Effects of staple size, tissue thickness, and precompression time on staple shape in side-to-side jejunocecal anastomosis in specimens obtained from healthy horses at an abattoir

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Objective—To determine effects of staple size, precompression time, and tissue thickness on staple shape and tissue approximation in side-to-side jejunocecal anastomosis in equine specimens.

Sample—Cecum, ileum, and jejunum specimens obtained from 18 healthy horses at an abattoir.

Procedures—Specimens were allotted into 2 groups. Anastomoses were stapled with 4.8- or 3.8-mm staples. Precompression time was 15 seconds for both groups. Staple lines were cut into proximal, middle, and distal sections. Thickness of intestinal walls was measured with a calibrated tissue micrometer, photographs were obtained, and intestinal tissues were digested. An investigator measured staples and assessed the shape of staples on high-definition digital images. Number of optimally shaped staples and staple height were compared among sections and between groups.

Results—Use of 4.8-mm staples resulted in poor approximation of tissues in the distal sections of anastomoses. The percentage of optimally shaped staples was 538 of 551 (97.6%) and 616 of 634 (97.2%) for 4.8- and 3.8-mm staples, respectively. The percentage of optimally shaped staples did not differ significantly between groups for the same sections. There was a lower percentage of optimally shaped staples in the distal sections than in the proximal and middle sections of each group. Mean staple height did not differ significantly among sections of each group.

Conclusions and Clinical Relevance—Use of 3.8-mm staples with an adequate precompression time for jejunocecal anastomosis in horses resulted in proper staple shape. These findings could be used to improve the technique and outcome for stapled jejunocecal anastomoses in horses. (*Am J Vet Res* 2014;75:680–684)

Stapled side-to-side jejunocecal anastomoses are commonly performed in horses, although the use of staples has a higher risk of complications than for hand-sewn techniques.[1] The reasons for this increased risk have not been fully evaluated and could depend on multiple factors, including hemorrhage and leakage from the anastomotic site,[1] which have been suspected in some cases.

These complications are recognized in stapled anastomoses in humans and may be related to mechanical or tissue properties or to ischemic damage.[2] Among mechanical or tissue-related causes of complications, staple shape is a main factor. Properly formed surgical staples ideally have a B shape, and the correct shape is essential to result in an effective anastomosis, reduce hemorrhage, and avoid leakage.[3] Proper staple formation depends on tissue thickness, appropriate staple dimensions, and precompression time.[3–5]

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Information from 2 surgical stapler manufacturers[6,7] indicates the size of staple that should be used for tissues of various thickness. Furthermore, some manufacturers advise the use of a 15-second precompression time before insertion of the staples.[5–7] Precompression time is the interval between closure of a stapler's jaws on the tissue and insertion of the staples. During this period, fluid escapes from the tissue located between the stapler's jaws, which reduces tissue thickness and improves staple formation.[3–5] Use of precompression before insertion of the staples improves staple shape,[3] results in less hemorrhage,[4] and increases mechanical strength of the anastomosis.[5] This can also be used on tissue thicker than that recommended for the chosen staple size.[3–5]

Standardized anastomosis protocols have been described for use in humans, and these protocols are safe, although they do not always ensure absolute efficacy.[3] Linear cutting staplers can be used with staples of 3 heights (2.5, 3.8, and 4.8 mm). Size selection depends on the thickness of the tissues to be stapled (thin [eg, vascular tissue], normal [eg, normal jejunum], or thick [eg, edematous jejunum or the stomach wall]). In horses, the use of 4.8-mm staples is commonly recommended, without consideration of tissue health and thickness.[8–10] However, to our knowledge, there have been no reports defining guidelines for the stapling technique or assessing the optimal size of staples to use for jejunocecal anastomosis in horses.

Therefore, the objective of the study reported here was to evaluate the effect of precompression time, staple size, and tissue thickness on staple shape and tissue approximation in intestinal tissues obtained from healthy horses. We hypothesized that 3.8-mm staples could achieve an optimal shape and height with good tissue approximation in an in vitro assessment of stapled side-to-side jejunocecal anastomoses in horses.

Materials and Methods

Sample—Intestinal specimens comprising the cecum, ileum, and 3 m of jejunum were collected from 18 horses immediately after the horses were killed at an abattoir. Horses ranged from 18 to 30 months of age (mean, 24 months), with a body weight of 300 to 500 kg (mean, 400 kg).

Procedures—A random-number generator was used to assign specimens to each of 2 groups. Staples were used to create 80-mm-long side-to-side jejunocecal anastomoses. Anastomoses were performed in 9 intestinal segments by use of a linear cutting stapler[a] loaded with 4.8-mm staples. Identical anastomoses were performed in 9 other intestinal segments by use of the same stapling device[a] loaded with 3.8-mm staples. Anastomoses were performed in accordance with a technique described elsewhere.[11] Briefly, the transected end of the jejunum (ie, jejunal stump) was closed with a Parker-Kerr suture pattern and apposed with 2 stay sutures to the wall of the cecum at a point between the dorsal and medial tenia. Two 1-cm-long enterotomies were performed to allow insertion of the stapler's jaws into the intestinal lumens. Once the stapler was positioned, it was held closed for 15 seconds (precompression time) before insertion of the staples, as previously described.[5,7] Enterotomies were then closed with 2-0 polyglactin 910[b] in a Cushing pattern.

Macroscopic appearance—Intestinal segments were connected to a manometer and inflated to a pressure of 8 mm Hg by means of a compressor that provided 1 L of air/min.[12] This pressure was chosen because a preliminary study conducted by our laboratory group revealed it resulted in the best possible shape of the anastomosis without causing stretching or disruption. Photographs of the staple lines for gross anatomic comparisons were obtained with a digital camera.

Intestinal wall thickness—Each staple line was divided into three 2.5-cm sections (proximal, middle, and distal). Intestines were incised longitudinally at a point on the lumen opposite the anastomosis. Each author separately measured the wall thickness of the jejunum and cecum 3 times in the center of each section. Measurements were obtained by use of a calibrated tissue micrometer[c] at a location adjacent to the anastomosis. The tissue micrometer was set at a pressure of 8 N/m^2, which is the pressure reported for use with surgical stapling devices.[2]

Tissue digestion and analysis of staples—Sections of each staple line were identified and placed separately in a digestion solution of 20% NaOH in distilled water; sections were incubated at 40°C for 72 hours. After intestinal tissues were completely digested, the staples were cleaned in distilled water, dried, and stored in plastic tubes. Subsequently, each set of staples was aligned on graph paper, and high-definition digital images were obtained for analysis. Each digital image was imported into imaging software[d] for measurement analysis. The image scale was recalibrated for each staple. An investigator (GG) who was not aware of the tissue source of the staples characterized the shape macroscopically as optimal (B, R, or D shape) or suboptimal (C, X, or U shape).[3] Optimal was defined as closed staple tips, and suboptimal was defined as open staple tips.[3] That same investigator measured the maximal height of each staple; only optimally shaped staples were used for height measurements.

Statistical analysis—Mean tissue thickness was compared between groups by use of an unpaired *t* test with Welch correction. The number of optimally shaped staples was determined. The percentage of optimally shaped staples in each group was expressed as an overall percentage. Percentages were compared between groups with the Fisher exact test. The number of optimally shaped staples in each section (proximal, middle, and distal) for each group was determined and expressed as a percentage. Percentages for each section were compared between groups and among sections within each group by use of the Fisher exact test. Mean height of optimally shaped staples was evaluated among sections (proximal, middle, and distal) of each group with a 1-way ANOVA.

All analyses were performed by use of commercially available statistical software.[e] For all tests, values were considered significant at $P < 0.05$.

Results

Intestinal specimens—There were no episodes of device malfunction during the study. One anastomosis performed with 4.8-mm staples was discarded because of technical error at the time of tissue harvest; thus, 17 jejunocecal anastomoses were used for evaluation. Broken or damaged staples were excluded from the analysis. During division of the staple lines, 161 of 1,346 (12.0%) staples were broken or damaged; thus, only 1,185 (88.0%) staples were analyzed.

Macroscopic appearance—Macroscopically, the use of 4.8-mm staples resulted in poor apposition of tissues in the distal section of the anastomoses, which was evident as wide spaces between the tissue edges (**Figure 1**). This was considered poor placement of staples. By contrast, there was much better tissue apposition with the 3.8-mm staples.

Intestinal wall thickness—Mean tissue thickness did not differ significantly between the 2 groups (**Table 1**). Mean ± SD combined thickness of the jejunum and cecum walls was 3.43 ± 0.48 mm for the 4.8-mm group and 3.41 ± 0.30 for the 3.8-mm group.

Analysis of staples—The overall percentage of optimally shaped staples exceeded 97% in both groups and was not significantly different between groups. Percentages of optimally shaped staples for the same sections did not differ significantly between the 2 groups. However, within each group, there was a significantly lower percentage of optimally shaped staples in the distal section, compared with the percentage of optimally shaped staples in the proximal and middle sections (**Table 2**).

Mean height for optimally shaped staples did not differ significantly among the 3 sections within each group (**Table 3**). However, mean height for optimally shaped staples differed significantly between the 2 groups.

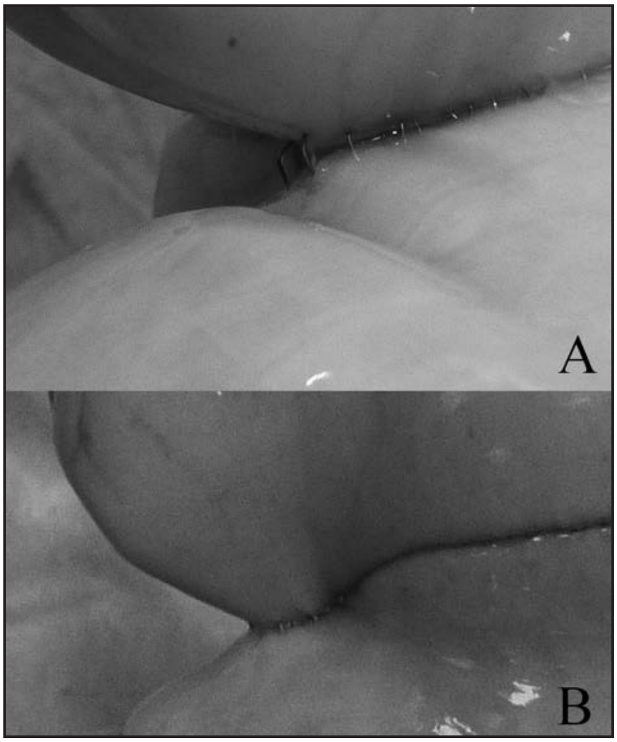

Figure 1—Photographs of the distal section of a representative staple line for a jejunocecal side-to-side anastomosis performed with 4.8-mm staples (A) or 3.8-mm staples (B) in specimens obtained from a healthy horse at an abattoir. Distal is to the left, and proximal is to the right. Notice that several 4.8-mm staples are not optimally shaped and there is a large space between the tips of the staples that could lead to improper hemostasis. By contrast, the 3.8-mm staples provide better tissue apposition

Table 1—Intestinal tissue thickness (mm) for jejunocecal side-to-side anastomoses performed with 2 sizes of staples in specimens obtained from 18 healthy horses at an abattoir.

Group*	Jejunum	Cecum	Total†
4.8 mm (n = 8)‡	1.52 ± 0.16	1.68 ± 0.43	**3.43 ± 0.48**
3.8 mm (n = 9)	1.32 ± 0.21	1.62 ± 0.31	**3.41 ± 0.30**

Values reported are mean ± SD; thickness was measured by use of a micrometer with pressure set at 8 N/m^2.

*Size of staples. †Combined thickness of jejunum and cecum. ‡One anastomosis in this group was discarded because of technical error at the time of tissue harvest.

Discussion

The study reported here was conducted to compare the effectiveness of staples of different size for jejunocecal anastomosis in horses. We focused on staple shape, which is essential to yield an anastomosis that does not leak, and staple height, which is essential for preservation of tissue integrity and hemostasis.

Macroscopically, 4.8-mm staples resulted in a loose apposition of tissue on the distal section of the anastomosis, whereas the 3.8-mm staples more closely apposed the tissues (Figure 1). This loose apposition for the 4.8-mm staples could lead to anastomotic leakage or hemorrhage.[2] We did not detect leakage of air from inflated anastomoses, although evaluation of leakage was not a purpose of this study.

Intestinal wall thickness is used to determine the appropriate staple size and influences proper staple shape once a staple is inserted. In the present study, the mean combined thickness of the jejunal and cecal walls was > 3.4 mm and exceeded the recommended thickness for use of both staples (2 and 1.5 mm for 4.8-mm and 3.8-mm staples, respectively). The percentages of optimally shaped staples did not differ for the same sections between the 2 groups, which indicated that tissue thickness was not excessive for either group. For moderately edematous tissue, use of precompression would allow formation of an optimal staple shape, although the use of staplers on edematous or thickened intestinal tissues is typically contraindicated.

Staple shape was considered optimal or suboptimal on the basis of whether the tips of a staple were closed or open, respectively.[3] Proper apposition of tissue is not achieved if the tips of a staple are open.[2,3] The percentage of optimally shaped staples in the distal section of the staple line was significantly lower than that in the proximal and middle sections in both groups. This difference may have been attributable to cartridge-to-anvil deflection, misalignment, or improper advancement of the stapler handle. This fact was confirmed by the macroscopic appearance of the distal section of the sta-

Table 2—Number and percentage of optimally and suboptimally shaped staples for each section and each group for jejunocecal side-to-side anastomoses performed with 2 sizes of staples in specimens obtained from 18 healthy horses at an abattoir.

	Proximal				Middle				Distal				Total			
Group*	Optimal	Suboptimal	Total	%	Optimal	Suboptimal	Total	%	Optimal	Suboptimal	Total	%	Optimal	Suboptimal	Total	%
4.8 mm (n = 8)†	165	2	**167**	98.8[a]	203	0	**203**	100[a]	170	11	**181**	93.9[b]	538	13	**55**	97.6
3.8 mm (n = 9)	190	0	**190**	100[a]	207	1	**208**	99.5[a]	219	17	**236**	92.8[b]	616	18	**634**	97.2

Optimal was defined as closed staple tips, and suboptimal was defined as open staple tips.

†One anastomosis in this group was discarded because of technical error at the time of tissue harvest.

[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$).

See Table 1 for remainder of key.

Table 3—Staple height (mm) for each 2.5-cm section of jejunocecal side-to-side anastomoses performed with 2 sizes of staples in specimens obtained from 18 healthy horses at an abattoir.

Group*	Proximal	Middle	Distal	Total†
4.8 mm (n = 8)‡	1.73 ± 0.07	1.69 ± 0.07	1.75 ± 0.12	**1.73 ± 0.08**
3.8 mm (n = 9)	1.46 ± 0.06§	1.45 ± 0.10§	1.50 ± 0.05§	**1.47 ± 0.08§**

Values reported are the mean ± SD; only optimally shaped staples were measured.

†Represents the mean value for the 3 sections. §Within a column, value differs significantly ($P < 0.05$) from the value for the 4.8-mm group.

See Table 1 for remainder of key.

ple line and is in accordance with results of another study.[12]

We did not detect a significant difference in the percentage of optimally shaped staples between the 2 groups. This indicated similar behavior for staples of both sizes in the intestinal specimens of the present study.

Resulting staple height corresponded to the manufacturer's specifications[7] for the 3.8-mm staples, whereas the resulting height for the 4.8-mm staples was lower than expected[7] (manufacturer's specifications, approx 2 mm). This finding may have resulted from thin tissue between the staple tips that allowed formation of an optimal staple shape but with a lower height. This could partially overcome the problem of choosing a cartridge with staples that have a larger size than that recommended for a specific tissue thickness, but it would likely not be sufficient to avoid hemorrhage and result in appropriate tissue apposition.

One limitation of the present study, which was similar to a limitation in another study,[3] was that we did not consider tissue damage or ischemia caused by the use of smaller staples and precompression. Nevertheless, it has been found that a precompression time < 1 minute did not cause ischemic damage in pigs[4] and eventually improved anastomotic mechanical strength.[5] However, further in vivo studies in horses are needed.

Correct use of a stapling device is essential.[3] However, methods for determining appropriate settings are inconsistent and differ among surgeons, even among human surgeons.[5]

Information about methods to improve techniques for stapled anastomoses in humans has been reported[3]; however, to our knowledge, there have been no similar reports for improvement of these techniques in horses despite the fact mechanical stapling devices are widely used for equine abdominal surgery. These devices are available with various staple sizes that are used in accordance with the thickness of tissues to achieve proper staple shape.[3] The use of only larger (4.8 mm) staples has been advocated in horses,[8–10] without regard for the size of the horse or thickness of the tissues.

We are not aware of any description for use of precompression in stapled anastomoses of horses. However, the use of precompression is commonly recommended in human surgery.[2–5,13]

Linear cutting staplers are considered to be precompression devices[3] because allowing a brief period between closing the jaws of a stapler and insertion of a staple improves staple line formation.[2–6,14] Because of the biphasic behavior of intestinal tissue, there is a noticeable change in tissue thickness during the first 15 to 20 seconds of precompression, which then plateaus.[2] Precompression prepares the tissue for staple insertion, reduces stress on the tissue prior to staple insertion, minimizes tissue blood flow, and helps to avoid serosal tears.[2] Thus, precompression might be useful when performing surgery on healthy or edematous tissue by causing expulsion of excessive fluid from the tissue and diminishing tissue thickness.[2] In the present study, a precompression time of 15 seconds was sufficient to allow a high percentage of optimally shaped staples in both groups, despite the fact that the combined tissue thickness exceeded the manufacturers' recommendations. To our knowledge, the study reported here was the first in which a precompression time of at least 15 seconds, followed by insertion of 3.8-mm staples, was used for jejunocecal anastomoses in specimens obtained from horses and resulted in optimal staple shapes. Nevertheless, we cannot exclude the possibility that shorter precompression times could also result in optimal staple shapes in horses. Further evaluation of the use of 3.8-mm staples without application of precompression would be warranted. Use of 3.8-mm staples without application of precompression has been evaluated in other species.[2–5,13]

It is desirable to select a cartridge on the basis of wall thickness of the intestines and to use an adequate precompression time, which could easily be accomplished in open abdominal surgeries by measuring intestinal wall thickness with a micrometer before choosing the size of the staples. As an alternative, state-of-the art staplers, such as linear cutting staplers with selectable staple size,[f,g] are commercially available, although one of these staplers[g] is currently available only for endoscopic surgical procedures. These staplers, along with the appropriate staples, could be used to improve tissue apposition and hemostasis in many situations without changing the staple cartridge.

Although further in vivo studies are needed, we concluded that when performing jejunocecal anastomosis in horses with 4.8-mm staples, surgeons must take particular care to examine for leakage or hemorrhage after completion of an anastomosis, as suggested by the guidelines for stapled side-to-side anastomosis in humans.[4,14] Equine surgeons should be aware that methods and technologies are available to improve the performance of stapled side-to-side jejunocecal anastomoses. Surgeons are advised to choose staples of an appropriate size and to apply precompression before insertion of staples to potentially reduce complications related to use of these techniques.

a. Autosuture GIA Multifire 80, Covidien Italia, Segrate, Italy.
b. Vicryl, Ethicon, Johnson and Johnson Italia, Milan, Italy.
c. Micrometer Mitutoyo M110-25, Mitutoyo Italiana srl, Lainate (MI), Italy.
d. ImageJ, National Institutes of Health, Bethesda, Md.
e. GraphPad InStat, version 3.5 for Windows, GraphPad Software Inc, San Diego, Calif.
f. Ethicon NTLC linear cutting stapler, Johnson and Johnson Italia, Milan, Italy.
g. Autosuture Tri-staple, Covidien Italia, Segrate, Milan, Italy.

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