Use of a hyaluronate membrane for jejunal anastomosis in horses

Randy B. Eggleston, DVM; PO. Eric Mueller, DVM, PhD; Jane E. Quandt, DVM, MS; Lisa Neuwirth, DVM; Barry G. Harmon, DVM, PhD; Wayne R. Waguespack, DVM; Mark E. Rainbow, DVM

**Objective**—To compare the outcomes of double-layer inverting anastomosis (DIA), single-layer anastomosis (SLA), and single-layer anastomosis combined with a hyaluronate membrane (SLA+HA-membrane) with respect to stomal diameter, adhesion formation, surgery time, and anastomotic healing in horses.

**Animals**—18 adult horses.

**Procedure**—Midline celiotomy and end-to-end anastomoses were performed. In control horses (n = 6), DIA was performed; in treated horses, SLA was performed (6) or SLA+HA-membrane was performed (6). Horses were euthanatized 21 days after surgery. Abdominal adhesions were evaluated grossly and histologically. Stomal diameters were measured ultrasonographically and compared with adjacent luminal diameters. Anastomotic healing was evaluated histologically for fibrosis and inflammation, tissue alignment, and inversion. Surgery times were recorded for the anastomotic procedure and compared among groups.

**Results**—There were significantly more adhesions in the SLA group, compared with the DIA and SLA+HA-membrane groups. Reduction in stomal diameters in the DIA group was significantly greater than the SLA and SLA+HA-membrane groups. Surgery times for the DIA group were significantly greater than the SLA and SLA+HA-membrane groups. Histologic findings of fibrosis, inflammation, and mucosal healing were similar among groups. There was significant tissue inversion in the DIA group, compared with the 2 treatment groups. Tissue alignment was not different among groups.

**Conclusions and Clinical Relevance**—Use of a SLA+HA-membrane was an effective small intestinal anastomotic technique. This technique was faster to perform and resulted in a larger stomal diameter, compared with the DIA technique and significantly fewer perianastomotic adhesions, compared with the SLA technique. (Am J Vet Res 2001;62:1314–1319)

Diseases of the small intestine requiring surgical intervention are important problems in horses admitted to referral hospitals for acute abdominal disease. Earlier studies reported survival rates of 24 to 52% in horses undergoing surgery for small intestinal disease. Despite improvements in diagnostic, anesthetic, and surgical techniques, more recent studies report survival rates of 52 to 73%. Commonly reported postoperative complications that contribute to the poor survival rate of horses with small intestinal disease include intra-abdominal adhesions, anastomotic leakage, ileus, and peritonitis.

Numerous techniques have been described for small intestinal anastomoses in horses. A hand-sewn end-to-end anastomotic technique results in the most anatomically correct alignment of intestinal segments and usually consists of a simple continuous pattern in the mucosa oversewn with a continuous Cushing or Lembert in the seromuscular layer. The hand-sewn end-to-end inverting technique provides a tight seal and low incidence of adhesion formation, compared with described appositional techniques. Inverting suture patterns, however, often result in greater reduction in stomal diameter at the anastomosis than the appositional or everting suture patterns.

Numerous treatments aimed at prevention of abdominal adhesions have been evaluated in humans, laboratory animals, sheep, ponies, and horses. Only a limited number of these treatments have been evaluated for use in abdominal surgery in horses. It has been reported that sodium carboxymethylcellulose (SCMC), a high molecular weight hydrophilic polysaccharide polymer solution, significantly decreased abdominal adhesions in mice, rats, ewes, and ponies. Precoating of serosal surfaces prior to intestinal manipulation results in more favorable results than coverage of serosal lesions after manipulation. It has also been reported that SCMC reduces the incidence of intra-abdominal adhesions in horses with no adverse effect on incisional wound healing, postoperative complications, or short- and long-term survival.

Sodium hyaluronate is a naturally occurring hydrophilic anionic polysaccharide polymer in connective tissue, skin, cartilage, synovial fluid, and vitreous and is a major component of the extracellular matrix. It has been reported that sodium hyaluronate...
significantly decreased adhesion formation in a rat cecal abrasion model.17,33,34 It also has no apparent adverse affects on anastomotic wound healing.35

More recently, SCMC and sodium hyaluronate have been combined in the form of a bioresorbable hyaluronate membrane. When applied to moist tissue, the membrane hydrates and adheres rapidly to the serosal surface. It is cleared from the abdomen in 7 days and is completely excreted through the kidneys within 28 days.35 Studies involving use of the membrane in rats, rabbits, humans, and horses have reported a significant decrease in the incidence and severity of experimentally induced postoperative abdominal adhesions.36,37,38 It was demonstrated in a more recent study that there were no adverse affects on intestinal or peritoneal wound healing in horses.39

An ideal small intestinal anastomotic technique for use in horses would provide a maximal stomal diameter without increasing the incidence of perianastomotic adhesions, allow accurate alignment of tissue layers, and be simple and quick to perform. We hypothesized that a single-layer appositional small intestinal anastomosis covered by an HA-membrane (SLA+HA-membrane) would provide an anastomosis with a larger stomal diameter, compared with a double-layer inverting anastomosis (DIA) technique and fewer peri-anastomotic adhesions, combined with a single-layer appositional anastomosis (SLA) technique. The objective of the study reported here was to compare a 2-layer, hand-sewn inverting anastomotic technique, a single-layer simple continuous appositional technique, combined with an HA-membrane in the small intestine of horses, with respect to stomal diameter, adhesion formation, surgery time, and anastomotic healing.

Materials and Methods

Experimental protocol—Eighteen healthy adult horses ranging in age from 1 to 20 years (mean, 8.4 years) and weighing between 341 and 568 kg (mean, 475 kg) were used in the study. An institutional Animal Care and Use Committee approved experimental procedures and animal care. Horses (n = 6/group) were randomly assigned to 1 of 3 groups, as determined by the type of anastomoses performed. Blood samples were collected from each horse for hematologic and biochemical analyses before surgery.

Food was withheld for 12 hours before surgery. One hour before induction of anesthesia, potassium penicillin G (22,000 U/kg of body weight, IV, q 6 h), gentamicin sulfate (6.6 mg/kg, IV, q 24 h), and flunixin meglumine (1.1 mg/kg, IV, q 12 h) were administered. Each horse was anesthetized with xylazine hydrochloride (2.2 mg/kg, IV), and anesthesia was maintained with sevoflurane in oxygen in a semiclosed system. Lactated Ringer’s solution (10 ml/kg h, IV) was administered during the surgical procedure. Horses were positioned in dorsal recumbency and prepared for abdominal surgery.

Ventral midline celiotomy and systematic exploration of the abdominal cavity were used to facilitate examination of the viscera. The jejunum was exteriorized and examined from the ileocecal orifice to the duodenocolic ligament. Sterile saline (0.9% NaCl) solution was used to lubricate the intestine during manipulation. In all horses, an end-to-end jejunal anastomosis was performed 10 meters proximal to the ileocecal orifice. A 1-meter length of jejunum was isolated from the abdomen with moistened sterile towels, and the jejunum was incised perpendicular to the mesenteric border. In DIA horses, the mucosa was apposed with 3-0 polydioxanone, using a simple continuous pattern. The serosal layer was inverted with 3-0 polydioxanone, using a continuous Cushing pattern. Each suture line was interrupted and tied at the mesenteric and antimesenteric borders to prevent a purse-string effect.

In SLA horses, the mucosa and seromuscular layers were apposed with a simple continuous suture pattern, using 3-0 polydioxanone. The mucosa was meticulously folded below the seromuscular layer with each throw to minimize mucosal exposure. The suture line was interrupted and tied at the mesenteric and antimesenteric borders.

In SLA+HA-membrane horses, a single layer end-to-end appositional anastomosis was performed. Once the anastomosis was complete, it was liberally lavaged with sterile saline solution and patted dry with a sterile gauze sponge. Half of a sheet of self-adherent HA-membrane (6.5 × 7.6 cm) was then applied to the jejunum to completely cover the anastomosis and adjacent mesentery (Fig 1).

Surgery times were recorded for each anastomosis. Transection of the jejunum marked the start time; stop time was marked when the last suture of the anastomosis was tied in DIA and SLA horses or the HA-membrane was applied to the anastomosis in SLA+HA-membrane horses. The linea alba was closed with size-3 polyglaclatin 910 in a simple continuous suture pattern, and the subcutaneous tissues and skin were apposed with 2-0 polydioxanone, using a continuous subcuticular suture pattern.

Postoperative care and monitoring—After recovery, horses were allowed access to water ad libitum and were gradually returned to full feed during the next 36 hours. Treatment with antibiotics and flunixin meglumine was continued for 48 hours. Each horse was monitored every 6 hours for food consumption, pulse and respiratory rate, rectal temperature, signs of pain, and swelling or drainage associated with the incision. After the initial 48-hour postoperative period, the monitoring interval was decreased to every 12 hours. Any horse that had clinical signs of abdominal pain after surgery was examined and treated appropriately. Horses were housed in individual stalls for 14 days after surgery and then turned out to pasture for 7 days.

Postmortem examination—Horses in all groups were euthanatized by administration of an overdose of pentobarbital.
bital sodium solution (87 mg/kg, IV) 21 days after surgery. The abdominal incision, peritoneal cavity, anastomoses, and all abdominal organs were evaluated. Adhesions were recorded according to location and severity. Each anastomosis, including 20 cm of jejunum proximal and distal to the anastomosis, was harvested for determination of stomal diameter and histologic evaluation of anastomotic healing.

Stomal diameter—Ultrasonographic images of segments of jejunum containing the anastomosis were obtained to determine stomal diameter.1 Each anastomosis was lavaged with isotonic saline solution to remove all feed material. Each end of the jejunum was fixed to a grooved No. 9 rubber stopper, which was fitted with a centrally placed pipette. One end of the jejunal segment was connected to a manometer2 to record intraluminal pressure; the other end was used to infuse lactated Ringer’s solution to an intraluminal pressure of 20 mm Hg (27.2 cm H20). The intestinal segments were placed in a lactated Ringer’s solution bath to obtain optimal contact with the ultrasound probe (Fig 2). Ultrasonographic images were acquired in the transverse plane from the intestinal segment 10 cm proximal and distal to the anastomosis and at the anastomosis, using a C7.5 MHz transducer.3 The same ultrasonographer (LN) performed all measurements without knowledge of the group assignment. The mean of the proximal and distal luminal diameters served as controls. To prevent errors in the measurements caused by obliquity, the ultrasound probe was positioned perpendicular to the serosal surface of the intestinal segment. The best circular cross section was subjectively obtained by visual inspection of the ultrasound image. Luminal and stomal diameter and circumference were obtained, using an internal ellipse function fit to the internal mucosal margin. The stomal diameter was calculated internally from the ellipse function measurements (Fig 3). Images were taken in triplicate, and data were recorded on radiographic film. The stomal diameter of each anastomosis was expressed as a mean (± SD) percentage of the control.

Histologic evaluation—Tissues from the anastomotic sites were fixed in neutral-buffered 10% formalin. Three sections from each anastomosis were cut at right angles to the anastomotic sites, and the specimens were processed routinely and stained with H&E in routine fashion. The same pathologist (BGH) evaluated all samples without knowledge of the group assignment. Histologic sections of anastomotic sites for all 3 groups were evaluated for the distance between apposed muscularis, thickness of serosal fibrosis, and tissue inversion and alignment. The distance between apposed muscularis and the thickness of serosal fibrosis at anastomotic sites were measured with a micrometer. The mean value for each variable was calculated from 3 measurements. In addition, the intensity of serosal inflammation was subjectively evaluated.

Statistical analyses—A 1-way ANOVA was performed to determine the effects of the 3 surgical procedures on the variables of age, percentage diameter reduction, surgery time, distance between apposed muscularis, and thickness of serosal fibrosis at the anastomoses. Orthogonal contrasts were used for those instances in which significant differences were detected among the surgical procedures. The effect of age versus adhesion (yes or no) was analyzed by use of an unpaired t-test. The 3 × 2 contingency table of surgical procedure versus adhesion (yes or no) was analyzed, using the Fisher exact test. The relative risk of adhesion formation associated with each surgical procedure was calculated by use of a standard odds ratio. For the anastomotic healing variables inversion and alignment, a generalized linear model (yes or no) was used to determine the effects of the 3 procedures. There was no apparent overdispersion in the model; therefore, no adjustments were made to scale the deviance. Orthogonal contrasts were used for those instances in which significant differences were detected among the surgical procedures. Values of P < 0.05 were considered significant.

Results

Significant differences regarding age or weight were not detected among groups (P = 0.85 and 0.44, respectively). Hematologic and serum biochemical values in samples obtained before surgery from all horses were within reference ranges for our laboratory. All horses recovered from surgery without complications.

One horse each from the SLA and SLA+HA-membrane groups had signs of mild abdominal pain 2 and 5 days after surgery, respectively. Both horses responded well to medical management, which consisted of nasogastric intubation, administration of IV fluids, and a single dose of flunixin meglumine (500 mg, IV).
Signs of abdominal pain resolved in both horses within 3 hours of treatment.

**Adhesion formation**—One horse in the DIA group developed a 1 x 1-cm adhesion from the anastomosis to the adjacent mesentery. The adhesion did not result in stricture of the jejunal lumen.

Five horses in the SLA group developed adhesions to the anastomosis involving 60 to 80% of the anastomotic circumference. Adhesions were to the adjacent mesentery (n = 4) and omentum (1). One adhesion formed a mesenteric band, creating a potential space for intestinal incarceration. In 3 horses, adhesions caused a reduction in jejunal luminal diameter that was grossly evident at necropsy.

One horse in the SLA+HA-membrane group developed a 1 x 1-cm adhesion to the adjacent mesentery. The adhesion did not result in stricture of the jejunal lumen. The remaining 5 horses in the SLA+HA-membrane group had no adhesions or stricture associated with the anastomosis (Fig 4). A significant (P = 0.039) association between surgical procedure and adhesion formation was identified. This statistical comparison infers that the SLA group was 25 times more likely to develop adhesions than either the DIA or SLA+HA-membrane group. Significant differences were not detected among the mean (± SD) age of all horses in which adhesions formed (8.3 ± 7.0 years) and the mean age of all horses in which adhesions did not form (8.5 ± 4.6 years; P = 0.95).

**Stomal diameter**—Percentage reduction in stomal diameters for the DIA, SLA, and SLA+HA-membrane groups were 44.0 ± 9.6, 35.6 ± 6.3, and 28.0 ± 8.3%, respectively. Percentage reduction of stomal diameters in the DIA group were significantly (P = 0.007) greater than the SLA and SLA+HA-membrane groups (Fig 5).

**Surgery time**—Mean surgery times for the DIA, SLA, and SLA+HA-membrane groups were 32.8 ± 3.6, 25.0 ± 1.3, and 27.3 ± 3.7 minutes, respectively. Significant (P = 0.012) differences were detected between the DIA group and the other groups; the time required to perform the DIA technique was significantly longer than the time required to perform the SLA and the SLA+HA-membrane techniques.

**Anastomotic healing**—In all groups, there was mature fibrous connective tissue between the closely apposed muscularis at the anastomotic sites, a common finding at this stage of intestinal healing. The distance between apposed muscularis at the anastomotic sites among the 3 groups did not differ significantly (P = 0.37).

The serosal surfaces of all anastomotic sites were sealed with mature fibrous connective tissue. The thickness of serosal fibrous tissue varied among sections from the same horse and between horses and did not differ significantly (P = 0.59) among groups. There was no evidence of remaining HA-membrane at any of the anastomoses. The mucosal epithelium was continuous across the mucosal surface at all anastomotic sites examined, except for focal microscopic ulcerations in 2 sections in SLA and 1 section in SLA+HA-membrane groups. In all instances, the focal mucosal ulcer was associated with suture material placed in close proximity to the mucosal surface. Focal aggregates of mucosal cells displaced into the submucosa were detected in 3 sections of anastomotic sites from DIA and in 1 section from SLA+HA-membrane groups. The serosal and mural inflammatory response was similar in sections from all 3 groups and was associated with the sutures. Typically, there was an intense infiltration of neutrophils around sutures with eosinophils, macrophages, and plasma cells surrounding the neutrophils. In some sections, an extensive area of concentric fibrosis surrounded the inflammatory infiltrates associated with the sutures.

Significant (P = 0.0001) differences were detected between the DIA group and the other groups with regard to tissue inversion; the DIA technique caused significantly more inversion of tissues than the SLA technique. Regarding alignment, significant differences were not detected among groups (P = 0.12).

**Discussion**

In the present study, the single-layer appositional suture pattern used in combination with an HA-mem-
brane resulted in an acceptable technique for small intestinal anastomoses in horses. The single-layer suture pattern allowed for maximal luminal diameter and minimal surgery time, and application of the HA-membrane prevented the formation of perianastomotic adhesions associated with single-layer appositional anastomoses.

Conditions that predispose patients to postoperative adhesion formation include peritonitis, intestinal ischemia or desiccation, bacterial contamination, and exposed mucosa or foreign material.20-22 Previous studies have revealed that use of the DIA technique for small intestinal anastomoses in horses result in minimal perianastomotic adhesion formation.11,24 Inverting techniques provide a tight serosal seal that minimizes leakage of intestinal contents. Serosal inversion also minimizes exposure of the peritoneal cavity to bacteria-laden mucosa. In contrast, the single-layer suture patterns, including the Gambee, crushing, and simple interrupted, result in varying degrees of mucosal exposure, exposed suture material, and anastomotic leakage, which may lead to adhesion formation.11,14 Adhesions associated with intestinal anastomoses may result in intestinal stricture or volvulus with intestinal obstruction as a common sequel. The recurrent signs of abdominal pain associated with adhesions often necessitate repeat celiotomy or euthanasia.23 Use the SLA+HA-membrane technique may prevent perianastomotic adhesion formation and decrease the incidence of postoperative complications associated with small intestinal anastomoses in horses.

The findings of the present study are similar to previous studies11,14 in that only 1 horse in the DIA group developed adhesions, whereas 5 horses in the SLA group developed adhesions associated with the anastomoses. Adhesions in SLA horses formed to the adjacent intestinal mesentery and distant omentum. However, when the SLA+HA-membrane technique was performed, only 1 horse developed 1 adhesion to the adjacent mesentery. The HA-membrane functions as a temporary mechanical barrier that prevents serosal-serosal or serosal-peritoneal contact during the immediate postoperative peritoneal repair phase, thereby preventing adhesion formation.11,22 In the present study, application of the HA-membrane to the SLA site significantly decreased the incidence of adhesions, compared with performing SLA alone.

Although standard DIA provide a tight serosal seal, the internal mucosal cuff produced as a result of tissue inversion considerably decreases intestinal stomal diameter.11,14 This decrease in stomal diameter may cause intestinal obstruction and recurrent signs of abdominal pain.11-14,26 Single-layer appositional suture patterns, including the Gambee, simple continuous, and simple interrupted, result in maximal intestinal luminal diameters, more accurate tissue alignment, and absence of the internal mucosal cuff.26 Previous studies revealed there may be > 40% reduction in stomal diameter with DIA, compared with < 33% reduction in stomal diameter with SLA.25

Contrast radiography and ultrasonography with varying intraluminal pressures have been used to evaluate stomal diameter in previous studies.9-14 In our study, intestinal segments were distended to an intraluminal pressure of 20 mm Hg in an attempt to more accurately represent the clinical situation.4 This amount of distention did not appear to artificially increase the stomal diameter measurements. Use of the internal ellipse function of the ultrasound gave accurate and consistent measurements of all intestinal segments. Anastomoses from the SLA and SLA+HA-membrane groups caused significantly less reduction in stomal diameter, compared with anastomoses in the DIA group. Although not significant, the SLA technique resulted in a greater reduction in stomal diameter than the SLA+HA-membrane technique. At necropsy, adhesion formation and fibrosis causing intestinal distortion and stricture was apparent in 3 of the anastomoses in the SLA group. This may have accounted for the greater mean reduction in stomal diameter observed in the SLA group.

Decreasing surgery and anesthetic time is always a concern in large animal surgery. The effects of prolonged anesthesia in the face of systemic illness, endotoxemia, and dorsal recumbency play a role in survivability.24 Horses requiring intestinal resection and anastomosis often require a prolonged surgery time, compared with those horses with simple intestinal obstructions. With the advent of intestinal stapling instrumentation in the early 1980s, surgery time was reduced substantially for certain procedures. There was, however, no difference in surgery time in a study in which hand-sewn end-to-end and stapled side-to-side jejunal anastomoses were compared.26 In our study, there was a significant difference in the time required to perform a DIA versus an SLA.

In the present study, the SLA+HA-membrane technique resulted in an effective alternative small intestinal anastomotic technique in horses. This technique resulted in greater stomal diameter, compared with the DIA technique, and significantly fewer perianastomotic adhesions, compared with SLA technique alone. Performing the SLA technique was also faster than the DIA technique. Use of this novel anastomotic technique may reduce the morbidity and mortality associated with small intestinal surgical diseases in horses.

References

5. Reeves MJ, Hilbert BJ, Morris RS. A retrospective study of


