. The width, extent, and the apparent maturity of fibrous connective tissue bands were similar in both groups. The adjacent tissue had the histologic appearance of normal skeletal muscle with some small areas of adipose tissue.

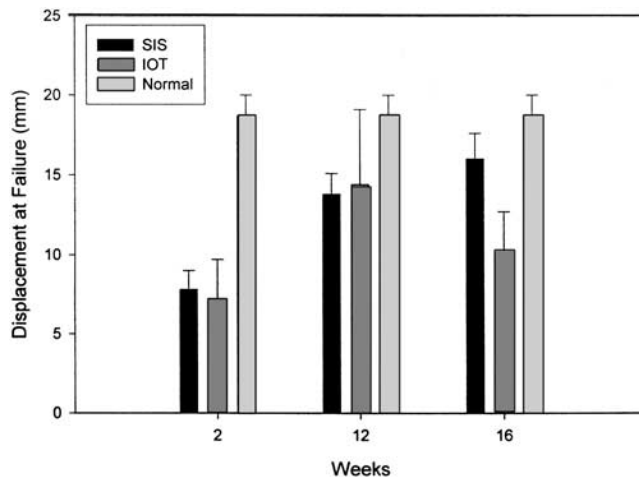


Fig 10. Mean (\pm SEM) of the lateral displacement at 2, 12, and 16 weeks comparing normal pelvic diaphragms to that of the SIS herniorrhaphy and IOT herniorrhaphy.

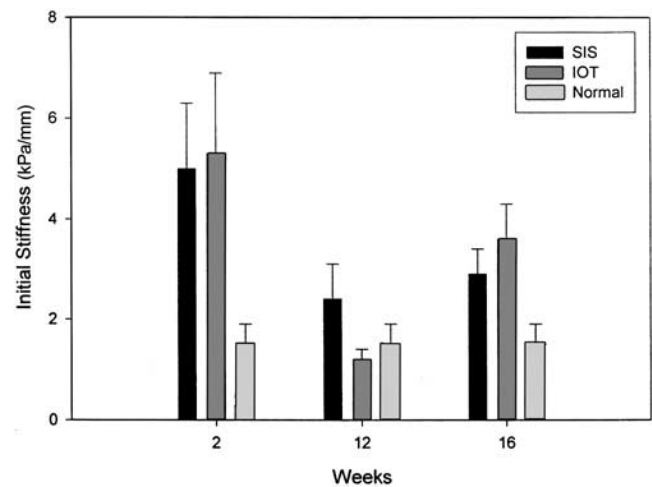


Fig 11. Mean (\pm SEM) of the initial stiffness at 2, 12, and 16 weeks comparing normal pelvic diaphragms to that of the SIS herniorrhaphy and IOT herniorrhaphy.

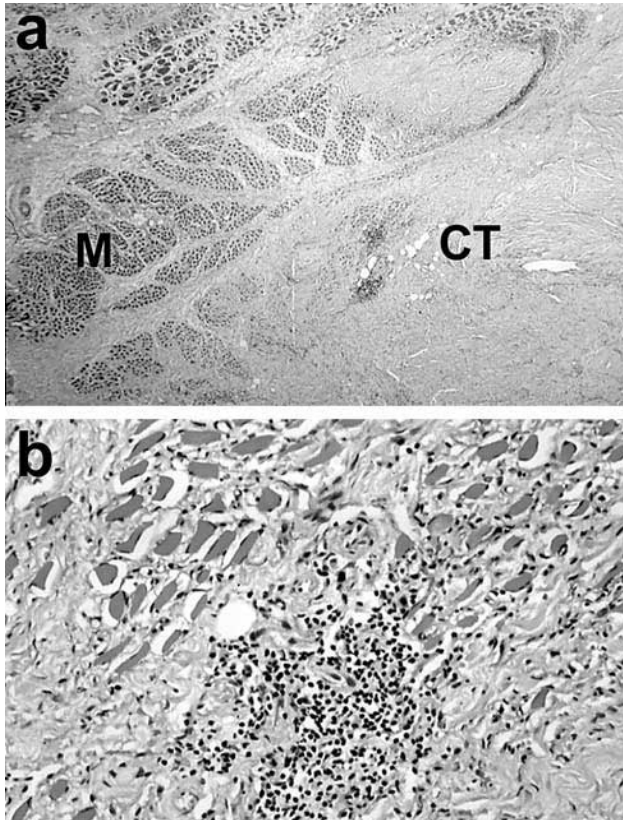


Fig 12. (A) Perineal hernia repair site using IOT at 2 weeks. There is a marked proliferation of fibrous connective tissue that extends into and separates bundles of skeletal muscle fibers (H & E, original magnification $\times 20$). (B) Higher magnification of the IOT repair site showing aggregates of mononuclear inflammatory cells, principally lymphocytes (H & E, original magnification $\times 20$). CT, connective tissue; M, muscle.

DISCUSSION

Perineal hernia is a common problem that most commonly affects middle-aged to older, intact male dogs.⁹ Reported complications for herniorrhaphy techniques are tenesmus (10% to 25%), sciatic nerve injury (<5%), rectal prolapse (2% to 13%), fecal incontinence (<10%), wound infection and abscess formation (6.4% to 26%), urinary incontinence, positional neuropraxia, rectocutaneous fistula, anal sacculitis, and recurrence (5% to 46%)^{3,12,18-21,29} Recurrence rates were found to decrease significantly with increasing experience of the surgeon.¹⁸ However, the need still exists for a technique that would provide superior results to the current techniques, augment current techniques, and serve as a salvage procedure for cases of recurrence.¹²

Porcine SIS is a biomaterial derived from the

jejunum of pigs that is used as a xenograft for vascular grafts, dura mater grafts, urinary bladder augmentation, Achille's tendon repair, abdominal wall herniorrhaphy, and other applications.^{22,23,25,27,28,30,33} There is no reported evidence of immunologic rejection of SIS in humans, dogs, or pigs.^{22-28,30-33} SIS is an acellular extracellular matrix that is primarily composed (90%) of type I collagen³⁴ and contains vascular endothelial growth factor (VEGF) and fibroblast growth factor.³⁵ These factors contribute to tissue synthesis and differentiation and may partially explain the site-specific structural and functional remodeling that occurs with SIS.²⁴

We chose to compare the SIS graft herniorrhaphy technique with the most commonly performed herniorrhaphy technique, the internal obturator muscle transposition. Our model, surgical excision of the

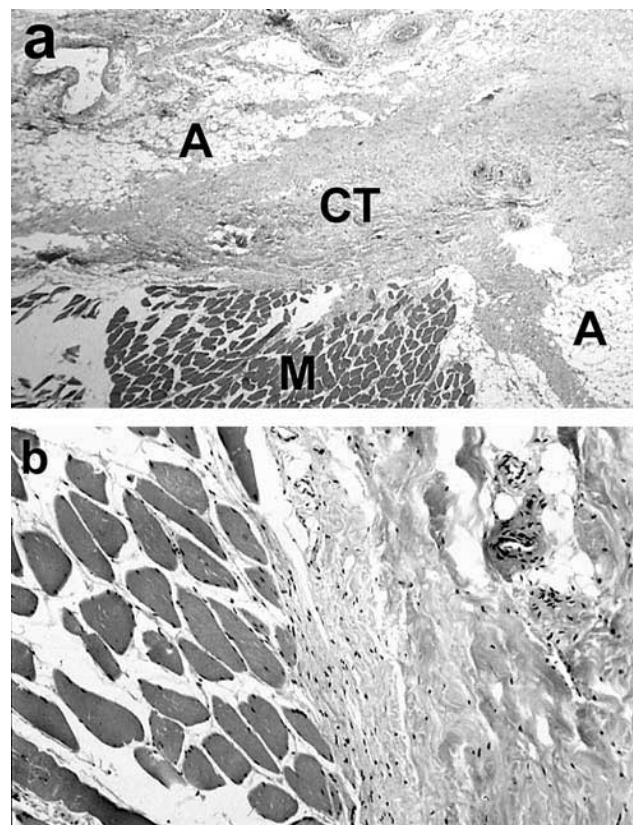


Fig 13. (A) Perineal hernia repair site using an SIS implant at 2 weeks. There is a narrow band of mature, collagenous connective tissue between the skeletal muscle and adjacent adipose tissue (H & E, original magnification $\times 20$). (B) Higher magnification of the SIS repair site. The zone of collagenous connective tissue is largely devoid of inflammation (H & E, original magnification $\times 20$). CT, connective tissue; M, muscle; A, adipose tissue.

levator ani muscle, was designed to simulate the naturally occurring disease and minimize confounding variables such as defect type, concurrent problems, disease duration, and extent of involvement. The model was designed to address the technical, biological, and biomechanical properties of the repair techniques, not those factors associated with the cause of this disorder. The biomechanical testing apparatus that we designed for this study allowed for complete occlusion of the pelvic cavity while securely holding the specimens in place, thus allowing for ideal testing of the pelvic diaphragm. The balloons used in the biomechanical testing allowed the pelvic diaphragm to be tested under extreme load by conforming to the contact area. This made it possible to immediately observe and identify the weak point or failure site in the pelvic diaphragm.

Strength of repair tissue is one of the most important properties to assess when determining the usefulness of a graft or implant. MPF_{\max} allowed identification of the pressure at which herniorrhaphy failed, thus providing an evaluation of the strength of the repair tissue. In this study, SIS herniorrhaphy was biomechanically as strong as internal obturator transposition herniorrhaphy and normal pelvic diaphragm. This provides evidence that 4-ply SIS can be used as a herniorrhaphy substrate in the dog.

Determining lateral displacement allowed for evaluation of the repair tissue's elastic properties. There were no significant differences in lateral displacement between the SIS and IOT groups at any time point. However, there was less displacement in operated dogs than in normal dogs. This is not an unexpected finding. Both the SIS and IOT groups had evidence of reparative fibrous connective tissue present that was not found within normal skeletal muscle. Although there were differences in the stiffness of the repair tissue, from clinical observation this did not affect the ability of these dogs to defecate normally postoperatively.

Lateral displacement of SIS herniorrhaphies was less at 2 weeks than at the other time points. These data appear relevant with respect to tissue ingrowth and remodeling associated with SIS grafts. Histologically, there was evidence of neovascularization, cell infiltration, and fibroplasia associated with the SIS grafts at 2 weeks after implantation. At 12 and 16 weeks after SIS implantation, tissue at the graft site contained increasing amounts of skeletal muscle. These findings are consistent with previous studies

using SIS grafts in that early tissue and vascular ingrowth is followed by remodeling, replacement, or regeneration such that the reparative tissue possesses morphological and functional features that closely resemble those of the host tissue.^{22-28,30} These biomechanical and histologic findings are important for understanding the temporal nature of SIS-induced tissue repair and the resultant implications with respect to clinical indications, postoperative management, and expected outcomes.

Tissue stiffness is an evaluation of a tissue's stress-strain characteristics. Stiffness in this study was reported in terms of pressure (kPa) over displacement (mm). Pressure-displacement curves had two linear segments, one that occurred between 0 to 15 kPa and the other between 60 to 100 kPa (Fig 7). The initial linear segment was evaluated so that the initial stiffness could be calculated. There were no significant differences detected between the SIS, IOT, or normal groups. The stiffness associated with the second linear segment would determine the characteristics of the stretched tissue.

Histologically, the lack of identifiable SIS at 12 and 16 weeks postoperatively is consistent with reports of SIS used for Achille's tendon repair.³⁰ The vascular and connective tissue response and the temporal disappearance of SIS are consistent findings in other studies where SIS was used.^{22-28,30} The IOT group had an inflammatory response at 2 weeks that was not present in the SIS group. In addition to inflammation, areas of necrosis were identified in the IOT group that were not present in the SIS group. These areas of inflammation and necrosis could be associated with areas of suture placement and result from disruption of the vascular supply to the muscle flap or could be a consequence of the larger tissue volume that required revascularization when compared with the SIS repair. In addition, SIS has been shown to induce an immunologic response of accommodation without signs of rejection because it is an acellular matrix.^{23,27} Histologically, the SIS repairs consistently had areas that were subjectively more vascular at 2 weeks than the IOT repairs. However, this neovascularization response did not appear to be as prominent as that reported for other applications of SIS.^{28,30,31} The suture granulomas present in one sample from each group were small and not considered clinically important. Histologic differences noted at 2 weeks may be of minimal consequence as histologic findings in both groups were indistinguishable at 12 and 16 weeks.

SIS was technically easy to handle and implant. SIS is available in a variety of forms, including single and multilaminar sheets. We chose to use the 4-ply SIS based on our clinical experience and other studies that have evaluated body wall defects.²⁸ Cutting the SIS to shape before rehydration makes handling much easier. Because the SIS is cut to fit the defect, it allowed closure of the dorsal aspect of the defect that can be difficult to achieve in some clinical cases using an internal obturator flap alone. The SIS procedure was no more difficult to perform than the IOT. In fact, the authors believe that it was less difficult than the internal obturator transposition. Although the reported surgical times for SIS were not significantly shorter than the IOT, the surgery time for SIS was less in all but 2 dogs. Surgical time for herniorrhaphy may have clinical importance, especially when performing bilateral repairs or repairs in compromised patients. In addition to the benefits elucidated in this study, the SIS technique avoids donor site morbidity associated with muscle flap herniorrhaphy techniques.

SIS has many advantages over other substrates that have been used for herniorrhaphies, including the lack of residual implant material and decreased infection rates.³¹ Substrates such as synthetic meshes have been used for perineal hernia repair in humans.³⁶ However, introducing a prosthetic implant increases the risks of infection, excessive granulation tissue, and seroma formation.³⁷ Such complications were not noted in this study. There was no evidence of excessive granulation, seroma, or bacterial infection clinically or histologically in any of our SIS repairs. SIS appears to offer defense mechanisms against infection that are believed to be secondary to early vascularization and delivery of host defenses to local tissues.²⁶

Results from our study indicated that 4-ply SIS maintained adequate strength and stiffness while allowing and encouraging appropriate repair tissue ingrowth in this experimental model with no signs of inflammation or rejection. This suggests that SIS could be used in the primary repair of perineal hernia, as an augmentation in cases where the internal obturator muscle is thin and friable, and as a salvage procedure in cases of recurrence in the dog.

This study provided evidence that 4-ply SIS can serve as a satisfactory substrate for perineal herniorrhaphy in dogs without causing a significant inflammatory reaction. SIS is as strong as a traditionally accepted technique and has significant clinical advantages over other substrates. The perineal hernia model

and mode of testing that we reported are acceptable methods for future testing of the pelvic diaphragm. Preliminary testing showed that both the SIS and IOT are not significantly different from the normal pelvic diaphragm in terms of strength. The SIS technique was easy to perform and has less potential complications than the IOT technique. However, further testing is necessary to determine the exact strength of the normal pelvic diaphragm. Also, further studies on clinical cases should be performed to elucidate the role of SIS in repair of naturally occurring perineal hernia.

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