Comparison of Tensile Strength and Early Healing of Self-Locking and Surgeon's Knots for Closure of Ventral Midline Celiotomy in Horses

by

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Abstract

Characteristics of surgical closure are associated with incisional complications. The surgical knot has been shown to be the weakest point of the suture line. Self-locking knots, such as the forwarder (F) and Aberdeen (A) knot, have been investigated in vitro and ex vivo, supporting their superior knot strength and higher bursting pressures. We hypothesized that a ventral midline celiotomy (VMC) closure using a forwarder-Aberdeen (F-A) self-locking knot combination would have greater tensile strength and improved wound healing when compared to the traditional surgeon's-surgeon's (S-S) knot combination in vivo.

Fourteen horses underwent a VMC, closed with either F-A or S-S knot combination. Incisions were subjectively graded for healing quality. Biomechanical testing was performed and histologic grading from each subject following humane euthanasia ten days post VMC.

Statistical analysis showed no difference between groups for tensile strength (p=0.97) location of failure (p=0.24), and wound healing (p=0.60). However, the F-A group failed more frequently (15/21, 71%) along the rectus sheath compared to S-S (6/21, 26%), consistent with normal linea alba.

Similar tensile strength and histologic healing shows either knot combination provides secure closure in horses for the early post-operative period following VMC. Results support a randomized prospective clinical trial to evaluate performance of the F-A self-locking knot combination in clinical cases with long-term follow-up to further support clinical use.

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List of Abbreviations

VMC	Ventral Midline Celiotomy
SEM	Standard error of the mean
SD	Standard deviation
F	Forwarder knot
A	Aberdeen knot
S	Surgeon’s knot
F-A	Forwarder – Aberdeen knot combination
S-S	Surgeon’s – Surgeon’s knot combination
RKS	Relative knot security
KHC	Knot holding capacity
PCV	Packed cell volume
TP	Total protein
IV	Intravenous
IM	Intramuscular
USP	United States Pharmacopoeia
lb	Pound
NaCl	Sodium Chloride
°F	Degrees Farenheit
N	Newtons
cm	Centimeters
N/cm^2	Tensile strength per unit area
FF	Fibrin formation
LMM	Linear mixed models
GLMM	Generalized linear mixed models

I. Introduction and Literature Review

Colic, or abdominal discomfort, is one of the most prevalent causes of mortality in the domestic equine population accounting for 28% of reported horse deaths annually.[1] Most colic episodes that occur in the general population resolve spontaneously or following medical treatment while approximately 8% of episodes will require surgical treatment or euthanasia.[2] Surgical management and knowledge of pathophysiology and epidemiology of colic has improved significantly through the years, however post-operative complications remain commonplace. As these complications carry significant considerations for our patients that often have expectations of future athletic careers; further advancements in surgical technique need to be pursued in order to provide better patient care and outcome.

A. Ventral Midline Celiotomy

A longitudinal incision on the ventral midline, through the linea alba, is the most common approach to the equine abdomen, allowing exteriorization of 75% of the intestinal tract.[3] The linea alba is the anatomical junction of aponeuroses of the external and internal abdominal obliques with the transverse abdominal muscles (Figure 1).[4] The tissue is characterized by dense mature collagen bundles coursing in a cranial to caudal fashion.[5] Thus, this approach creates minimal hemorrhage, is extendable, and contains strong fibrous tissue for closure.[6] However, the avascular linea alba has a comparatively slow rate of healing in comparison to other tissues, thus the integrity of closure depends entirely on the technique utilized by the surgeon.[5] The linea alba also has a positional predisposition to dehiscence due to the forces applied along the ventral abdominal wall.[7] This is of special significance in the equine patient due to the large mass of abdominal viscera and distracting forces of occasional violent movements, such as anesthetic recovery or post-operative colic.[7]

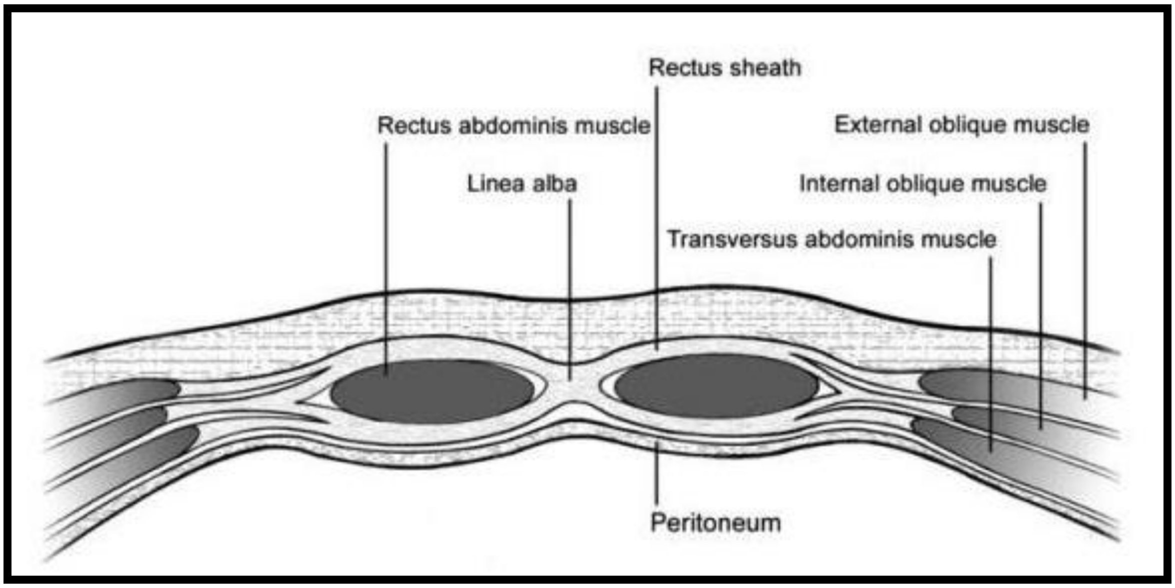


Figure 1. The muscle layers of the abdominal wall taken from Yarwood et al. 2010

B. *Biodynamics of Linea Alba*

Studies have been performed to describe the biomechanics of the equine linea alba following celiotomy and provide clinical significance to research investigating tissue strength, suture materials, and closure techniques. Kirker-head et al performed a study utilizing liquid metal strain gauges implanted surgically on the cranial, middle, and caudal linea alba. The study included 8 horses and data was collected during various routine activities such as recumbency under general anesthesia, anesthetic recovery, standing, rectal palpation, vocalization, and exercising at the walk, trot, and canter.[7] The max peak stress, which is the measurement of force applied to a cross-sectional area, and max peak strain, which is the deformation of material, were measured.[7]

Results of the study showed that the dynamic properties of stress and strain were not homogenous throughout the length of the incision. Results differed significantly between various locations, with the middle and caudal linea alba experiencing greater stress and strain than the cranial location (Figure 2A and 2B). In addition, max peak stress and greatest change in strain occurred during anesthetic recovery and the canter.[7] Understanding the stress and strain placed on the intact linea alba, allows for approximation of similar stress and strain on incised linea alba during early wound healing of this fibrous tissue.

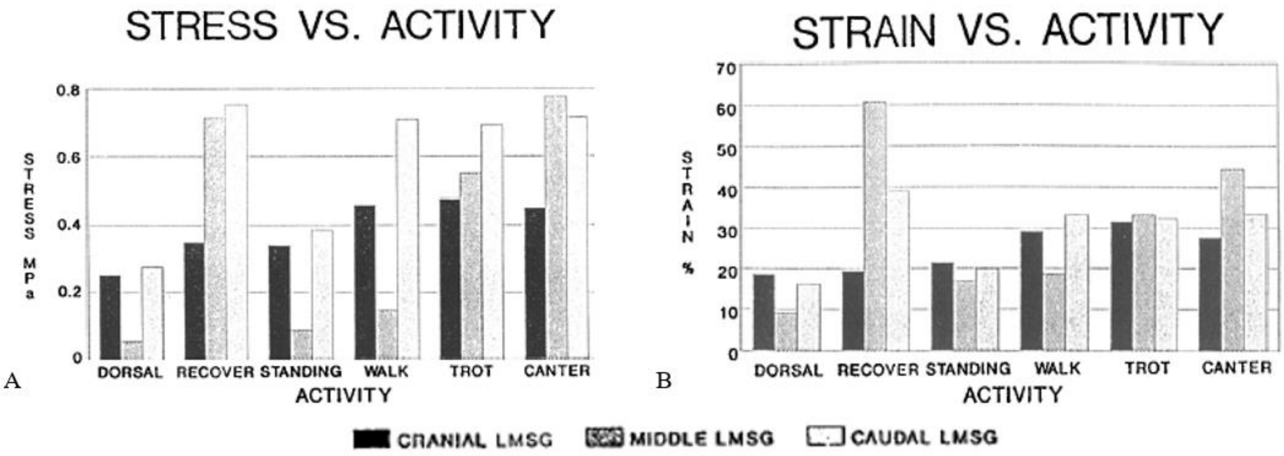


Figure 2. Bar graphs illustrating mean peak stress (A) and strain (B) at three ventral midline locations during various activities taken from Kirker-Head et al. 1989

C. *Testing Methods*

Two parameters for measuring the strength of body tissues include tensile strength and bursting strength.[8] Tensile strength is defined as the load or force per cross-sectional area at point of failure.[8] This is measured by applying a single unilateral load to failure. Testing most often utilizes a material testing apparatus, such as an Instron Universal Testing System (Figure 3), with various clamp attachments to secure the material to be tested. Load can then be applied in either a vertical or horizontal direction based on the required configuration. Clamp types can include commercially available grips or custom built clamps that utilize hydraulics[9], pneumatics[10], cryoclamps[5,11], or bolted grips[12] to secure the tissue and reduce slippage at the point of contact. Studies that evaluated equine linea alba tensile strength have utilized the cryoclamp system with horizontal load making this an accepted method.[5,11] Limitations of this tensile strength model includes single load to failure and unidirectional force, spurring investigation into ex vivo and in vivo testing.

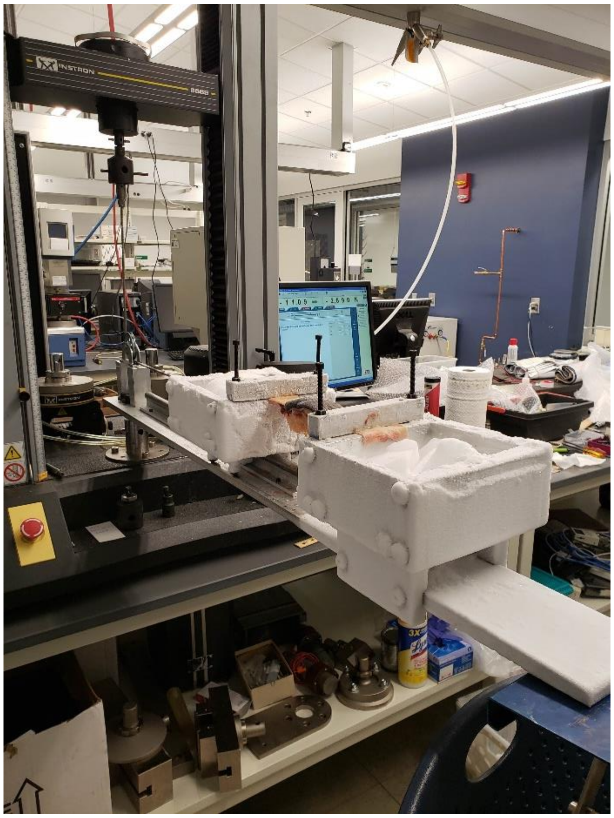


Figure 3. Picture illustrating an example of a universal materials testing apparatus with custom-made cyroclamps for tensile strength testing.

Bursting strength is defined as the amount of pressure required to rupture a viscus.[8] An ex vivo cadaver model was first developed in humans to assess abdominal pressures then further suture strength.[13] The model consisted of a rubber bag placed in the peritoneal cavity in humans via a midline or paramedian incision with tubing exiting via separate stab incisions. The midline or paramedian incision was closed, the bag inflated with an oxygen cylinder, and pressures at rupture were recorded. The model has since been converted for use in rats, cats, and horses to determine optimal abdominal closure methods.[14–22]

The model as described in horses (Figure 4), consists of a 200-L polyurethane bladder inserted through a ventral midline incision.[20,22–24] The ingress and egress tubing exit through stab incisions in the 14th intercostal space on both the right and left sides. The ingress tubing would be attached to an air compressor and flow meter while egress tubing connected to a pressure transducer. Following closure of the midline incision, the bladder would then be inflated at 40L/min until failure of the construct.

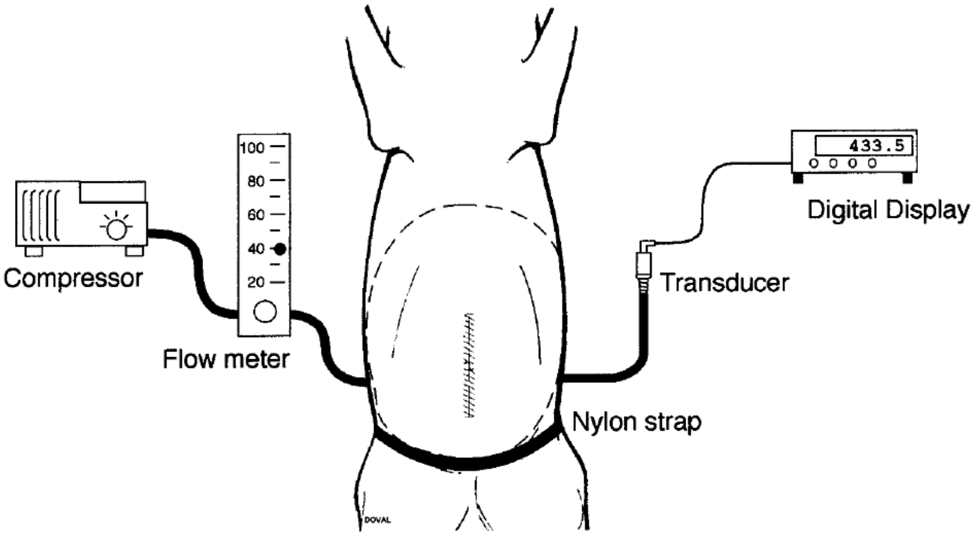


Figure 4. Schematic diagram of the bursting strength ex vivo equine cadaver model, as originally described by Magee et al.[23] The dotted line represents the 200-L polyurethane inflatable bladder.

This model is limited by the ex vivo nature as it does not fully represent the dynamic environment of a live patient and utilizes supraphysiologic pressures.[20,22–24] The effects of respiratory rate and effort, lung volume, and vigorous activity have been shown to change intra-abdominal pressures and forces along the ventral midline.[7,25,26] While intra-abdominal pressure during recovery from general anesthesia is currently unknown, it is the time at which the linea alba is under highest stress and strain.[7] This cadaver model is considered to simulate in vivo conditions more accurately than other models due to application of an even stress to the construct in a three dimensional fashion which reflects potential post-operative conditions.

D. Wound Healing

Wound healing is a dynamic process involving cellular and biochemical events that are similar across species and involved structures. The process has been divided into three phases – acute inflammatory, proliferative, and remodeling (Figure 5). The phases overlap and are also associated with collagen synthesis, especially at the 1 to 2 week mark when proliferation is ending and remodeling is beginning.

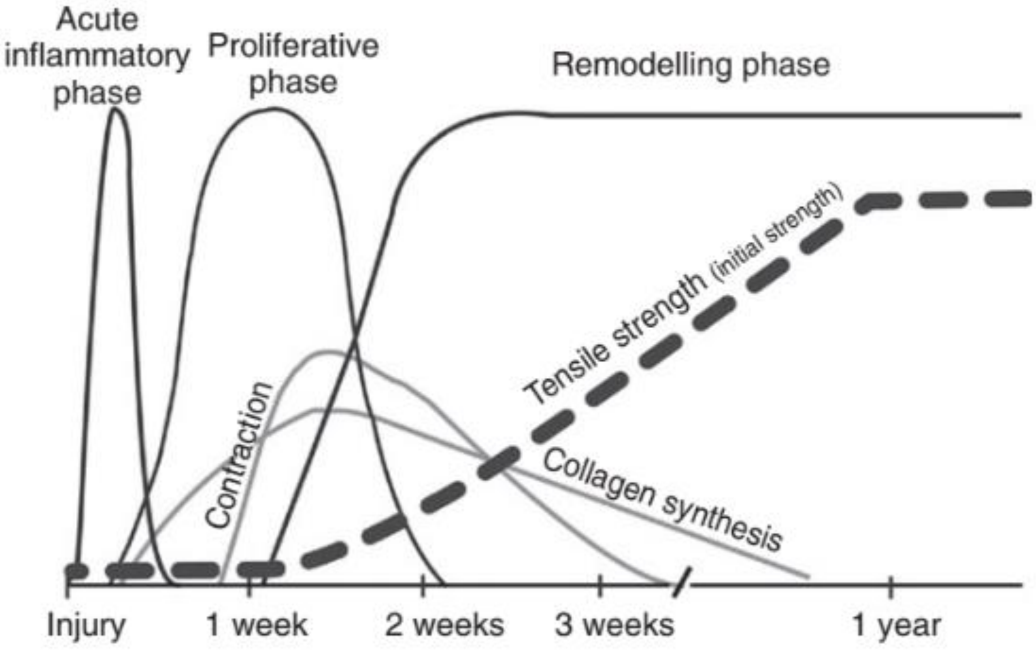
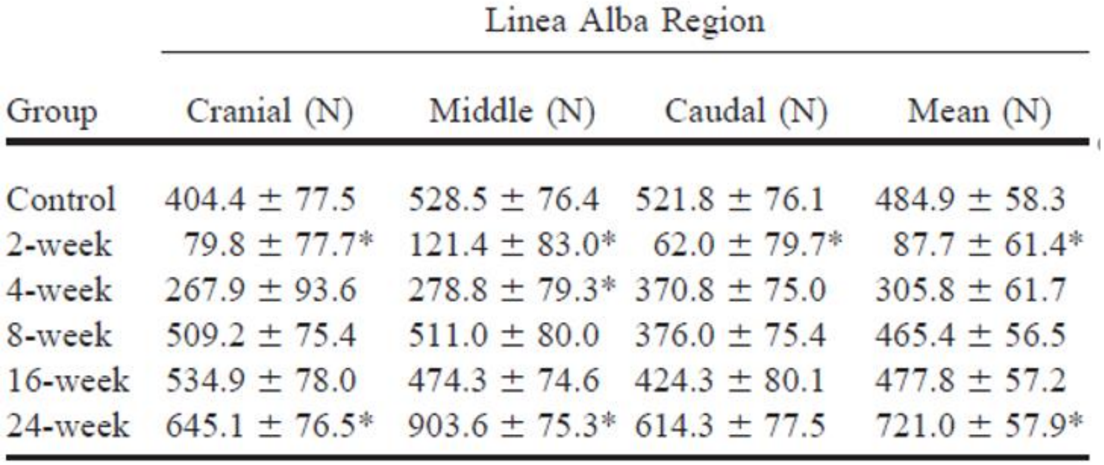


Figure 5. Phases of wound healing and gain in tensile strength post wounding.[6]

Chism et al in 2000 performed a controlled study to evaluate the normal healing of incised equine linea alba – in particular the temporal changes in tensile strength and morphological changes following ventral midline celiotomy.[5] This information was to be used as a basis for recommendations on returning horses to exercise, but also provides important data that can be used for similar studies comparing different closure techniques. 15 horses were included in the study, with linea alba samples collected at 2, 4, 8, 16, and 24 weeks for comparison against control samples for tensile strength, thickness, as well as wound and collagen morphology. Control linea alba was collected from 3 additional horses euthanized for reasons other than the study with no history of abdominal disease.[5] Important to note, in this study, all suture was removed prior to biomechanical testing and was not a factor in tensile strength determination.

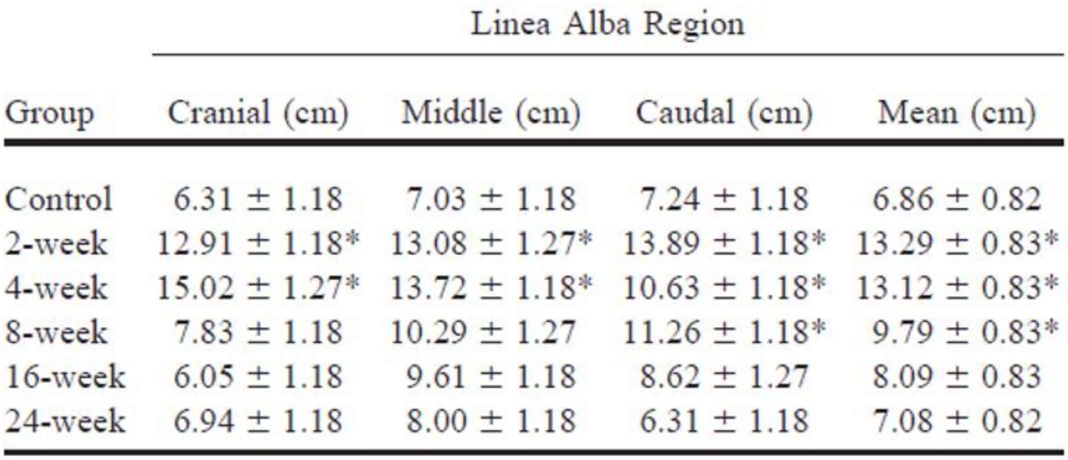
Distraction was performed utilizing an Instron materials testing system and cryoclamps, where a seal between the tissue and clamp was achieved through freezing via liquid nitrogen. The linea was distracted with a single cycle to failure. Results showed the mean tensile strength of the control group was significantly stronger than the 2-week healing specimens, with 484.9 +/- 58.3N for controlled verses 2 weeks of healing with 87.7 +/- 61.4N required to distract the linea (Figure 4).[5] There was no statistical difference at 4, 8, and 16 weeks; however at 24 weeks the strength was significantly greater than controls at 721+/- 57.9 (Figure 6).[5] The week 24 strength increase was thought to be due to mature scar tissue formation.[5]



	Linea Alba Region			
Group	Cranial (N)	Middle (N)	Caudal (N)	Mean (N)
Control	404.4 ± 77.5	528.5 ± 76.4	521.8 ± 76.1	484.9 ± 58.3
2-week	79.8 ± 77.7*	121.4 ± 83.0*	62.0 ± 79.7*	87.7 ± 61.4*
4-week	267.9 ± 93.6	278.8 ± 79.3*	370.8 ± 75.0	305.8 ± 61.7
8-week	509.2 ± 75.4	511.0 ± 80.0	376.0 ± 75.4	465.4 ± 56.5
16-week	534.9 ± 78.0	474.3 ± 74.6	424.3 ± 80.1	477.8 ± 57.2
24-week	645.1 ± 76.5*	903.6 ± 75.3*	614.3 ± 77.5	721.0 ± 57.9*

Figure 6. Least squared means (± SEM) of tensile strength of healing linea alba in horses at selected times after ventral midline celiotomy, taken from Chism et al. 2000

In regards to tissue (linea alba) thickness with 2, 4, and 8 weeks of healing, linea alba specimens were significantly thicker than controls, whereas at 16 and 24 weeks tissue thickness returned to normal (Figure 7).[5] Histologically, the 2 week samples were primarily composed of granulation tissue, unlike the dense mature collagen of control samples. Immature collagen that had not formed bundles was present at 4 weeks and was mature by 8 weeks. At 8, 16, and 24 weeks abundant mature collagen fibers formed into bundles, was present.[5] Based on these findings, we can conclude that the incision is at its weakest point 2 weeks following celiotomy and, for an uncomplicated case, the linea alba would be comparable with non-incised tissue in both strength and morphology by week 8.

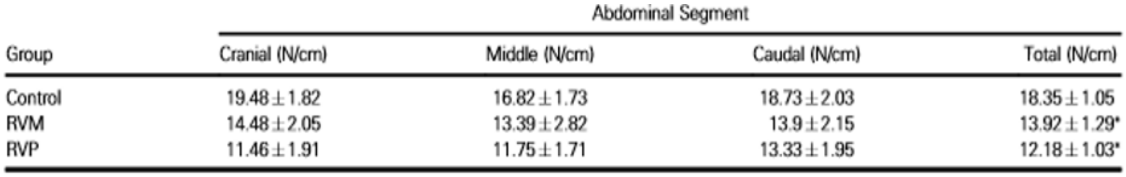


	Linea Alba Region			
Group	Cranial (cm)	Middle (cm)	Caudal (cm)	Mean (cm)
Control	6.31 ± 1.18	7.03 ± 1.18	7.24 ± 1.18	6.86 ± 0.82
2-week	12.91 ± 1.18*	13.08 ± 1.27*	13.89 ± 1.18*	13.29 ± 0.83*
4-week	15.02 ± 1.27*	13.72 ± 1.18*	10.63 ± 1.18*	13.12 ± 0.83*
8-week	7.83 ± 1.18	10.29 ± 1.27	11.26 ± 1.18*	9.79 ± 0.83*
16-week	6.05 ± 1.18	9.61 ± 1.18	8.62 ± 1.27	8.09 ± 0.83
24-week	6.94 ± 1.18	8.00 ± 1.18	6.31 ± 1.18	7.08 ± 0.82

Figure 7. Least mean squared (± SEM) thickness of healing linea alba in horses at selected times after ventral midline celiotomy, taken from Chism et al. 2000

In 2014, Boone et al took a different approach to qualifying the healing characteristics of the ventral midline incision with the perspective of cases requiring acute repeat surgery during the initial early wound healing phase.[11] Occurrence of incisional complications increases after repeat celiotomy, understanding the underlying cause is important to improve patient care. This increase could be related to healing characteristics of the approach which is performed either through the previously made ventral midline incision or a separate incision, through the abdominal musculature (ventral paramedian).

Overall a significant difference was observed between control horses and the treatment groups (repeat ventral median [RVM], repeat ventral paramedian [RVP]), however, no difference between the treatment groups or the three abdominal segments (cranial, middle and caudal) was observed (Figure 8).[11] Both RVM and RVP had lower tensile strength supporting Chism et al's findings of incised linea alba being weaker than normal tissue in the first 2 weeks post-operatively. In addition to tensile strength, incisions were subjectively scored daily for incisional complications (edema, drainage, and dehiscence) and abdominal segments were submitted for histopathology following euthanasia to assess healing. Histologically the RVM incisions had higher healing scores, while there was no difference in daily subjective incision scores.[11] This information supports best practices, with surgeon's using clinical judgement and integrity of the original wound to make their abdominal approach decision and provided further comparative information for future areas of research.



	Abdominal Segment			
Group	Cranial (N/cm)	Middle (N/cm)	Caudal (N/cm)	Total (N/cm)
Control	19.48 ± 1.82	16.82 ± 1.73	18.73 ± 2.03	18.35 ± 1.05
RVM	14.48 ± 2.05	13.39 ± 2.82	13.9 ± 2.15	13.92 ± 1.29*
RVP	11.46 ± 1.91	11.75 ± 1.71	13.33 ± 1.95	12.18 ± 1.03*

Figure 8. Mean (±SD) tensile strength per unit length of abdominal segments obtained from control, repeat ventral median (RVM) horses and right ventral paramedian (RVP) horses, taken from Boone et al. 2014

E. Incisional Complications

Incisional complications following ventral midline celiotomy (VMC) have been correlated with increased morbidity and mortality in the equine population.[2] These vary from mild, self-limiting conditions to those that increase duration of hospitalization and costs incurred by the client. While more severe complications can even be life-threatening or prevent return to athletic function. Common complications include edema occurring in 74% of cases, drainage and or infection in 11-42%, herniation 0.8-18%, and dehiscense 0.3-4%.[2] The wide range in prevalence is due to the variable definitions of such complications like surgical site infection. However, it can still be appreciated that incisional complications are common and an important area of continued work.

A number of studies have looked at methods of preventing incisional complications. One of the methods includes prophylactic antimicrobials, although best practice of drug administration is still debated in terms of dosing and optimal timing.[27] Human medicine recommends administration 60 min prior to surgical incision and re-dosing when surgery time surpasses 2 half-lives which has been extrapolated for veterinary medicine.[27] A retrospective study out of a large academic hospital identified that antibiotic use often did not follow these recommendations and there is wide variation in use making it difficult to ascertain true impact of antibiotics on SSI in the equine population.[27,28] In addition to antibiotics, reducing contamination of the surgery site either by the environment in recovery or through the nature of the surgical procedure (resection and anastomosis) has been investigated.[29] Abdominal bandages have been recommended both to prevent contamination of the incision as well as to reduce incisional edema however the studies vary in regards to closure technique, types of bandages utilized, frequency of bandage changes and duration of use making interpretation of results difficult.[2,30,31] In addition, it has been well-documented that prolonged anesthetic duration is associated with greater risk of incisional complications thus utilizing best practices to decrease overall anesthetic time is important.[29] Another important contributing factor in incisional complications however is the suture itself.

F. *Suture Characteristics and Contributions*

Ventral midline celiotomy incisions in the horse are prone to complications with the most devastating being complete incisional dehiscence. Incisions have been reported to fail because of infection, suture slippage or failure, knot slippage or failure, and/or suture tearing through the tissues holding the implanted suture.[19,32] In two ex vivo studies by Magee et al and Fierheller et al, 71% of sutures failed at the knot when evaluating closure with simple continuous pattern and 98.7% of sutures failed rather than the surrounding tissue with 90.4% of disruptions occurring at the knot-suture junction. The knot is under significant shear stress which decreases breaking strength by 30-35% making it more vulnerable to failure.[33,34] This work suggests that the knot may be a limiting factor for a secure celiotomy closure.[19,32] The optimal suture pattern and knot technique that minimizes the risks of dehiscence, infection, and herniation of the linea alba in the horse has not been established. However, the ideal closure would consist of a knot that is both secure and supports a high load to failure.

Suture itself is a foreign material that stimulates the inflammatory response of the body while supporting the stress and strain placed on the ventral abdominal wall.[6,35] The reactive tissue surrounding suture leads to complications such as suture sinus formation, abscess formation, incisional infection, and incisional failure.[35] Thus minimizing the amount of suture within the incision would be ideal to decrease stimulation local reaction, with the goal of providing as secure a closure as possible.

Method of abdominal closure historically has been based on surgeon’s preference with considerable variation in regard to suture material, bite size, and suture pattern used for closure of the equine linea alba.[3,36] Textbooks and previous studies recommended tissue bites anywhere from 4mm to 15mm from the edge of the linea alba.[33] As incisional failure could be related to improper selection based on suture material and the tissue being apposed, Trostle et al in 1994 looked at various tissue bite sizes as well as suture materials and sizes in order to provide controlled objective data to support surgical decisions.

The abdominal wall was harvested from cadavers, sectioned, and incised along the linea alba.[33] Suture bites were placed at varying distances from the linea then mounted in a materials testing apparatus for linear distraction testing as described earlier.[33] Tissue bite size had a significant effect on the suture breaking strength in a logarithmic fashion (Figure 9).[33] As shown in the figure below, breaking strength increased with increased bite size up to 15mm from the incision.[33] At this point the curve plateaus and large bites did not produce any additional security. It was suggested that this is due to the mechanical transition zone of the fibrous equine linea stress and strain curve being located between 12-15mm from the edge.[33]

In regard to suture material, failure mode was not affected by type with all 56 suture loops failing before complete fascial disruption and 52/56 (93%) failing at the knot. However, breaking strength was affected by material used (Figure 10). Size 5 USP polyester (Ethibond) had the greatest strength, however it is nonabsorbable thus more likely to be implicated in suture sinus and infection. The next strongest materials were size 3 USP polyglactin 910 (Vicryl) and 2 polyglycolic acid (Dexon) which are both absorbable multifilament materials with similar properties and strength.[37] Polygalactin 910 has been shown to retain 75% of tensile strength once implanted by 2 weeks, whereas polyglycolic acid retention is 65%.[37]

Stiffness is a measure of the force required to deform a material.[32] Stiffness of suture materials is attributed to the loss of mobility under a bending force in the fibers of the suture and usually increases with the suture size.[38,39] Stiffness is also related to the chemical constituents of the materials, which determines the magnitude of increase with size.[38,39] Stiffness determines the handling characteristics of a suture strand, such as softness and ease of knot-tying.[39] Polyglactin 910 is thought to have better handling properties than polyglycolic acid and in other studies showed greater knot-breaking strength.[37] However both the size 3 USP polyglactin 910 and 2 polyglycolic acid exhibited similar stiffness and strength in this study likely due to similar chemical composition (Figure 10).[37] It is important to choose suture materials with high stiffness for abdominal closure due to the forces applied to the equine linea alba.[37] Recommendations based on this cadaveric study are 15mm bite size with a large gauge, absorbable suture such as 3 Polyglactin 910, which have been adopted by most equine surgeons.[3,36]

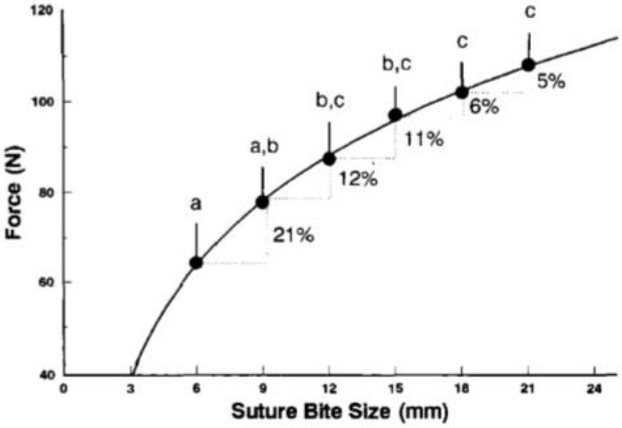
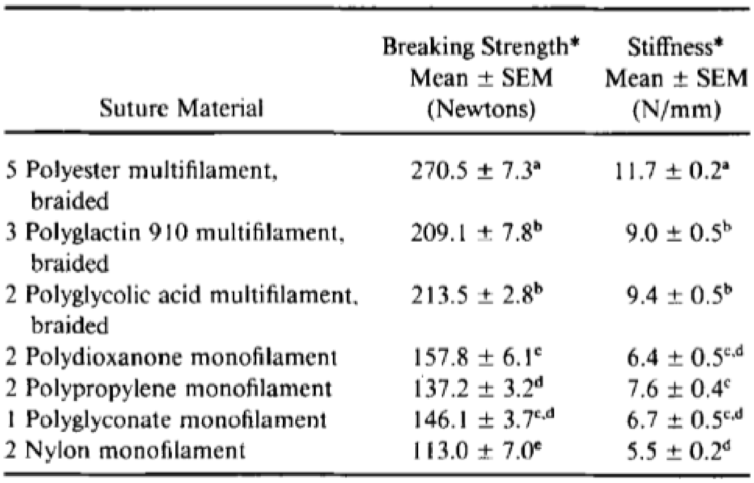


Figure 9. The effects of tissue bite size on breaking strength (mean ± SEM) of the adults equine linea alba, taken from Trostle et al. 1994.The dotted lines represent the percentage change in force between respective tissue bite sizes. The means with different letters are significantly different from each other.

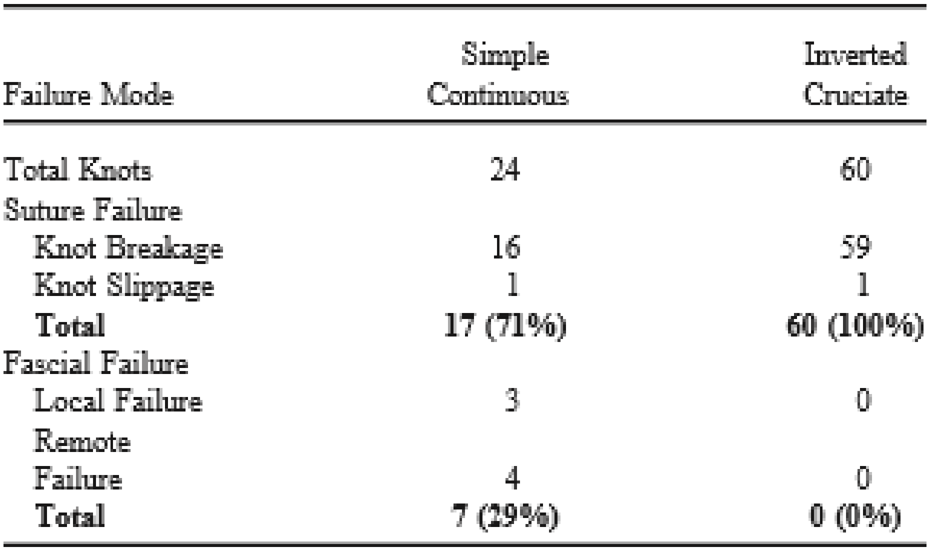


Suture Material	Breaking Strength* Mean ± SEM (Newtons)	Stiffness* Mean ± SEM (N/mm)
5 Polyester multifilament, braided	270.5 ± 7.3^{a}	11.7 ± 0.2^{a}
3 Polyglactin 910 multifilament, braided	209.1 ± 7.8^{b}	9.0 ± 0.5^{b}
2 Polyglycolic acid multifilament, braided	213.5 ± 2.8^{b}	9.4 ± 0.5^{b}
2 Polydioxanone monofilament	157.8 ± 6.1^{c}	6.4 ± 0.5^{c,d}
2 Polypropylene monofilament	137.2 ± 3.2^{d}	7.6 ± 0.4^{c}
1 Polyglyconate monofilament	146.1 ± 3.7^{c,d}	6.7 ± 0.5^{c,d}
2 Nylon monofilament	113.0 ± 7.0^{e}	5.5 ± 0.2^{d}

Figure 10. Breaking strength and stiffness of commercially available suture materials in adult equine linea alba, taken from Trostle et al. 1994

While Trostle et al guided suture placement and material selection, their work could not comment on ideal pattern. Magee et al in 1999 built on the previous clinical retrospective studies and in vitro work to provide information to reduce disruption of abdominal incisions. The study was also used to evaluate the intact cadaver model described earlier for assessment of incisional closure methods in horses.

Magee and colleagues found that the simple continuous pattern had significantly higher bursting pressure than the inverted cruciate pattern. This higher bursting pressure indicates that the continuous pattern may offer greater wound security than the interrupted inverted cruciate as it increased strength by 17%.[19] While in vitro work in horses previously showed no difference, the intact cadaver model has been reported to more accurately stimulate in vivo forces and the findings here are in agreement with similar studies in laboratory animals.[14,15] In addition, the continuous pattern utilized less suture material than the interrupted pattern, which reduces the amount of reactive foreign material within the surgical wound. Additionally, as previously mentioned Magee et. al found that 71% of knots in the simple continuous pattern failed with only 29% withstanding bursting pressure with fascial failure, contrasted by 100% of the cruciate knots failing (Figure 11).[19]



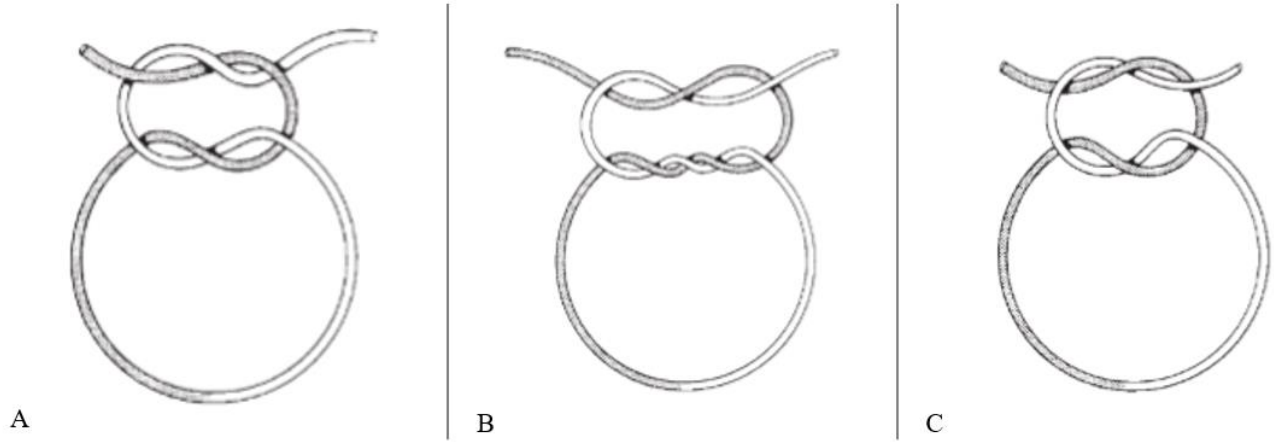
Failure Mode	Simple Continuous	Inverted Cruciate
Total Knots	24	60
Suture Failure		
Knot Breakage	16	59
Knot Slippage	1	1
Total	**17 (71%)**	**60 (100%)**
Fascial Failure		
Local Failure	3	0
Remote Failure	4	0
Total	**7 (29%)**	**0 (0%)**

Figure 11. Mode and type of failure of equine linea alba incisions closed with 3 Polygalactin 910 in a simple continuous or inverted cruciate pattern, taken from Magee et al. 1999

G. Surgical Knots

A consistent theme of suture characteristics is the contribution of the surgical knot to failure of the incisional closure. Within a line of suture, the surgical knot comprises the highest density of foreign material, while decreasing the tensile strength of the suture strand by 30-40%.[33,40,41] In addition, there is repeatable evidence both in vitro and ex vivo that the suture loops breaks most often at the suture knot interface.[42–44] The knot is under significant shear stress which decreases suture breaking strength by 30-35% making it more vulnerable to failure.[33,34] The knot is also affected by slippage of suture material through the knot as well as the suture elongation as the knot is formed and tightened.[39] Surgical knot performance is evaluated in vitro through assessing knot holding capacity (KHC) and relative knot security (RKS). KHC is defined as the breaking point of the suture or the maximum force that can be applied before breakage or slippage.[45–47] RKS is defined as the knot's ability to resist slippage and breakage as a load is applied.[47–50] RKS is calculated as a percentage of the load to failure, or KHC, of the suture material.[47] This is affected by the structural configuration of the knot, number of throws, suture end length, and the type and diameter of suture material.[37] Additionally, RKS is affected by the stiffness and coefficient of friction of a material, which is affected by bodily fluids during surgery.[37]

In the equine surgical field, the most common types of knots used include a square and surgeon's knot (Figure 12A, Figure 12B).[51] A knot is constructed of two suture ends combined with at least two throws on top of each other which are then tightened.[37] The differentiation between the correct square knot, and incorrect alternative "granny knot" is the direction of the throws (Figure 12C).[37] For a square knot, the direction is reversed each throw for the desired number.[37] The "granny knot" is not an ideal surgical knot as it is prone to slippage which is considered undesirable.[37]



Figures 12 A, B, C. Schematics of a square knot (A), surgeon's knot (B), and granny knot (C) taken from Equine Surgery, 5th edition.[6,37]

H. Self-Locking Knots

The forwarder knot (F) is a self-locking knot with a unique combination of throws, bearing resemblance to the fishing half-blood knot that can be used to start a continuous line of suture (Figure 13).[22,42,52,53] The knot is a unique combination of throws encircling the working end of the suture and needle drivers before the free end is grasped by the needle drivers and pulled through the loops. Based on in vitro testing in both dry and biologic conditions under linear distraction testing, the F knot had greater KHC and RKS for all suture materials and throws put through biomechanical testing than traditional S knots.[52,53] Therefore, even the weakest F knot still provided a significantly higher KHC and RKS than the strongest surgeon's or square knot. A particular aspect of this knot, is the RKS was 80% of the original tensile strength of the suture strand which contrasts the 50-60% of tensile strength the surgeons and square knots exhibited.[52,53] When the number of throws to achieve superior KHC and RKS was evaluated, a F knot with 4 throws using 3 polyglactin 910 was determined to be the most efficient knot.[52,53]

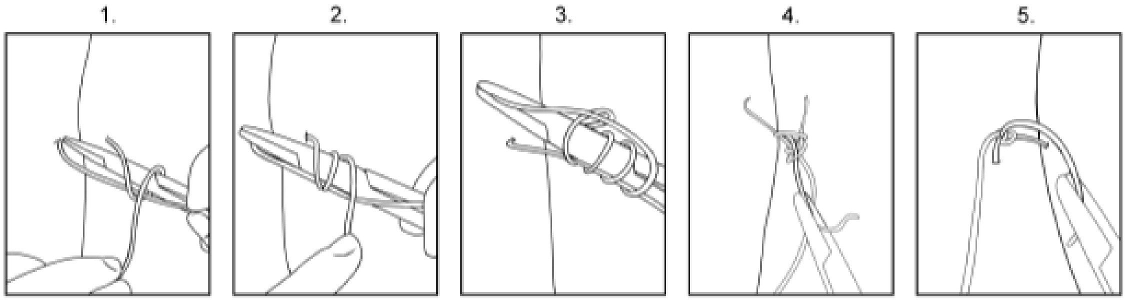


Figure 13. Schematic depiction of the formation of a forwarder knot, taken from McGlinchey et al. 2018

The Aberdeen knot (A) is a self-locking knot that consists of a combination of unique throws and turns that is used to secure the end of a continuous suture line (Figure 14).[22,42,54,55] Suture loops are passed through a loop of suture in the tissue known as throws until the end of the suture is passed through to secure the knot known as the turn.[56] This knot can be easily performed as a hand-tie as well. The study by Gillen et al in 2016 utilized linear distraction testing as described earlier under dry conditions comparing KHC and RKS of A knots to surgeons' knots. The study produced positive results similar to what has been found in the human and companion animal literature.[56–59] Bariatric surgeons recommend use of the Aberdeen knot with monofilament suture to end a continuous line for its improved RKS and decreased use of materials.[56,59] Gillen found that the A knots had higher KHC and RKS than surgeon's or square knots. Additionally, none of the Aberdeen knots unraveled, while a portion of square and surgeon's knots with <7 throws did. Aberdeen knots had a smaller volume and weight than both surgeon's and square knots with equal numbers of throws. The knot with the combined highest RKS and smallest size and weight was an Aberdeen knot with 4 throws using size 3 USP polyglactin 910. A major difference in Gillen's work was the recommendation of a multifilament versus monofilament suture material. This recommendation is due to the inherent forces applied to the equine ventral abdomen.[7,54] Multifilament suture, as shown by Trostle et al, is more suited for these forces applied along the equine ventral abdominal wall.[33,54]

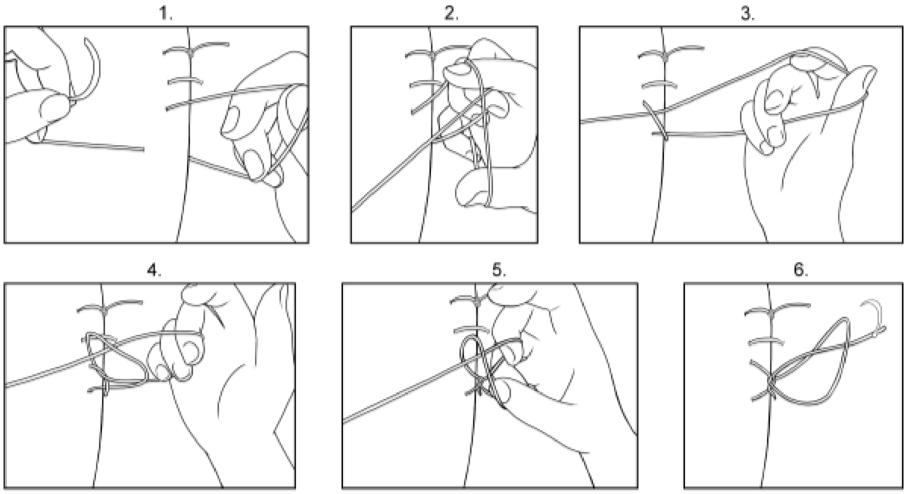


Figure 14. Schematic depiction of the combination of throws and turn to complete an Aberdeen knot, taken from McGlinchey et al. 2018

A limitation of the two previous studies was testing of the knot under dry conditions which does not reflect the clinical environment. Schaaf and colleagues recognized the importance of testing under various conditions, such as liquefied canine fat, to better stimulate the knot tying environment.[60] Coleridge et al exposed the Aberdeen, surgeons, and square knots tied with large gauge suture to balanced electrolyte solution, 1% sodium carboxymethylcellulose, equine serum, and equine fat then performed the same biomechanical linear distracting testing that was used by Gillen et al.[55] In a similar study, McGlinchey et al used the same biological media to compare biomechanics of the forwarder knot.[53] The objective was to further investigate the knot strength of suture materials and determine the effect of exposure to media commonly encountered in equine abdominal surgery. The KHC and RKS was improved for all knot types, most significantly for the sliding self-locking knots.[53,55] This is due to the lubricating effect of the fluid media utilized changing the coefficient of friction improving the knot security for large and small gauge suture.[58]

McGlinchey et al also performed an ex vivo cadaver study with the bursting strength testing model described earlier, to determine the bursting strength of the self-locking knots in comparison to a surgeon’s start and end knot in a continuous suture line. Results indicated that a simple continuous suture pattern with a combination of the forwarder start and Aberdeen end knot compared to a traditional surgeons' start and end knot provided a more secure closure with significantly higher bursting pressures (Figure 15).[22] In addition, the most common mode of failure of the self-locking knots was adjacent to the incision or mid-suture line in contrast to the surgeons’ failure at the knot.[22] The hypothesis that the closure time would be increased using the self-locking knots was refuted as there was no statistical time difference between closure techniques.[22]

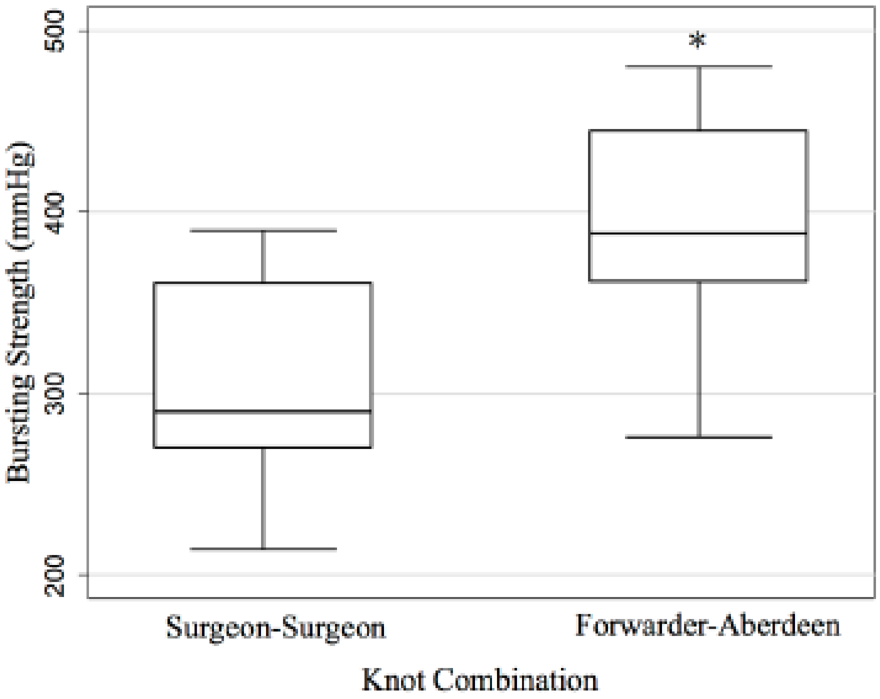


Figure 15. Box plots showing the difference in bursting strength of surgeon-surgeon and forwarder-Aberdeen knot combinations, taken from McGlinchey et al. 2018

Despite the consistent positive results of in vitro and ex vivo testing of self-locking knots utilizing large gauge suture in various conditions, significant limitations preclude the use of the knots in clinical cases. All testing was performed in a single plane of motion and single load to failure. As discussed earlier, the linea alba is a viscoelastic tissue that is uniquely suited to accommodate large cyclical changes in the stress and strain applied during events such as anesthetic recovery. It is also important to note that the sliding aspect of the self-locking knot will allow for progressive constriction of the avascular tissue, which may negatively affect wound healing. Therefore, in vivo testing where knots will be compared under dynamic conditions and healing quality can be assessed would be the next step towards transitioning to the use of self-locking knots in clinical equine abdominal closures.

II. Objectives

The objectives of in vivo testing of the self-locking knots for closure of a VMC are twofold. First, to determine the extent by which tensile strength of a VMC incision is affected by closure with a self-locking knot (F-A) combination compared to a surgeon's (S-S) knot combination. Second, to determine differences in early incisional healing between VMC incisions closed with either combination.

A. Hypotheses

We hypothesized that a VMC closure with a self-locking knot combination (F-A) will have greater tensile strength when compared to traditional closure (S-S). In addition, we also hypothesized that VMC closure with F-A will have improved tissue healing compared to closure with S-S.

III. Materials and Methods

All animal housing, handling, and procedures were approved by the Auburn University Institutional Committee for Animal Care and Use Committee (2018-3255).

A. *Study Population*

Healthy, adult horses (N=21) donated to the university for reasons unrelated to gastrointestinal disease were utilized for this study. All horses were determined to be free of abdominal wall defects with no history of previous celiotomy based on physical examination and ultrasound examination of the ventral abdomen by a board-certified radiologist (RC). Signalment and weight were recorded. Fourteen horses were randomly assigned to either the F-A knot group (N=7) or the S-S knot group (N=7) using a random number generator (www.random.org). Control horses (N=7) that did not undergo celiotomy and were euthanized for reasons unrelated to gastrointestinal disease were also included for biomechanical testing.

B. *Preoperative Care*

All horses were kept in individual stalls throughout the study and were acclimated to the hospital environment prior to entering the study. All horses were maintained on a diet of hay (coastal/alfalfa) and a complete pelleted feed.

Twelve hours before surgery, feed but not water, was withheld. The morning of surgery an IV catheter was placed in either the left or right jugular vein. Horses were administered flunixin meglumine (1.1 mg/kg IV), procaine penicillin G (22,000 IU/kg IM), gentamicin (6.6 mg/kg IV), and a tetanus toxoid (1 ml/horse IM) 15 minutes prior to anesthesia. A packed cell volume (PCV) and total protein (TP) were obtained for each horse prior to surgery.

C. Anesthesia

After sedation with xylazine hydrochloride (1.1 mg/kg IV), general anesthesia was induced with ketamine hydrochloride (2.2 mg/kg IV) and midazolam (0.05 mg/kg IV). Horses were orotracheally intubated with an appropriate sized endotracheal tube and isoflurane was delivered in oxygen for maintenance of anesthesia. Balanced polyionic fluids were administered IV (10 ml/kg) during the procedure. Mean arterial blood pressure was maintained above 70 mmHg with dobutamine at the discretion of the anesthesiologist. At the conclusion of the surgical procedure, horses were placed into a padded recovery stall in either lateral recumbency and administered acepromazine (0.01 mg/kg IV) and detomidine (0.01 mg/kg IV) to facilitate a smooth recovery. Horses were allowed to recover from anesthesia unassisted. Recoveries were under direct observation of an experienced veterinarian and/or anesthesiologist.

D. Surgical Technique

Anesthetized horses were positioned in dorsal recumbency and the ventral abdomen was clipped. The surgical site was prepared by scrubbing the skin for a minimum of 5 minutes with three alternating applications of 4% chlorhexidine gluconate surgical scrub and sterile saline. The surgical site was then draped for aseptic surgery. A 25 cm VMC was made beginning 2.5 cm cranial to the umbilicus and extending cranially (Figure 16).

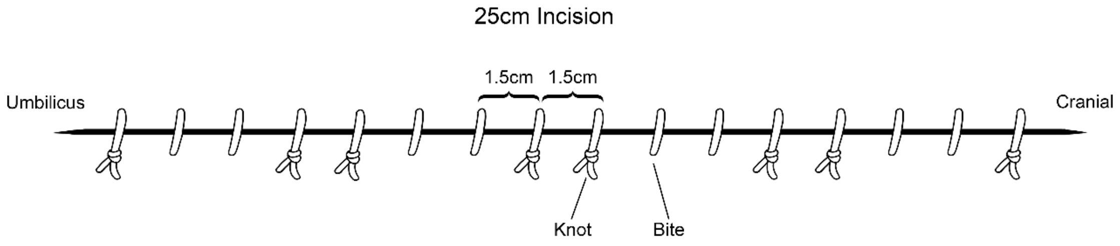


Figure 16. Illustration of the closure – 4 individual simple continuous runs with start-end knot determined by treatment group.

The peritoneum was digitally penetrated and enlarged, but abdominal exploration was not performed. The linea alba was apposed using four strands of 3 USP polyglactin 910 (Vicryl, Ethicon US, LLC, Somerville, NJ) in four separate runs of simple continuous with knot type determined by experimental group (Figure 17). The F-A start knot was tied with 4 throws and end knot tied with 3 throws and 1 turn.[22,52,54,55] The S-S start knot was tied with 5 throws and end knot was tied with 6 throws. [22,52,54,55] The F and S start and end knots were tied with 8” Mayo-Hegar needle drivers, while the A end knots were tied by hand.

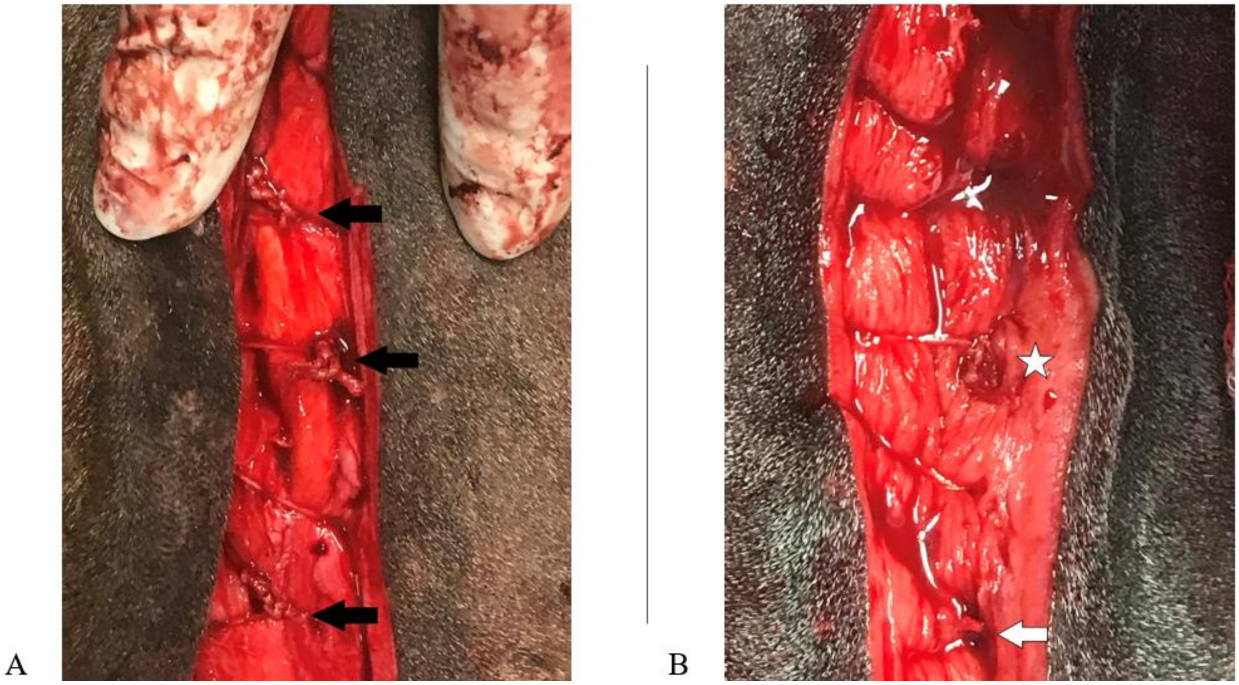


Figure 17. Incision closures in situ with the S-S combination on the left (A) and F-A combination on the right (B). Please note the size difference between the knots in the surgical field. Caudal is the top of the picture and cranial the bottom of the picture. Surgeon's knots, black arrows; Forwarder start knot, white arrow; Aberdeen end knot, white star.

All surgeries were performed by the same two surgeons (RH, KW) with incision and closure performed by one surgeon for all subjects (KW). A sterile ruler and tissue marker were used to ensure each bite of suture was 15 mm from the incision, 15 mm apart, and 15 mm from the next starting knot. A modified subcuticular pattern was used for the second layer including the subcutaneous tissue and dermis with 2-0 poliglecaprone 25 (Ethicon US, LLC, Somerville, NJ). Total surgical procedure and anesthetic time was recorded. Incisions were covered with sterile gauze overlaid with a betadine impregnated adhesive bandage (Ioban®, 3M, St. Paul, MN) for recovery.

E. *Postoperative Care and Monitoring*

Post-operatively, horses were administered a tapering course of flunixin meglumine (0.55 – 1.1 mg/kg, orally every 12 hours) for 72 hours following surgery. Horses were immediately allowed free access to water, while feed (coastal/alfalfa hay and pelleted feed) was reintroduced 6 hours after recovery from anesthesia.

Once the horses were returned to the stall, the protective covering was replaced by a sterile towel covering the incision, overlaid by an elastic adhesive abdominal support bandage (Elastikon®, Johnson & Johnson, New Brunswick, NJ). The bandage was maintained and monitored for strike-through for 3 days post-operatively and then removed for the remainder of the study period. Following removal, the incisions were monitored daily and subjectively evaluated for integrity, edema (absent, mild = portion of the incision, moderate = full length of the incision, severe = shelf visible from a distance), and drainage (location and character—absent, serous, purulent) by the same two evaluators (LB, FC) blinded to treatment (Figure 18). Any purulent drainage was sampled for culture and sensitivity.

Figure 18. Examples of peri-incisional edema scores of absent (A), moderate (B), and severe (C). Head is to the left and tail is to the right. Figure 16C also is an example of purulent incisional drainage.

Horses were hand-walked 15 minutes twice daily and monitored hourly for signs of colic throughout the study. Physical examinations were performed every 6 hours for the first 24 hours after celiotomy, then every 12 hours for the duration of the study. Rectal temperature, heart rate, respiratory rate, mucous membrane color, mucous membrane hydration, capillary refill time, intestinal borborygmi, fecal production, water consumption, and presence or absence of digital pulses were recorded.

F. *Abdominal Wall Specimen Collection*

All horses were euthanized 10 days post-celiotomy with an overdose of sodium pentobarbital (10ml/100lb IV). The celiotomy was examined grossly for signs of drainage, infection, or dehiscence. The abdominal wall spanning 13 cm to the right and left of midline (26 cm total width) and 3 cm cranial and caudal to the incision was collected *en bloc* for treatment and control horses. The incision was measured and divided into 4 equal segments (5 cm long x 26 cm wide) each containing a separate run of suture (caudal, middle caudal, middle cranial, cranial). The segments were examined to ensure there was no evidence of suture along the cut edges of the segment indicating that the segment was harvested through the continuous line of suture rather than between the start knot of one segment and the end knot of the adjacent segment. Three segments per horse were subjected to biomechanical testing. One randomized sample from each subject was selected for histologic evaluation and further divided into 3 equal (1.5 cm long x 4.5 cm wide) caudal [start knot], suture line, and cranial [end knot] segments. Control abdominal wall was collected, divided in the same fashion, and all four segments subjected to biomechanical testing.

G. *Mechanical Evaluation*

Methods for abdominal segment preparation, storage, and testing are similar to methods described previously.[61] The abdominal segments were mounted flatly to a piece of saline (0.9% NaCl) soaked cardboard using 1” 25g hypodermic needles, wrapped in a saline soaked surgical towel, and sealed in a plastic storage bag. Segments were stored at -80°C until mechanical testing.

Each abdominal segment was removed from the freezer and defrosted at room temperature prior to testing. The skin, subcutaneous tissues, and rectus abdominus muscle were dissected from the external rectus sheath ~10 cm from the edge of each abdominal segment leaving ~6 cm of full thickness tissue at the center of each sample. Samples were kept moist with saline solution and saline soaked towels during testing. Suture material was not removed from the abdominal segments during preparation. Three segments were available per test subject (N = 21 for F-A and S-S groups) and 4 segments available for control subjects (N = 28). One abdominal segment from each location (caudal, middle caudal, middle cranial, and/or cranial) was mounted in a material testing apparatus (Instron Universal Testing System, Instron®, Norwood, MA) using custom-engineered cryoclamps to prevent fascial slippage (Figure 19).[61] Each of the segments were tested to failure.

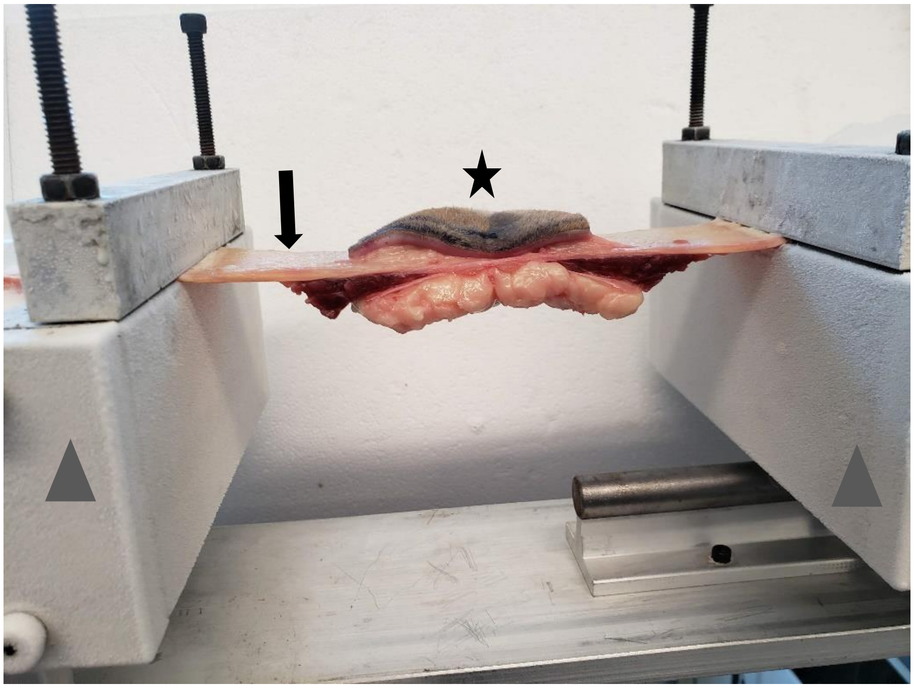


Figure 19. Abdominal segment mounted in a material testing apparatus using custom-engineered cryoclamps to prevent fascial slippage. Segment was placed in the testing apparatus such that the celiotomy incision was approximately 10 cm from either clamp. Rectus sheath, black arrow; linea alba, black star; cryoclamps, gray triangles

For all horses, each abdominal segment was placed in the testing apparatus such that the celiotomy incision was approximately 10 cm from either clamp (Figure 19). Dry ice was used to freeze the samples to the cryoclamps while the temperature of the external rectus sheath and celiotomy incision was monitored utilizing a thermal camera (FLIR 6, VWR, Radnor, PA) [Figure 20]. Once the temperature of the tissue adjacent to the cryoclamps reached below freezing point and a clear frost line could be observed, the tissue was distracted at 0.42 mm/s. The maximum load sustained was recorded in Newtons (N) and the tensile strength per unit area (N/cm^2) was calculated. Location of failure was also recorded as at the incision or rectus sheath (away from the incision) (Figure 21).

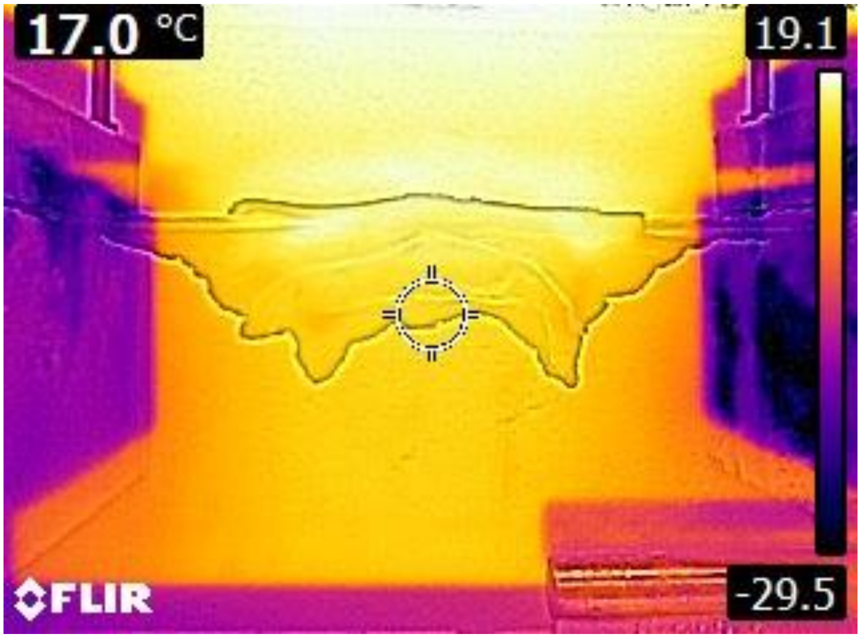


Figure 20. Temperature of the external rectus sheath and celiotomy incision was monitored utilizing a thermal camera while mounted in the custom-engineered cyroclamps. Color indicates temperature range between 19.1°C (yellow) to -29.5°C (deep purple).

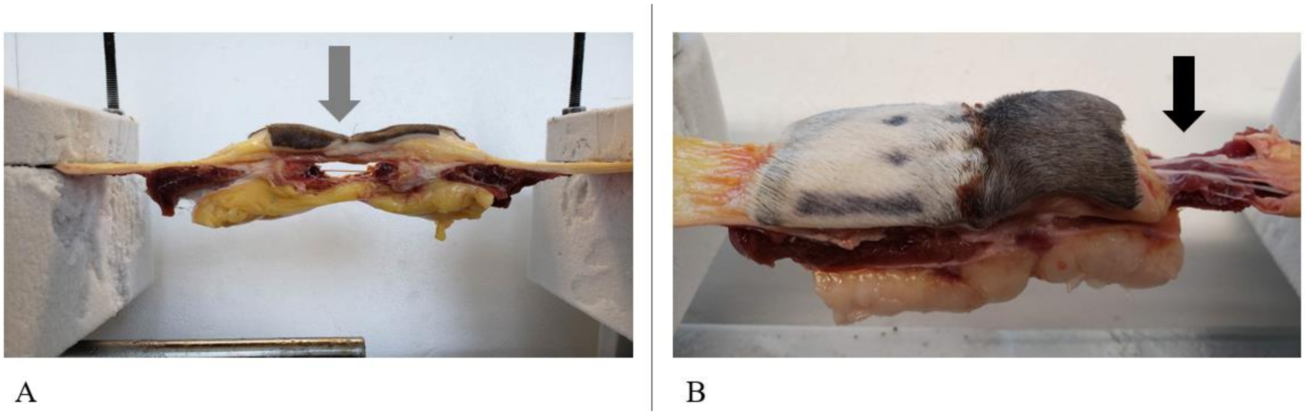


Figure 21. Locations of failure of abdominal segments under mechanical testing. Failure at the incision (A) denoted by gray arrow. Failure at the rectus sheath (B) denoted by black arrow.

H. Histologic Evaluation

Tissues for histologic evaluation were preserved in 10% buffered neutral formalin. Preserved specimens were processed routinely, paraffin embedded, and sections were stained. All sections were histologically graded by a boarded pathologist (RCC) for evidence of healing, inflammation, and infection using a previously described grading scale for VM celiotomies (Table 1).[61] In addition, incisions were evaluated for necrosis and focal accumulation of fibrin and hemorrhage. Fibrosis depth (cm) was measured from the subcutis to the peritoneal face of the linea alba at the site of celiotomy was measured.

Table 1. Histologic Grading System for Incisions[22]

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Healing[22]	Edges unopposed by fibrin clos/fistula formation	Edges opposed by fibrin clot	Early ingrowth of fibroblasts and capillaries/early granulation tissue	Collagen production within lattice of numerous small caliber blood vessels/organizing granulation tissue	Abundant collagen with scattered small caliber blood vessels/well organized granulation tissue/fibrous scar tissue
Inflammation[22]	None	Scant inflammation	Mild inflammation	Moderate inflammation	Marked inflammation and/or abscess formation
Infection[22]	No microorganisms seen	Rare	Small scattered colonies	Large colonies	Confluent colonies
Necrosis[22]	None	Mild (<25% of section)	Moderate (25-50% of section)	Severe (> 50% of section)	
Focal accumulations of fibrin and hemorrhage[22]	None	Mild (<25% of section)	Moderate (25-50% of section)	Severe (> 50% of section)	

I. *Statistical Analysis*

A priori power analysis with statistical software (G*Power V 3.1.94, Dusseldorf, Germany) was performed to ensure adequate sample size in light of economical and ethical constraints.[62] The effect size was calculated based on findings from previous in vivo work testing tensile strength of equine linea alba in early healing phase of 2 weeks.[61] With statistical significance set at $P < 0.05$ and statistical power set at > 0.08, a total sample size of 14, with sample size groups of seven each, would provide appropriate power.

All data analyses were performed using commercial statistical software (SAS V 9.4, Cary, NC). A threshold of $P < 0.05$ was used for statistical significance. Age, weight, PCV, TP, anesthesia and procedure time were compared between groups by student t-tests. The folded form F statistic was used to test if variances were equal between groups. Gender and breed proportions were compared between knot groups by Fisher's exact tests. Heart rate, temperature and respiratory rate were compared between knot groups by linear mixed models (LMM). The model had fixed factors of group, time and a group by time interaction.

Tensile strength and maximum load were compared between knot groups by LMMs. The first model had fixed factors of group, segment and a group by segment interaction. The second model had the same fixed factors and the addition of fixed covariates of age and weight to control for effects of age and weight. Multiple comparisons were adjusted for using Tukey's test. Location of failure was compared between F-A and S-S groups by a generalized linear mixed model (GLMM). The model had fixed factors of group and section. A multinomial distribution with a generalized logit link function was assumed.

Edema was compared between knot groups by a GLMM. The model had fixed factors of group, time, a group by time interaction and surgeon. A multinomial distribution with a cumulative logit link function was assumed. Drainage location was compared between knot groups by Fisher's exact tests for each time and surgeon. Drainage character was compared between F-A and S-S groups by a GLMM. The model had fixed factors of group, time and surgeon. A multinomial distribution with a generalized logit link function was assumed.

Healing, inflammation, necrosis and fibrin formation (FF)/hemorrhage were compared between knot groups by a GLMM and fibrosis thickness was compared between knot groups by a LMM. Each model had fixed factors of group, section and a group by section interaction. A binary distribution with logit link function was assumed for healing and necrosis. A multinomial distribution with a cumulative logit link function was assumed for inflammation and FF/hemorrhage. Simple effects of group were tested for each section.

All LMM and GLMMs had random intercepts for horse to account for within horse correlation. Model residuals for LMMs were examined to evaluate the assumption of normality. Satterthwaite degrees of freedom method were used in all LMM and GLMMs.

IV. Results

A. Study Population

There were two females and five castrated males in the control group; one female and six castrated males in the F-A group; and three females and four castrated males in the S-S group. Breeds were Thoroughbred (N = 4), Quarter Horse (1), American Paint Horse (1) and Warmblood (1) in the control group; Quarter Horse (3), Thoroughbred (2), Warmblood (1), and Mixed breed (1) in the F-A group; and Quarter Horse (2), Arabian (2), Mixed breed (2), and Warmblood (1) in the S-S group. Horse demographics per treatment group are listed in Table 2. Mean ± SD ages were: control group, 11.6 ± 4.7 years [range, 6-20]; F-A group, 13.4 ± 2.2 years [range, 4-20 years]; S-S group, 18.4 ± 6.9 years [range, 12-28 years]. Mean ± SD weights were: control group, 466.7 ± 102.4 kgs [range, 317.5 – 636.4 kgs]; F-A group, 498.9 ± 78.4 kg [range, 373.6 – 600 kg]; S-S group, 463.2 ± 110.9 kg[range, 353.6 – 686.4 kg]. There was no significant differences in age (*P* = 0.171), weight (*P* = 0.499), breed (*P* = 0.650) and gender (*P* = 0.559) between groups.

B. Physical Examination Findings

All 14 treatment horses recovered from anesthetic episodes without complications. There was no significant difference between PCV or TS pre-celiotomy (PCV *P* = 0.773, TS *P* = 0.675). No significant differences were detected in vital parameter variables between groups (temperature *P* = 0.698, pulse *P* = 0.777, respiration *P* = 0.843) during the study period. One F-A horse (horse 11) and two S-S horses (horses 4, 5) developed fevers (>101°F) post-celiotomy (Table 2). Horse 4 had a fever of 101.2-101.8°F that appeared to be related to the incision as there was significant peri-incisional edema and purulent discharge noted from the incision. Horses 11 and 5 had fevers (101.5°F and 101.3°F, respectively) within 24 hours of surgery that resolved within 6 hours classified as a transient post-operative fever and no further diagnostics were performed.

Table 2. Case details for study subjects with treatment group, surgical time, and abnormal post-operative vital parameters

Horse	Age (yrs)	Weight (kg)	Sex	Breed	Knot Type	Anesthesia time (min)	Procedure time (min)	Fever Post-op (>101°F)	Tachycardia Post-op (>48bpm)	Tachypnea Post-op (>24brpm)	Incision Drainage
1	22		G	Mix	S-S	87	53				
2	11		G	QH	F-A	102	68			Yes, 26brpm Days 8, 9	Yes, Purulent Day 6
3	8		G	WB	F-A	84	36				
4	12		G	Mix	S-S	95	63	Yes, 101.2-101.8°F, Days 3, 4	Yes, 52bpm Days 4, 8, 10	Yes, 28-30brpm Days 1, 4, 8, 9	Yes, Purulent Day 6
5	12		G	WB	S-S	77	38	Yes, 101.3°F Day 1			Yes Serosanguinous, Day8
6	16		G	TB	F-A	77	38				Yes, Serosanguinous, Day 6
7	17		G	TB	F-A	88	51		Yes, 52bpm Days 1, 3		Yes, Serosanguinous Day 7
8	26		F	Arab	S-S	76	45				
9	12		F	Arab	S-S	93	60				
10	17		F	QH	S-S	82	41				
11	4		F	Mix	F-A	78	37	Yes, 101.5°F Day 1			
12	18		G	QH	F-A	84	40			Yes, 30brpm Day 5	
13	20		G	QH	F-A	78	27				
14	28		G	QH	S-S	74	37				

F, female; G, gelding; Arab, Arabian; Mix, mixed breed; QH, Quarter Horse; TB, Thoroughbred; WB, Warmblood
yr, year; min, minutes
kg, kilograms
bpm, beats per minute; brpm, breaths per minute

Two horses, one from each group, had mildly elevated heart rates (>48 beats/min); while two horses from the F-A group (horse 2, horse 12) and one from the S-S group (horse 4) had elevated respiratory rates (>24 breaths/min) at one or more than one time point (> 12 hours post-operatively) during the study (Table 2). No other clinical signs of discomfort were present and vital parameters were normal at the following examinations thus no further diagnostics were performed. For horse 4, at one time point the increased heart rate and respiratory rate occurred at the same time point as increased temperature prior to purulent discharge being noted from the incision. One horse from the S-S group (horse 10) had decreased fecal output and reduced appetite following VMC necessitating palpation per rectum and nasogastric intubation with water once on day 3. Following intervention, feeding off the ground was also initiated due to significant carpal osteoarthritis which improved appetite dramatically.

C. *Procedure and Anesthesia times*

Anesthesia and procedure times per horse are listed in Table 2. Mean ± SD for anesthesia length were: F-A group, 84.4 ± 8.6 min [range, 102 – 77 min] and S-S group, 83.4 ± 8.4 min [range, 95 – 74 min]. Mean ± SD for procedure length were: F-A group, 42.8 ± 13.1 min [range, 68 – 27 min] and S-S group, 48.1 ± 10.6 min [range, 63 – 37 min]. There was no significant difference between anesthesia time ($P = 0.831$) or procedure time ($P = 0.423$) between groups.

D. Incisional Edema

Three (3/7, 42.8%) F-A horses had severe (grade 3, visible shelf) incisional edema compared to six (6/7, 85.7%) S-S horses for at least one day during the study. Mean ± SD incisional edema scores were: F-A group, 1.53 ± 0.78 (range, 0 – 3); S-S group, 1.89 ± 0.85 (range, 0 – 3) (Table 3). There was no difference in the probability of having worse incisional edema between F-A and S-S groups (*P* = 0.131).

Table 3. Mean (± SD) Incisional Scores, Histologic Grading, and Incisional Fibrotic Depth (cm) of Celiotomy Incisions Closed with either Forwarder-Aberdeen or Surgeon's-Surgeon's Knot Combinations

Group	Segment	Edema	Healing	Fibrotic Depth	Inflammation	Necrosis	FF/Hemorrhage Accumulation
F-A	Cranial		3.43 ± 0.49	2.26 ± 0.65	1.13 ± 0.78	0.57 ± 0.49	0.71 ± 0.45
	Suture Line		3.14 ± 0.35	1.90 ± 0.19	1.29 ± 0.45	0.86 ± 0.35	0.57 ± 0.49
	Caudal		3.57 ± 0.49	2.04 ± 0.45	1.00 ± 0	0.25 ± 0.43	0.43± 0.49
	All	1.53 ± 0.78	3.38 ± 0.49	2.07 ± 0.49	1.14 ± 0.56	0.52 ± 0.50	0.57 ± 0.49
S-S	Cranial		3.57 ± 0.49	1.89 ± 0.52	1.29 ± 0.45	0.57 ± 0.49	0.43 ± 0.49
	Suture Line		3.71 ± 0.45	2.06 ± 0.70	1.43 ± 1.18	0.43 ± 0.49	0.71 ± 0.70
	Caudal		3.43 ± -.49	1.90 ± 0.39	1.29 ± 1.38	0.43 ± 0.49	0.71 ± 0.45
	All	1.89 ± 0.85	3.57 ± 0.49	1.95 ± 0.56	1.33 ± 1.04	0.48 ± 0.50	0.62 ± 0.58

Forwarder-Aberdeen (F-A) group was closed with a forwarder start knot (caudal) and Aberdeen end knot (cranial). Surgeon's-Surgeon's (S-S) group was closed with a surgeon's start knot (caudal) and end knot (cranial).

E. *Incisional Complications*

There was no evidence of incisional dehiscence detected in any horse at any time point during the study. F-A horses had a slightly higher occurrence of incisional drainage (3/7, 42.8%) compared to S-S horses (2/7, 28.6%). There was no difference in drainage location between F-A and S-S groups ($P > 0.300$) and no difference in the probability of having worse discharge character between F-A and S-S groups ($P = 0.536$).

Of the three F-A horses (horses 2, 6, 7) that developed incisional drainage, horses 6 and 7 had serosanguinous discharge characterized by gross fluid accumulation in the form of fluid droplets from the incision either at the caudal (horse 6) or cranial (horse 7) aspects of the incision. Neither of these horses developed a fever and at time of collection for mechanical testing there was no gross evidence of infection therefore these incisions were not cultured. Horse 2 had serous discharge present starting on day 3 that progressed to purulent discharge on day 6 with evidence of infection at time of collection. Culture of this incision grew a mixed population (*Escherichia coli, Klebsiella pneumoniae, Bacillus species, coagulase negative Staphylococcus, Streptococcus species alpha haemolytic*) sensitive to majority of antibiotics tested. While horse 2 did not develop a fever post-operatively, the subject got loose during a hand-walk on day 3, resulting in exuberant exercise, incisional drainage commenced following return to the stall. Due to no further extraordinary circumstances it was elected to maintain the subject in the study.

Of the two S-S horses (horses 4, 5) that developed incisional drainage, horse 5 had serosanginous discharge cranially. Horse 5 had a mild fever (101.3°F) within 12 hours of surgery and at time of collection for mechanical testing there was no gross evidence of infection therefore was not cultured. Horse 4 had excessive cutaneous surgical bleeding during the celiotomy prompting use of active suction to facilitate closure. Active sanguineous drainage was present 24 hours post-operatively with no evidence of acute dehiscence. Additional adhesive elastic tape was applied over the initial abdominal bandage to provide further precautionary support to the incision. The horse then developed purulent discharge day 6 following an elevated rectal temperature day 3 and 4 with evidence of infection at time of collection for mechanical testing. Culture and sensitivity produced an *Escherichia coli* with a very narrow susceptibility profile (susceptible to enrofloxacin, imipenem, marbofloxacin, and polymyxin B).

F. Tensile Strength

Mean ± SD for tensile strength (N/cm^2) were: control group, 44.55 ± 9.94 (range, 21.06-66.69); F-A group, 23.79 ± 8.77 (range, 11.89-41.61); S-S group, 24.56 ± 7.64 (range, 10.57-43.28). (Table 4) There was no significant differences between the tensile strength of F-A and S-S groups ($P = 0.975$, $P = 0.455$ age and weight adjusted). Tensile strengths in the control group were significantly higher than both the F-A ($P < 0.001$) and the S-S group ($P < 0.001$) (Figure 22). There was no significant interaction of treatment group and abdominal segment [caudal, caudal middle, cranial middle, or cranial] ($P = 0.492$) which indicates that group differences were consistent between abdominal segments. Tensile strength decreased a small amount with age, average decrease of 0.5 N/cm^2 per year, which was not significant ($P = 0.107$). Tensile strength was not affected by weight with an average decrease of 0.16 N/cm^2 per 10 kg or 1.6 per 100 kg ($P = 0.466$).

Table 4. Mean (± SD) Tensile Strength per Unit Area of Abdominal Segments Obtained from Control Horses, F-A Horse, and S-S Horses

Study Group	Cranial Segments (N/cm^2)	Middle Cranial Segments (N/cm^2)	Middle Caudal Segments (N/cm^2)	Caudal Segments (N/cm^2)	Total (N/cm^2)
Control (no celiotomy)	44.11 ± 12.66	47.63 ± 11.94	43.75 ± 5.68	42.75 ± 6.85	44.55 ± 9.94
F-A	24.07 ± 3.95	22.56 ± 8.66	20.99 ± 7.64	24.08 ± 11.08	23.79 ± 7.64 *
S-S	28.87 ± 7.67	20.87 ± 4.55	22.79 ± 8.46	26.07 ± 6.44	24.56 ± 7.64 *

Total refers to the mean tensile strength per unit area of all abdominal segments within the group.
*Significantly different ($P < 0.05$) from control

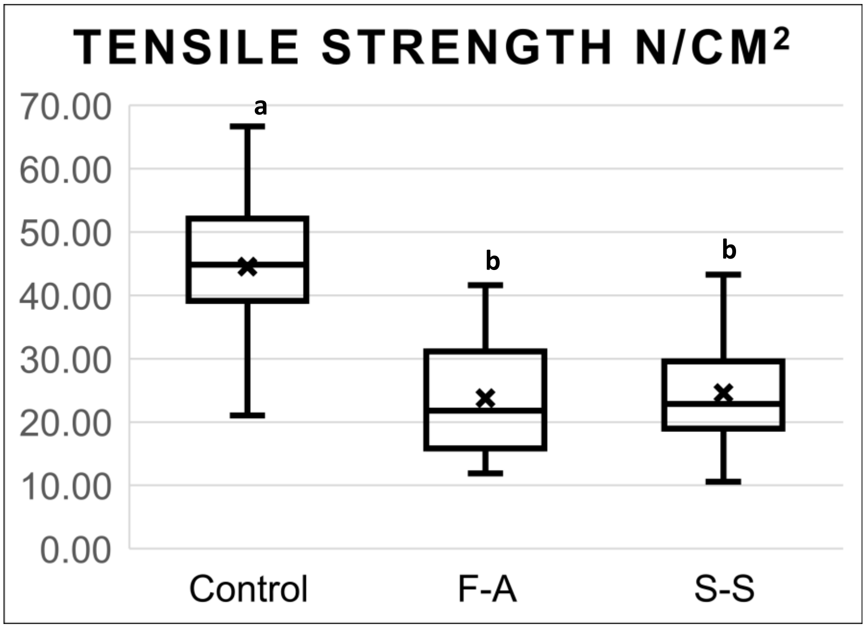


Figure 22. Box and whisker plot of tensile strength (N/cm^2) of control, forwarder-Aberdeen (F-A), and surgeons-surgeons (S-S) knot combination treatment groups. Different letters indicate significant difference between groups ($P < 0.05$).

G. *Location of Failure*

Statistically there were no differences in the location of failure between the F-A and S-S groups (*P* = 0.239) or between abdominal segments (*P* = 0.791). However the sections in the F-A group did fail more frequently (15/21, 71%) along the rectus sheath versus S-S (6/21, 26%), consistent with the control group (28/28, 100%) (Figure 23).

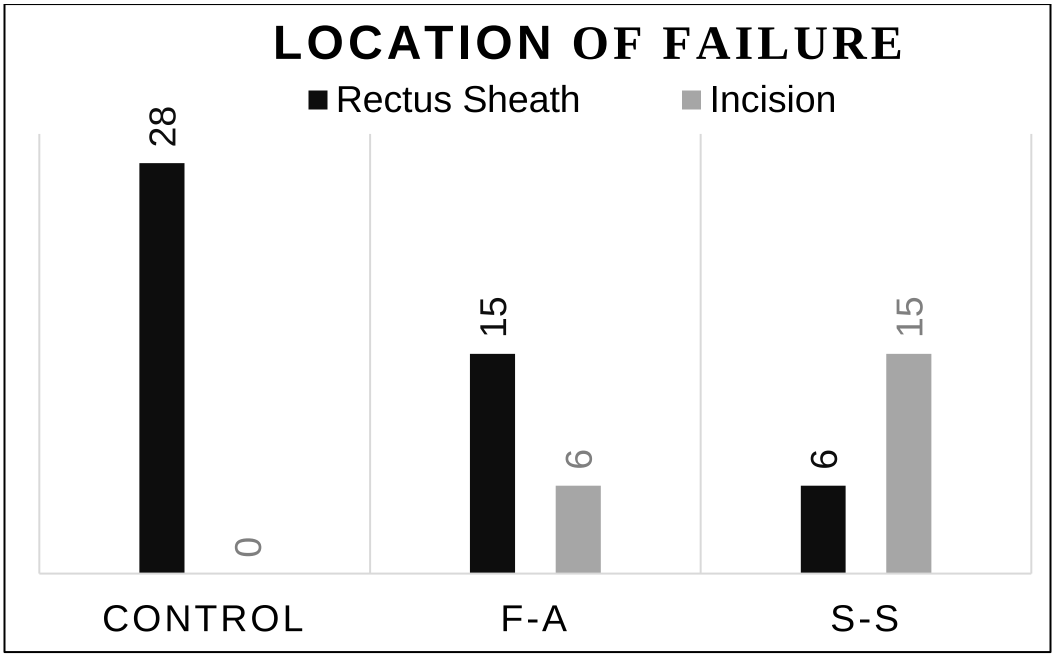


Figure 23. Frequency of location of failure per control group and F-A & S-S knot combination treatment groups. No statistical difference (p=0.24).

H. *Histologic Findings*

Histologic scores are presented in Table 3. There were no significant differences in the odds of having grade 3 vs 4 healing between groups in caudal [start knot] (*P* = 0.598) or cranial [end knot] (*P* = 0.649) section. The S-S group had marginally higher odds of having a grade 4 than 3 healing in the suture line section (*P* = 0.060). There were no significant differences in the odds of having a higher inflammation grade between groups (*P* = 0.762) [Figure 24]. There were no significant differences in the odds of having necrosis between groups in caudal [start knot] (*P* = 0.353), cranial [end knot] (*P* = 0.885) or suture line (*P* = 0.161) sections. There were no significant differences in the odds of having a higher FF/hemorrhage grade between groups (*P* = 0.866). There were no significant differences in fibrosis thickness between groups in caudal [start] (*P* = 0.635), cranial [end knot] (*P* = 0.226) or suture line (*P* = 0.602) sections.

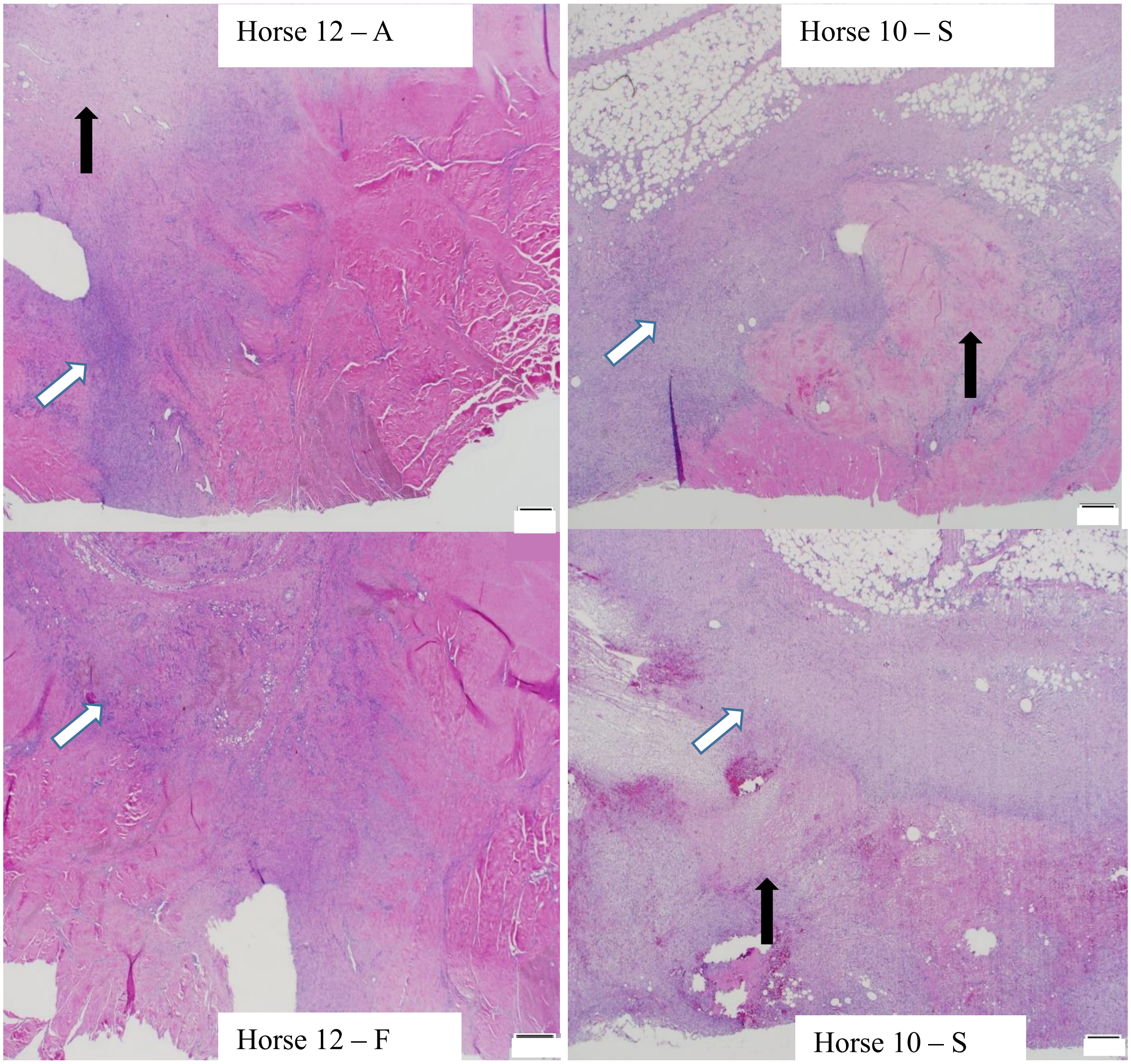


Figure 24. H&E stained histology samples of tissue surrounding the knot types, Horse 12, group F-A, and Horse 10, group S-S. Each sample showing fibrosis (black arrows) and inflammatory cell accumulation (white arrows), mostly mononuclear cell. All pictures magnified to ~400μm. A, Aberdeen; F, forwarder; S, surgeons.

V. Discussion

Our findings do not support our hypotheses that the F-A combination would result in greater tensile strengths and improved tissue healing. The tensile strength between the two test groups was similar, with both groups having significantly lower tensile strength than the control group without celiotomy. While this study failed to find statistical difference between the tensile strength of these knot combinations, the F-A had a greater tendency to fail in a similar manner as normal tissue along the rectus sheath.

VMC closed with a self-locking knot combination (F-A) resulted in similar tensile strength as the traditional closure method (S-S). Previous in vitro and ex vivo work have shown self-locking knots to be more secure with smaller weight and volume compared to surgeon’s knots, however testing was performed under single load to failure and under supraphysiologic conditions.[22,52,54,55] When tested in a single load to failure in this study, the location of failure of F-A segments was more commonly along the rectus sheath than at the incision consistent with body wall that had not undergone a celiotomy (control). Additionally, the study model consisted of physiologic conditions of recovery from general anesthesia and a ten-day post-operative period to mimic stresses and strains applied to these closures. Our findings indicate that either knot combination results in a secure closure that withstands the biodynamic forces and loads applied during anesthetic recovery and the early post-operative period.

More F-A horses developed incisional drainage, however more S-S horses had severe incisional edema and an equal number developed incisional infections (1 per group). No significant differences were observed in vital sign variables or incisional complications suggesting horses tolerated either closure technique. No differences were seen in histologic healing, inflammation, infection, and necrosis between F-A and S-S groups.

Reported incisional complications following VMC vary considerably due to wide variety of definitions used in the literature, which can make direct comparisons difficult. In this study we chose to classify incisional drainage based on its physical characteristic (hemorrhagic, serous, serosanguinous, or purulent) consistent with other publications.[29,30,61,63] Additionally, we chose to define incisional infection as purulent discharge from the incision in which case a sample of the surgical site discharge was obtained for bacterial growth.[31,64] Evidence of purulent discharge was present in only 2 cases, one from each treatment group. Both horses experienced outlying events including escape during hand walking (horse 2, F-A group) and excessive intra-operative cutaneous bleeding (horse 4, S-S group) that were not noted in any other horse during the study period. The excessive activity was the result of horse 2 getting loose during a hand-walk on day three, however the incision remained intact thus the horse was not removed from the study protocol. While bleeding during linea alba closure was encountered in other study subjects, this did not correlate with the concurrent anesthetic treatment groups and was more likely related to the short procedure times not allowing for adequate hemostasis. Horse 4 was the only patient that active suction was necessary to facilitate body wall closure.

The total percentage of horses in the study, from both treatment groups, that developed incisional drainage was 35.7% (5/14 Control; 3/7 F-A, 2/7 S-S). This is consistent with reported incisional infection/drainage of up to 42%.[2] However during the study period, an increased incidence of incisional drainage was noted among both research and hospital patients. As a result, a hospital wide change in cleaning protocol was initiated. Study subjects undergoing surgical intervention following implementation of the new cleaning protocol did not have any evidence of incisional drainage during the study time period, in contrast to the 5 earlier study subjects that did.

Tensile strength of healing linea alba has been shown to be comparable to control linea alba by 8 weeks with a substantial gain in strength at 2 and 4 weeks post celiotomy.[5] This emphasizes the need for a strong and secure closure during the initial 2 week post-operative period. Our study period of 10 days was chosen in order to compare tensile strength of the surgical knots during the timeframe under which the incision is most reliant on strength of the closure. In addition, evaluation of early wound healing with the self-locking knots has previously been lacking due to in vitro and ex vivo study designs.[22,52,54,55] Lack of histologic difference in wound healing between the treatment groups does not support concern of translation of intrinsic forces applied to the sliding self-locking knots resulting in poorer wound healing due to increased constriction of the knots.

Limitations of this study include the small sample size, the use of healthy subjects, and the short study period. The lack of difference in a small population could result in a type II error, however p-values did not approach marginal significance. While diseased subjects and longer study periods were beyond the scope of this project, these variables could influence tensile strength and incisional healing. Evaluation of histology and tensile strength at incremental time points could provide information regarding endogenous healing, however the purpose of the study was to evaluate tensile strength of the knot in addition to the tissue thus a period of 10 days was chosen based on the tensile strength of polyglactin 910 over time.[37] In addition, a number of decisions were made in regard to suture material chosen and use of abdominal bandages based on common practice within our hospital to improve clinical relevance of post-operative findings. Three USP polyglactin 910 is the largest gauge suture available within the Unites States and has been evaluated for RKS and KHC with the proposed self-locking knots. While the use of an abdominal bandage precluded observation of the incision during the first 72 hours following celiotomy, drainage was still identified when present based on strike-through (horse 4) and infections are generally delayed up to 3-14 days post-operatively.[2]

VI. Conclusions

This work provides objective and subjective assessment of the F-A self-locking knot combination in the early wound healing period following VMC in horses. We found no differences between tensile strength and early wound healing between the F-A self-locking knot combination and S-S traditional knot combination. In addition, there was no difference in incisional complications encountered in the early post-operative period between the knot combinations. Given these findings, in conjunction with the previous in vitro and ex vivo studies, it is reasonable to consider closure with the proposed self-locking knot combination for an acute VMC. However, further evaluation of the F-A knot combination in vivo, such as in a randomized, prospective clinical trials in patients with acute abdomen is warranted.

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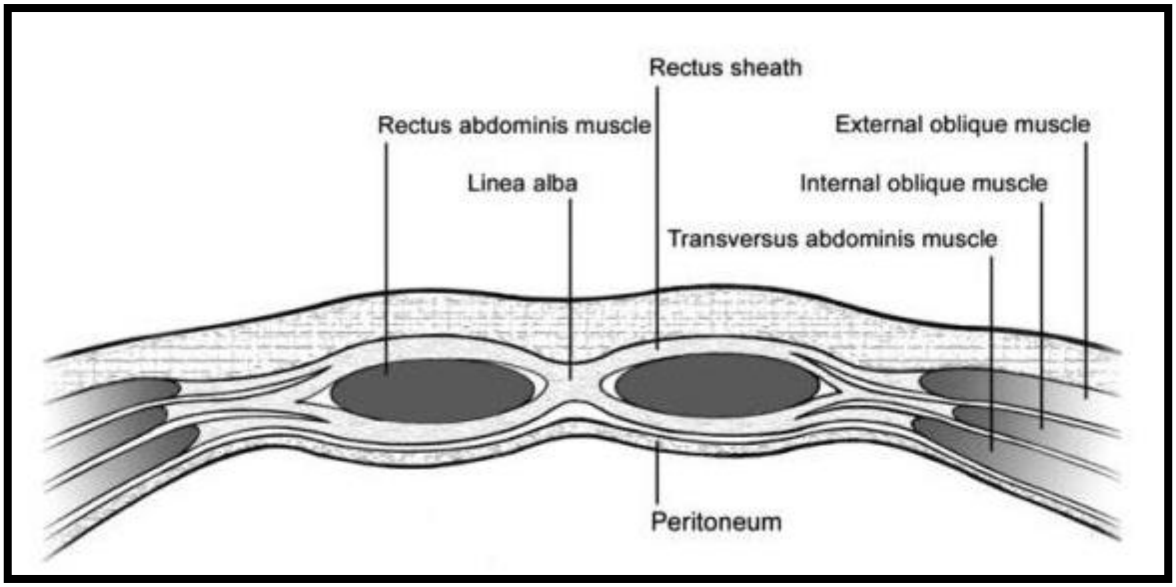
VIII. Appendix

Figure 1. The muscle layers of the abdominal wall taken from Yarwood et al. 2010



Figures 2A and B. Bar graphs illustrating mean peak stress and strain at three ventral midline locations during various activities taken from Kirker-Head et al. 1989

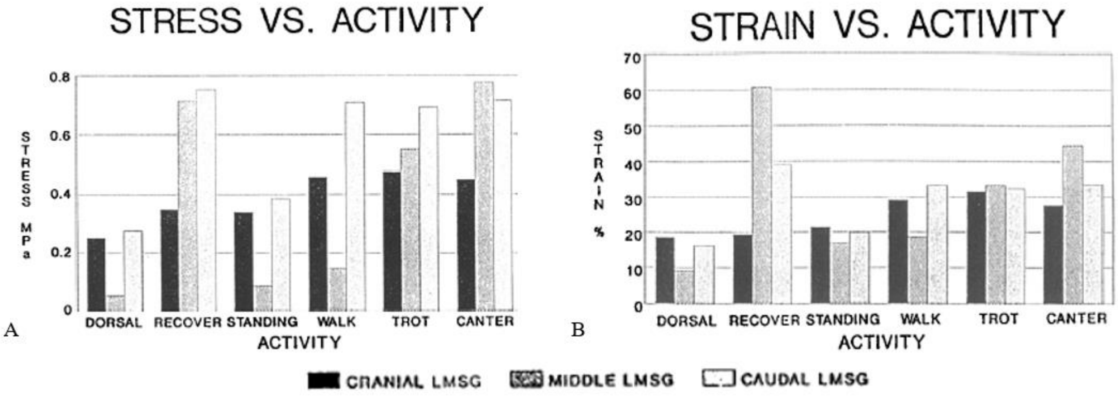


Figure 3. Picture illustrating an example of a universal materials testing apparatus with custom-made cyroclamps for tensile strength testing.

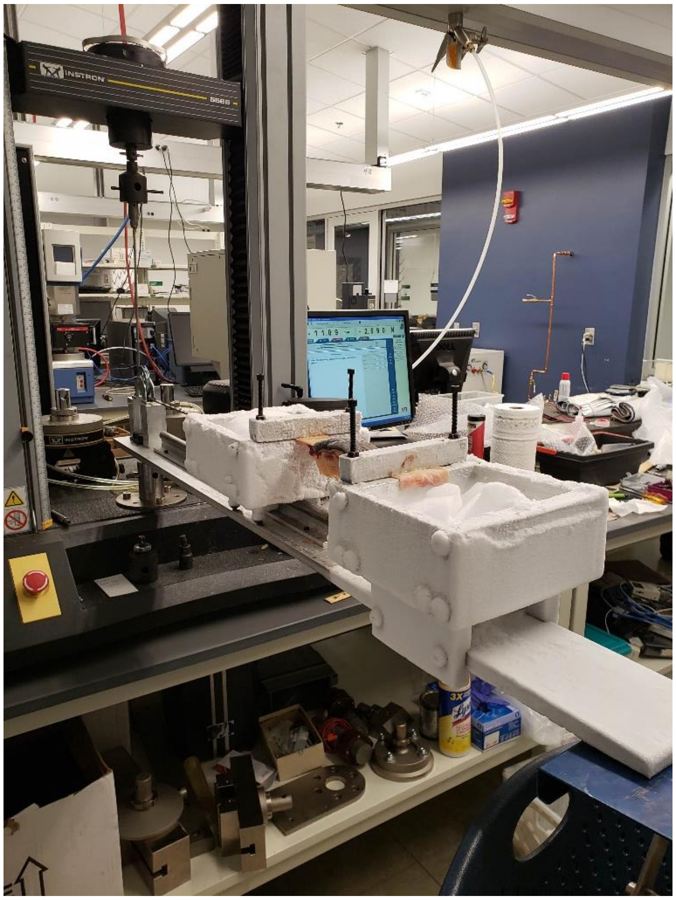


Figure 4. Schematic diagram of the bursting strength ex vivo equine cadaver model, as originally described by Magee et al.[23] The dotted line represents the 200-L polyurethane inflatable bladder.

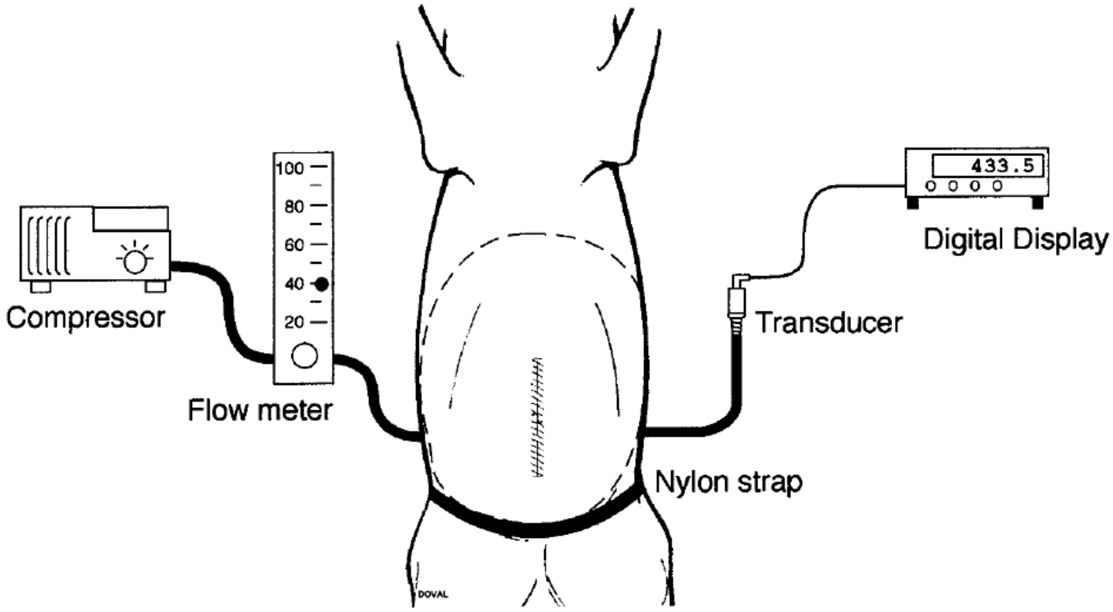


Figure 5. Phases of wound healing and gain in tensile strength post wounding.[6]

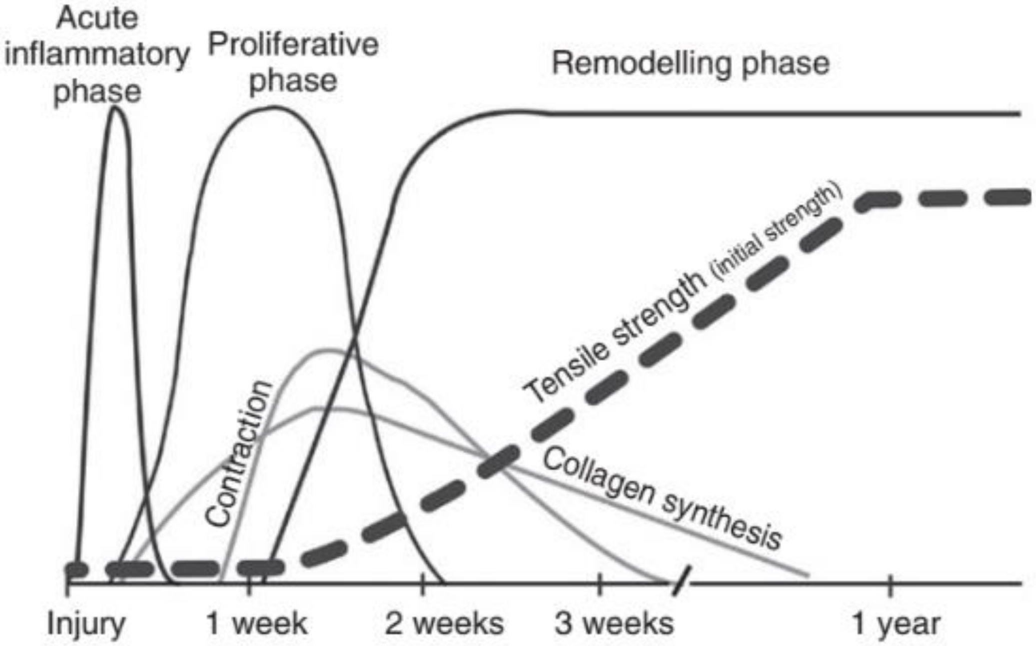
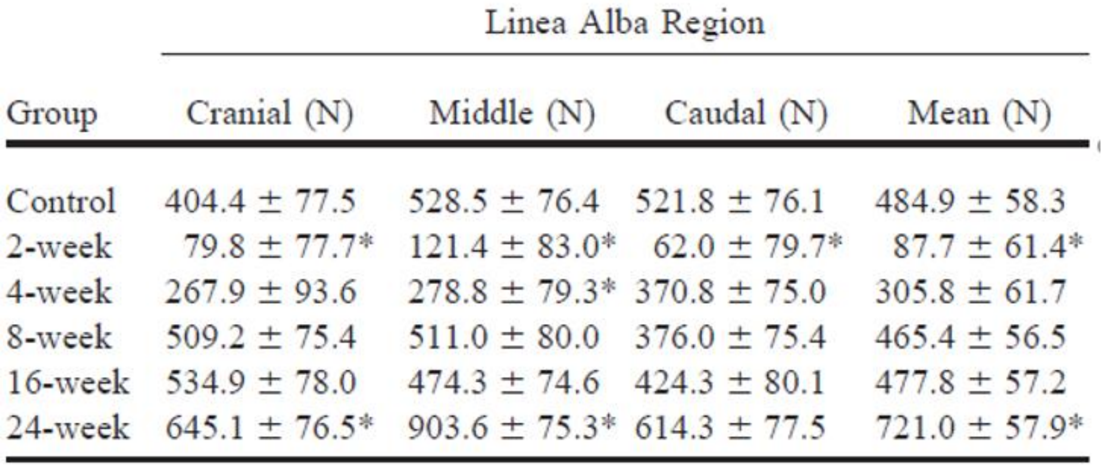
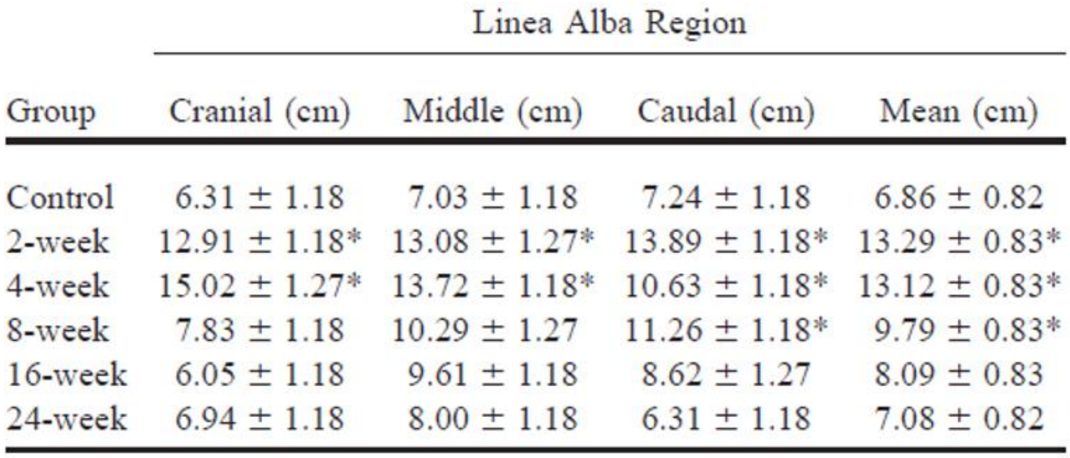


Figure 6. Least squared means (± SEM) of tensile strength of healing linea alba in horses at selected times after ventral midline celiotomy, taken from Chism et al. 2000



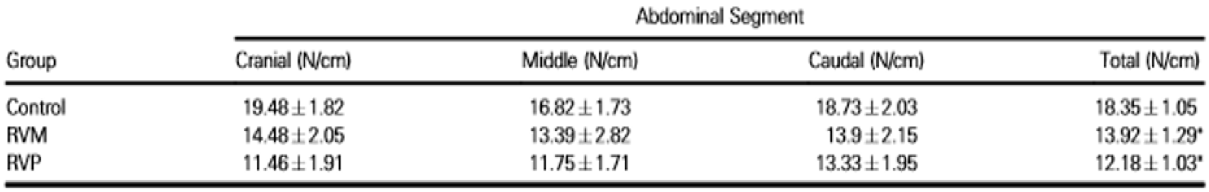
Group	Linea Alba Region Cranial (N)	Middle (N)	Caudal (N)	Mean (N)
Control	404.4 ± 77.5	528.5 ± 76.4	521.8 ± 76.1	484.9 ± 58.3
2-week	79.8 ± 77.7*	121.4 ± 83.0*	62.0 ± 79.7*	87.7 ± 61.4*
4-week	267.9 ± 93.6	278.8 ± 79.3*	370.8 ± 75.0	305.8 ± 61.7
8-week	509.2 ± 75.4	511.0 ± 80.0	376.0 ± 75.4	465.4 ± 56.5
16-week	534.9 ± 78.0	474.3 ± 74.6	424.3 ± 80.1	477.8 ± 57.2
24-week	645.1 ± 76.5*	903.6 ± 75.3*	614.3 ± 77.5	721.0 ± 57.9*

Figure 7. Least mean squared (± SEM) thickness of healing linea alba in horses at selected times after ventral midline celiotomy, taken from Chism et al. 2000



Group	Linea Alba Region Cranial (cm)	Middle (cm)	Caudal (cm)	Mean (cm)
Control	6.31 ± 1.18	7.03 ± 1.18	7.24 ± 1.18	6.86 ± 0.82
2-week	12.91 ± 1.18*	13.08 ± 1.27*	13.89 ± 1.18*	13.29 ± 0.83*
4-week	15.02 ± 1.27*	13.72 ± 1.18*	10.63 ± 1.18*	13.12 ± 0.83*
8-week	7.83 ± 1.18	10.29 ± 1.27	11.26 ± 1.18*	9.79 ± 0.83*
16-week	6.05 ± 1.18	9.61 ± 1.18	8.62 ± 1.27	8.09 ± 0.83
24-week	6.94 ± 1.18	8.00 ± 1.18	6.31 ± 1.18	7.08 ± 0.82

Figure 8. Mean (±SD) tensile strength per unit length of abdominal segments obtained from control, repeat ventral median (RVM) horses and right ventral paramedian (RVP) horses, taken from Boone et al. 2014



	Abdominal Segment			
Group	Cranial (N/cm)	Middle (N/cm)	Caudal (N/cm)	Total (N/cm)
Control	19.48 ± 1.82	16.82 ± 1.73	18.73 ± 2.03	18.35 ± 1.05
RVM	14.48 ± 2.05	13.39 ± 2.82	13.9 ± 2.15	13.92 ± 1.29*
RVP	11.46 ± 1.91	11.75 ± 1.71	13.33 ± 1.95	12.18 ± 1.03*

Figure 9. The effects of tissue bite size on breaking strength (mean ± SEM) of the adults equine linea alba, taken from Trostle et al. 1994

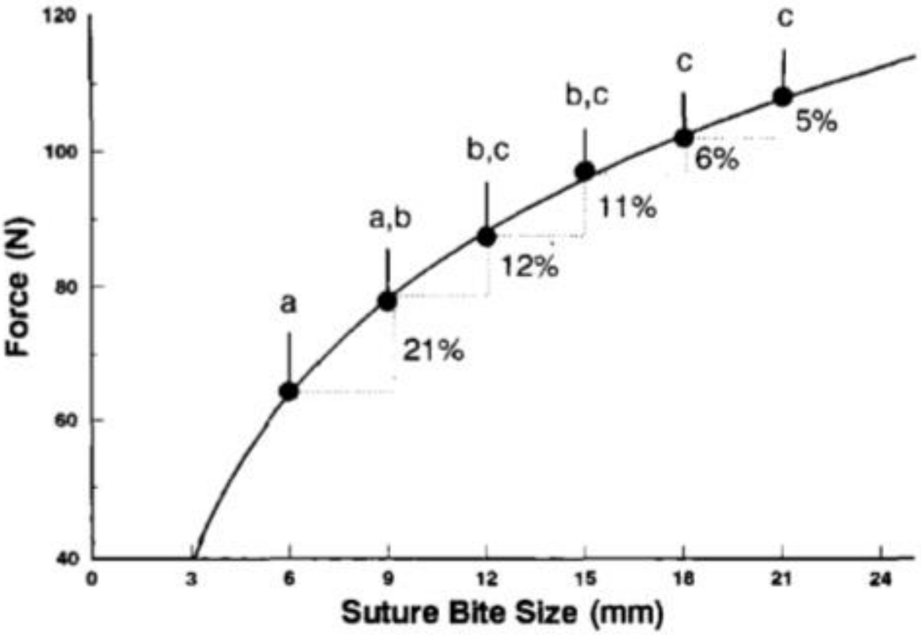
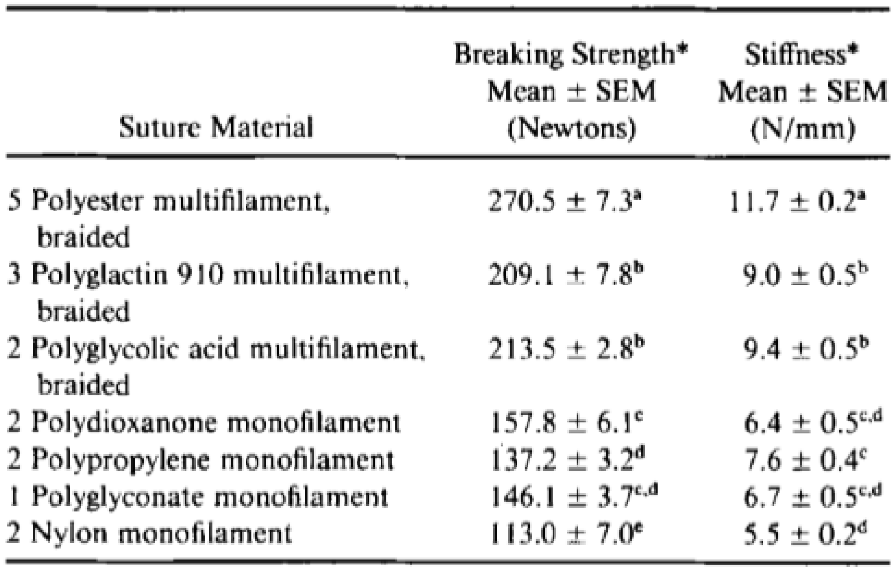
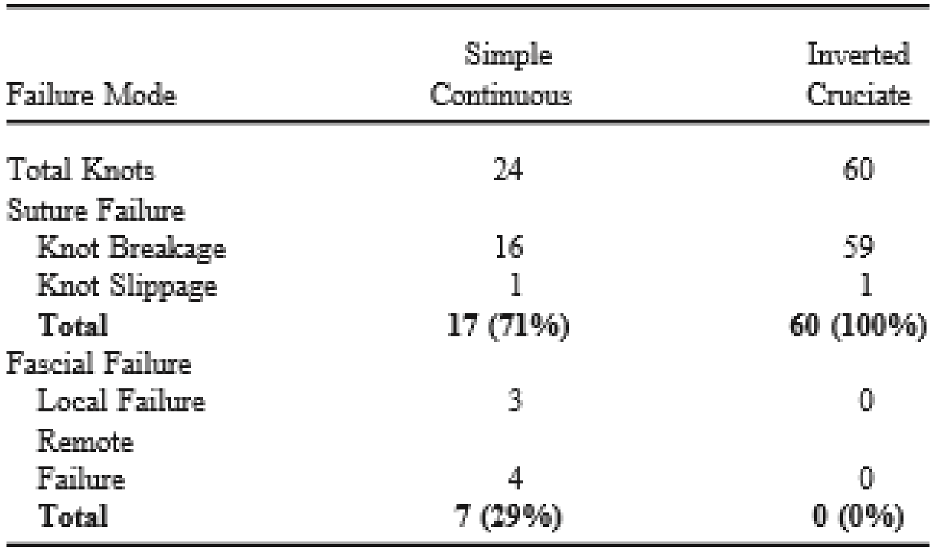


Figure 10. Breaking strength and stiffness of commercially available suture materials in adult equine linea alba, taken from Trostle et al. 1994



Suture Material	Breaking Strength* Mean ± SEM (Newtons)	Stiffness* Mean ± SEM (N/mm)
5 Polyester multifilament, braided	270.5 ± 7.3^{a}	11.7 ± 0.2^{a}
3 Polyglactin 910 multifilament, braided	209.1 ± 7.8^{b}	9.0 ± 0.5^{b}
2 Polyglycolic acid multifilament, braided	213.5 ± 2.8^{b}	9.4 ± 0.5^{b}
2 Polydioxanone monofilament	157.8 ± 6.1^{c}	6.4 ± 0.5^{c,d}
2 Polypropylene monofilament	137.2 ± 3.2^{d}	7.6 ± 0.4^{c}
1 Polyglyconate monofilament	146.1 ± 3.7^{c,d}	6.7 ± 0.5^{c,d}
2 Nylon monofilament	113.0 ± 7.0^{e}	5.5 ± 0.2^{d}

Figure 11. Mode and type of failure of equine linea alba incisions closed with 3 Polygalactin 910 in a simple continuous or inverted cruciate pattern, taken from Magee et al. 1999



Failure Mode	Simple Continuous	Inverted Cruciate
Total Knots	24	60
Suture Failure		
Knot Breakage	16	59
Knot Slippage	1	1
Total	**17 (71%)**	**60 (100%)**
Fascial Failure		
Local Failure	3	0
Remote Failure	4	0
Total	**7 (29%)**	**0 (0%)**

Figures 12 A, B, C. Schematics of a square knot (A), surgeon's knot (B), and granny knot (C)

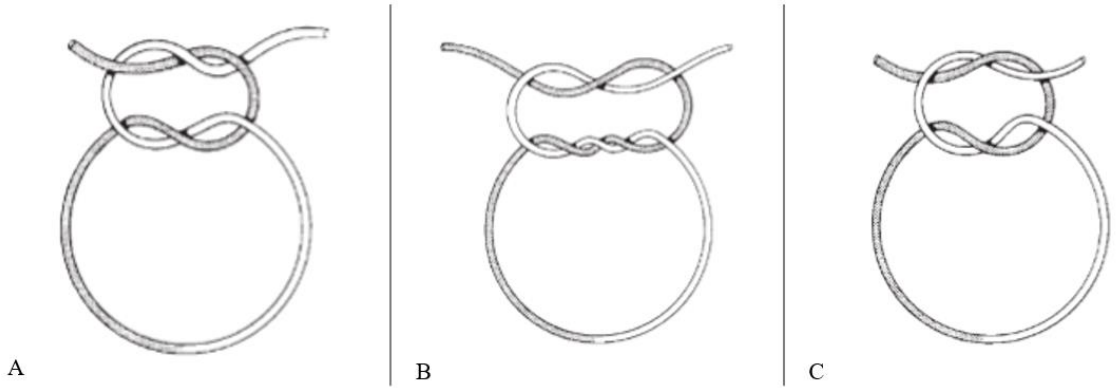


Figure 13. Schematic depiction of the formation of a forwarder knot, taken from McGlinchey et al. 2018

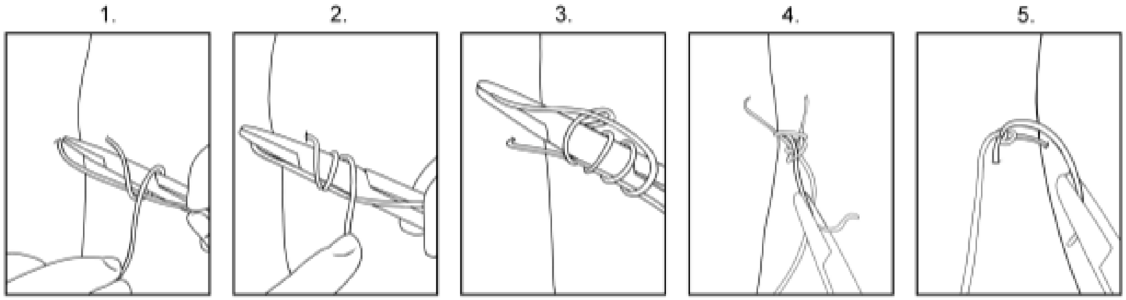


Figure 14. Schematic depiction of the combination of throws and turn to complete an Aberdeen knot, taken from McGlinchey et al. 2018

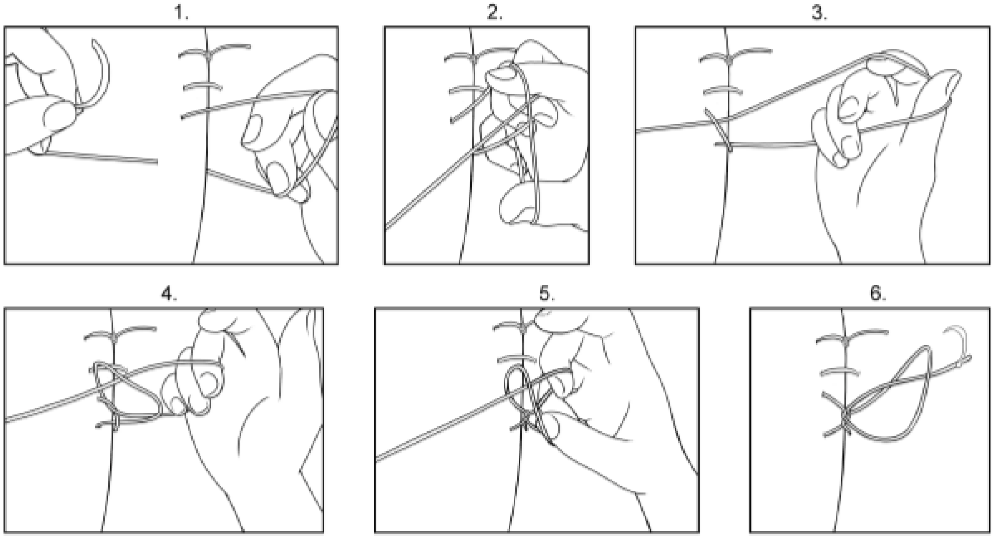


Figure 15. Box plots showing the difference in bursting strength of surgeon-surgeon and forwarder-Aberdeen knot combinations, taken from McGlinchey et al. 2018

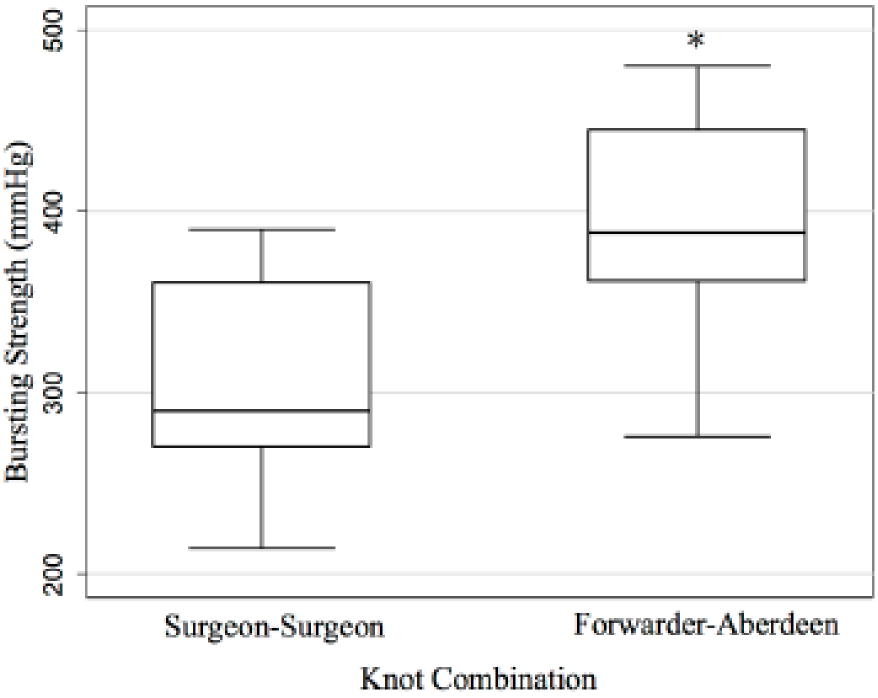
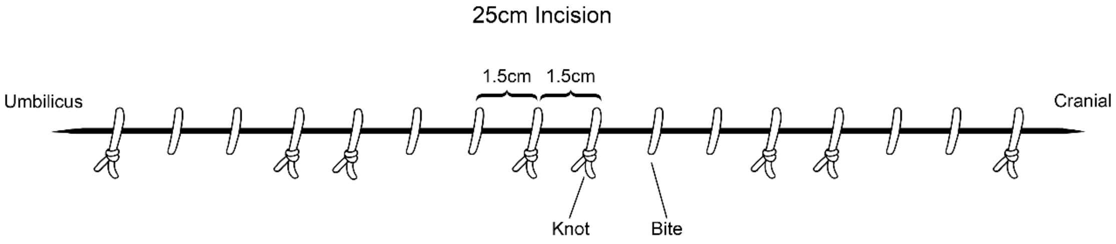
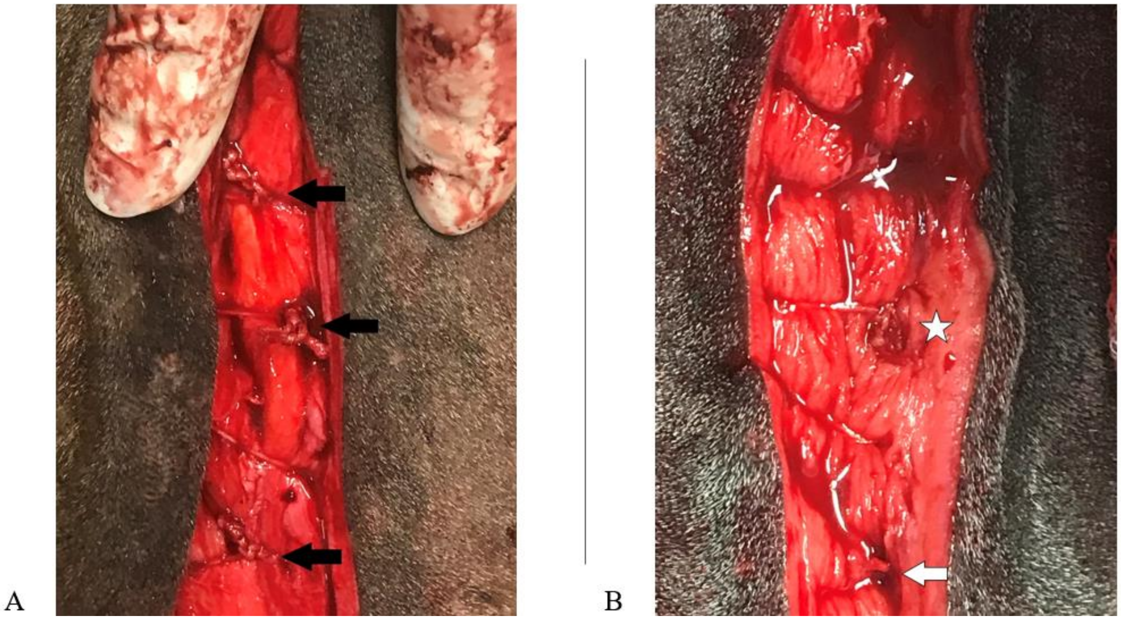


Figure 16. Illustration of the closure – 4 individual simple continuous runs with start-end knot determined by treatment group.



Figures 17. Incision closures in situ with the S-S combination on the left (A) and F-A combination on the right (B). Please note the size difference between the knots in the surgical field. Caudal is the top of the picture and cranial the bottom of the picture. Surgeon’s knots, black arrows; Forwarder start knot, white arrow; Aberdeen end knot, white star.



Figures 18. Examples of peri-incisional edema scores of absent (A), moderate (B), and severe (C). Head is to the left and tail is to the right. Figure 16C also is an example of purulent incisional drainage.

Figure 19. Abdominal segment mounted in a material testing apparatus using custom-engineered cryoclamps to prevent fascial slippage. Segment was placed in the testing apparatus such that the celiotomy incision was approximately 10 cm from either clamp. Rectus sheath, black arrow; linea alba, black star; cryoclamps, gray triangles

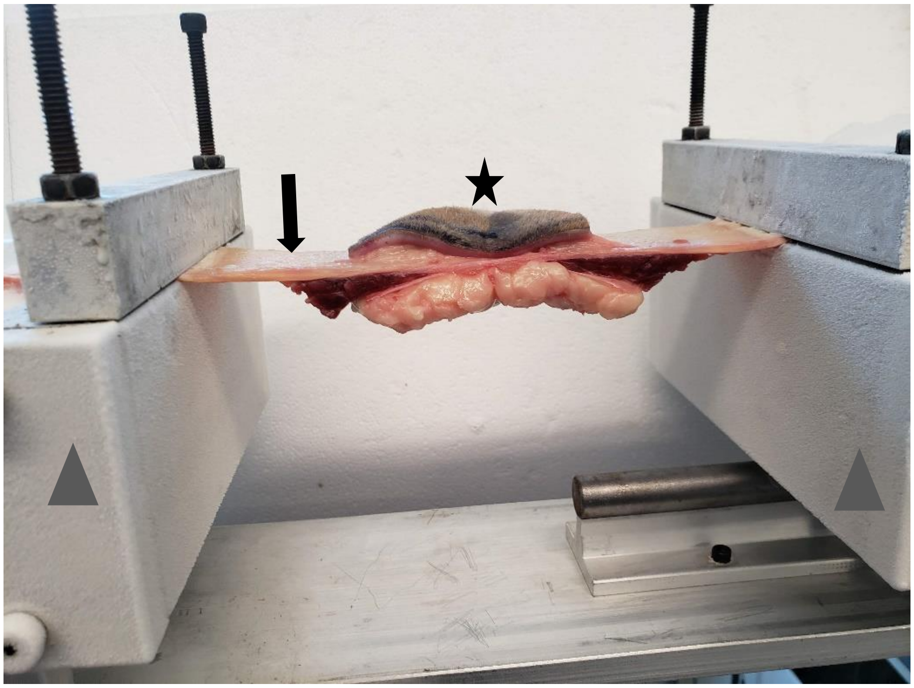
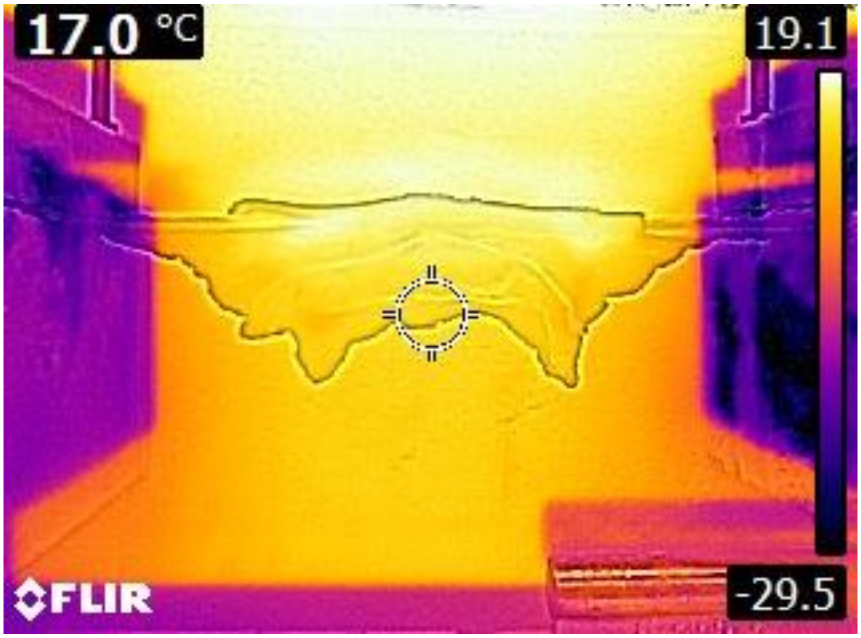


Figure 20. Temperature of the external rectus sheath and celiotomy incision was monitored utilizing a thermal camera while mounted in the custom-engineered cyroclamps. Color indicates temperature range between 19.1°C (yellow) to -29.5°C (deep purple).



Figures 21. Locations of failure of abdominal segments under mechanical testing. Failure at the incision (A) denoted by gray arrow. Failure at the rectus sheath (B) denoted by black arrow.

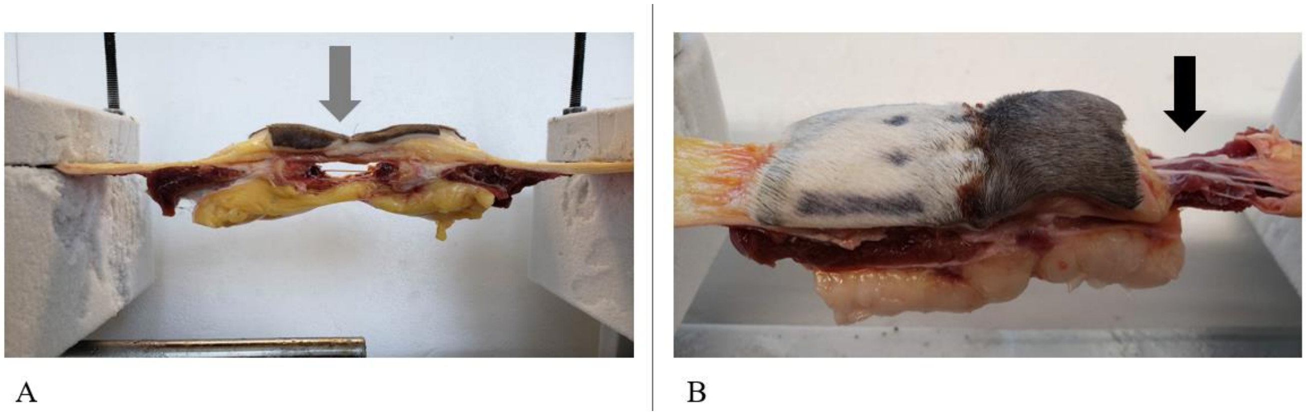


Figure 22. Box and whisker plot of tensile strength (N/cm^2) of control, forwarder-Aberdeen (F-A), and surgeons-surgeons (S-S) knot combination treatment groups. Different letters indicate significant difference between groups (*P* < 0.05).

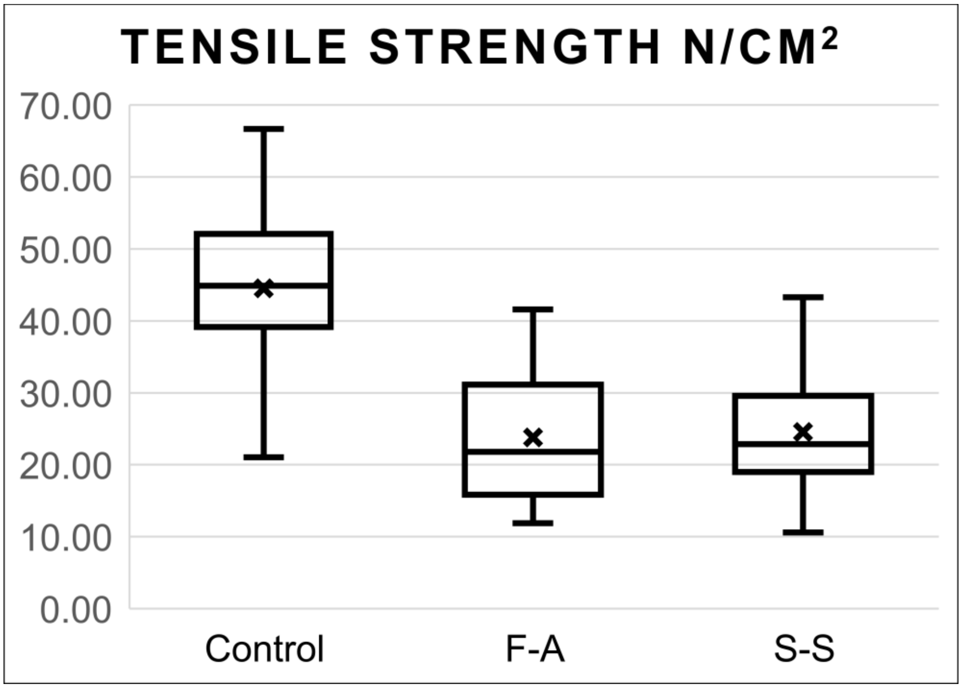


Figure 23. Frequency of location of failure per control group and F-A & S-S knot combination treatment groups. No statistical difference (p=0.24).

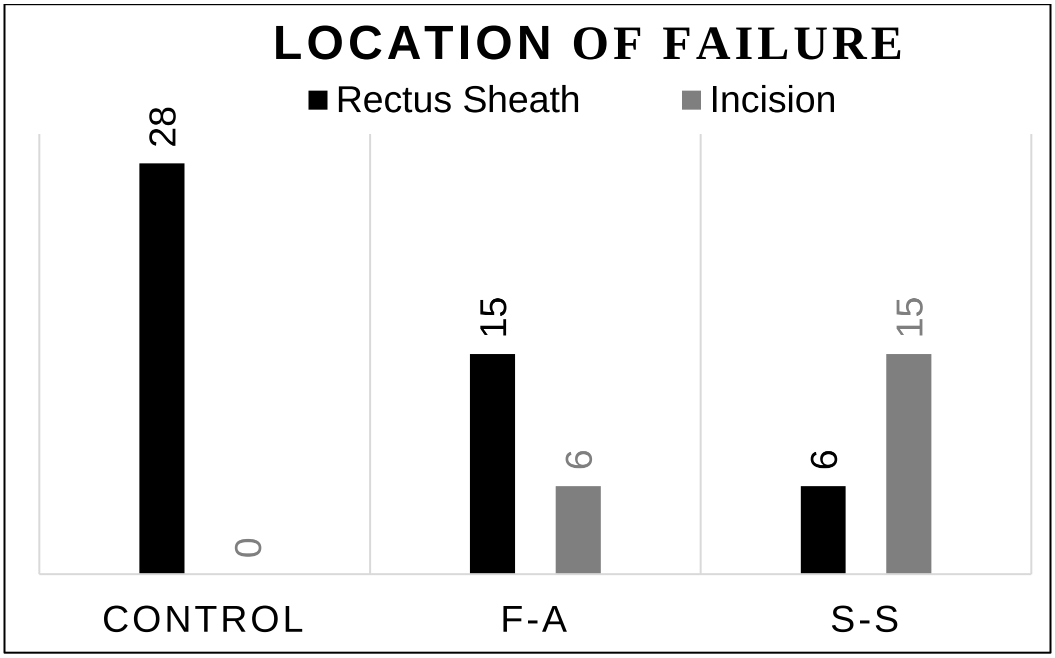


Figure 24. f

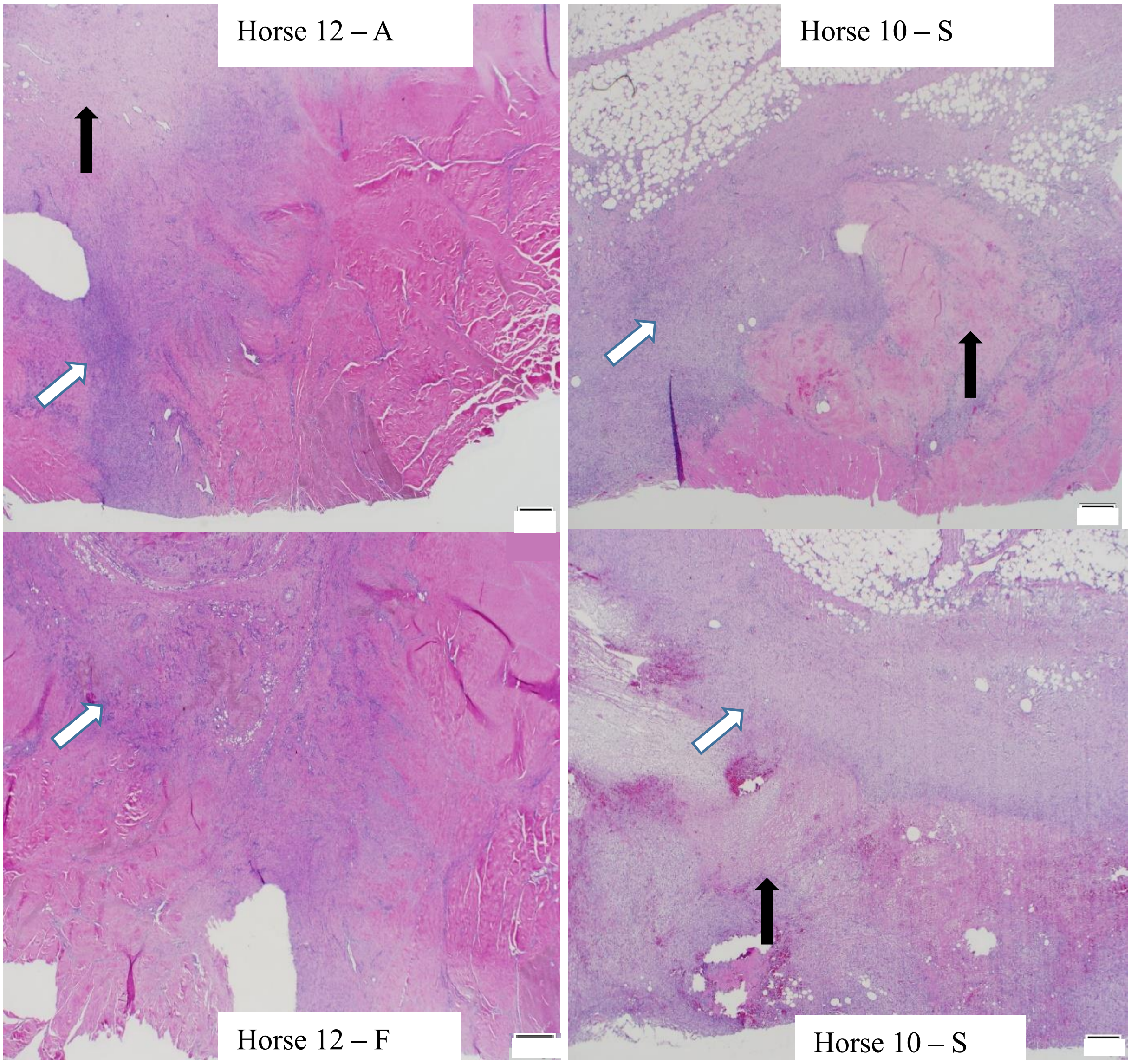


Table 1. Histologic Grading System for Incisions[22]

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Healing[22]	Edges unopposed by fibrin clos/fistula formation	Edges opposed by fibrin clot	Early ingrowth of fibroblasts and capillaries/early granulation tissue	Collagen production within lattice of numerous small caliber blood vessels/organizing granulation tissue	Abundant collagen with scattered small caliber blood vessels/well organized granulation tissue/fibrous scar tissue
Inflammation[22]	None	Scant inflammation	Mild inflammation	Moderate inflammation	Marked inflammation and/or abscess formation
Infection[22]	No microorganisms seen	Rare	Small scattered colonies	Large colonies	Confluent colonies
Necrosis[22]	None	Mild (<25% of section)	Moderate (25-50% of section)	Severe (> 50% of section)	
Focal accumulations of fibrin and hemorrhage[22]	None	Mild (<25% of section)	Moderate (25-50% of section)	Severe (> 50% of section)	

Table 2. Case details for study subjects with treatment group, surgical time, and abnormal post-operative vital parameters

Horse	Age (yrs)	Weight (kg)	Sex	Breed	Knot Type	Anesthesia time (min)	Procedure time (min)	Fever Post-op (>101°F)	Tachycardia Post-op (>48bpm)	Tachypnea Post-op (>24brpm)	Incision Drainage
1	22		G	Mix	S-S	87	53				
2	11		G	QH	F-A	102	68			Yes, 26brpm Days 8, 9	Yes, Purulent Day 6
3	8		G	WB	F-A	84	36				
4	12		G	Mix	S-S	95	63	Yes, 101.2-101.8°F, Days 3, 4	Yes, 52bpm Days 4, 8, 10	Yes, 28-30brpm Days 1, 4, 8, 9	Yes, Purulent Day 6
5	12		G	WB	S-S	77	38	Yes, 101.3°F Day 1			Yes Serosanguinous, Day8
6	16		G	TB	F-A	77	38				Yes, Serosanguinous, Day 6
7	17		G	TB	F-A	88	51		Yes, 52bpm Days 1, 3		Yes, Serosanguinous Day 7
8	26		F	Arab	S-S	76	45				
9	12		F	Arab	S-S	93	60				
10	17		F	QH	S-S	82	41				
11	4		F	Mix	F-A	78	37	Yes, 101.5°F Day 1			
12	18		G	QH	F-A	84	40			Yes, 30brpm Day 5	
13	20		G	QH	F-A	78	27				
14	28		G	QH	S-S	74	37				

F, female; G, gelding; Arab, Arabian; Mix, mixed breed; QH, Quarter Horse; TB, Thoroughbred; WB, Warmblood
yr, year; min, minutes
kg, kilograms

bpm, beats per minute; brpm, breaths per minute

Table 3. Mean (± SD) Incisional Scores, Histologic Grading, and Incisional Fibrotic Depth (cm) of Celiotomy Incisions Closed with either Forwarder-Aberdeen or Surgeon's-Surgeon's Knot Combinations

Group	Segment	Edema	Healing	Fibrotic Depth	Inflammation	Necrosis	FF/Hemorrhage Accumulation
F-A	Cranial		3.43 ± 0.49	2.26 ± 0.65	1.13 ± 0.78	0.57 ± 0.49	0.71 ± 0.45
	Suture Line		3.14 ± 0.35	1.90 ± 0.19	1.29 ± 0.45	0.86 ± 0.35	0.57 ± 0.49
	Caudal		3.57 ± 0.49	2.04 ± 0.45	1.00 ± 0	0.25 ± 0.43	0.43± 0.49
	All	1.53 ± 0.78	3.38 ± 0.49	2.07 ± 0.49	1.14 ± 0.56	0.52 ± 0.50	0.57 ± 0.49
S-S	Cranial		3.57 ± 0.49	1.89 ± 0.52	1.29 ± 0.45	0.57 ± 0.49	0.43 ± 0.49
	Suture Line		3.71 ± 0.45	2.06 ± 0.70	1.43 ± 1.18	0.43 ± 0.49	0.71 ± 0.70
	Caudal		3.43 ± -.49	1.90 ± 0.39	1.29 ± 1.38	0.43 ± 0.49	0.71 ± 0.45
	All	1.89 ± 0.85	3.57 ± 0.49	1.95 ± 0.56	1.33 ± 1.04	0.48 ± 0.50	0.62 ± 0.58

Forwarder-Aberdeen (F-A) group was closed with a forwarder start knot (caudal) and Aberdeen end knot (cranial). Surgeon's-Surgeon's (S-S) group was closed with a surgeon's start knot (caudal) and end knot (cranial).

Table 4. Mean (± SD) Tensile Strength Per Unit Area of Abdominal Segments Obtained from Control Horses, F-A Horse, and S-S Horses

Study Group	Cranial Segments (N/cm^2)	Middle Cranial Segments (N/cm^2)	Middle Caudal Segments (N/cm^2)	Caudal Segments (N/cm^2)	Total (N/cm^2)
Control (no celiotomy)	44.11 ± 12.66	47.63 ± 11.94	43.75 ± 5.68	42.75 ± 6.85	44.55 ± 9.94
F-A	24.07 ± 3.95	22.56 ± 8.66	20.99 ± 7.64	24.08 ± 11.08	23.79 ± 7.64 *
S-S	28.87 ± 7.67	20.87 ± 4.55	22.79 ± 8.46	26.07 ± 6.44	24.56 ± 7.64 *

Total refers to the mean tensile strength per unit area of all abdominal segments within the group.
*Significantly different ($P < 0.05$) from control