Ex vivo comparison of the bursting strength of surgeon's knot compared to self-locking knots for closure of ventral midline celiotomy in horses.

by

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Abstract

It is not uncommon for the equine population to experience abdominal pain referred to as 'colic'. It is estimated that approximately 9% of the general population of horses that experience colic require surgical intervention.[1] The most common approach to gain access to the equine abdomen is through the ventral midline, with the horse under general anesthesia.[2] Frequently, incisional complications occur post operatively with reported rates as high as 44%.[3] As a result, investigators strive to identify risk factors associated with morbidity and improve techniques and conditions for surgical exploration, abdominal closure, and post-operative care.[4-8] Previous studies have identified the surgical knot as the weakest part of the suture, with failure occurring at the knot 90-100% of the time.[9-13] Although the most commonly used knot is the surgeon's knot, *in vitro* studies examining self-locking knots revealed that they are stronger and more secure.[10-13]

The objective of this study was to compare the bursting strength and failure mode of ventral midline celiotomy closure using a simple continuous suture pattern with one of two knot combinations, a novel self-locking knot combination of forwarder start with an Aberdeen end knot (F-A) compared to a traditional knot combination of a surgeon's start with a surgeon's end knot (S-S). An *ex vivo* cadaver study was performed.

A 20-cm ventral midline celiotomy was created in 14 equine cadavers. Horses were assigned to celiotomy closure with a F-A or S-S knot combination. Prior to closure, a 200L inflatable bladder was placed in the abdomen and insufflated until failure of the celiotomy closure. The horses' signalment, weight, breed, age, knot combination type, mode of failure, closure time (minutes) and bursting strength in millimeters of mercury (mmHg) were recorded.

The median bursting strength of closure with F-A knot combination (388 mmHg) was significantly greater than the median bursting strength of closure with the S-S knot combination (290 mmHg) (P=0.035). The majority (6 out of 7) of F-A combinations failed along the fascia, in contrast to the majority (6 out of 7) of S-S combinations that failed at the knot. There were no significant differences in closure times (P=0.48).

The self-locking F-A knot combination had a significantly higher bursting strength compared to the traditional S-S knot. Our results indicate that closure of a ventral midline celiotomy with a F-A knot combination may offer a more secure closure than that of a traditional S-S knot combination, increasing bursting strength by an average of 25%.

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F-A	Forwarder knot – Aberdeen knot combination
S-S	Surgeon's – Surgeon's knot combination
mmHg	Millimeters of mercury
TP	Total protein
PCV	Packed cell volume
CFU	Colony forming units
USP	United States Pharmacopoeia
TS	Tensile strength
KHC	Knot holding capacity
RKS	Relative knot security
SL:WL	Suture length to wound length ratio
L	Liter
mm	Millimeters
cm	Centimeters

I. Introduction and Literature Review

It is not uncommon for the equine population to experience abdominal pain referred to as 'colic'. It is estimated that approximately 9% of the general population of horses that experience colic require surgical intervention.[1] The most common approach to gain access to the equine abdomen is through the ventral midline, with the horse under general anesthesia.[2] Frequently, incisional complications can occur post operatively with rates as high as 44%.[3] As a result, investigators strive to identify risk factors associated with morbidity and improve techniques and conditions for surgical exploration, abdominal closure, and post-operative care.[4-8] These will be discussed in detail to follow.

a. Ventral Midline Celiotomy Incision

i. Approaches to the Equine Abdomen

Surgical exploration of the equine abdomen may be performed to gain access to the abdominal or urogenital tract to diagnose and treat a variety of medical and surgical conditions. Colic surgery is the most common reason for requiring an emergency laparotomy with an estimated 9% of colic cases in the general population requiring surgical intervention.[14] Access to the abdomen is possible through a laparoscopic or a flank approach, through the paralumber fossa in the standing horse, but is typically performed under general anesthesia with the horse in dorsal recumbency. Approaches through the ventral abdominal wall include ventral midline, paramedian, inguinal, parainguinal and suprapubic.[2]

ii. Ventral Midline Approach

The most commonly used approach to gain access to the equine abdomen is through the ventral midline.[2] This approach allows exteriorization of ~75% of the intestinal tract, with the exception of the stomach, duodenum, distal ileum, dorsal body and base of the cecum, distal right dorsal colon, transverse colon and terminal small colon. This approach is performed with the horse in dorsal recumbency. When performed correctly the incision is made through the skin, subcutaneous tissues and finally the linea alba. The linea alba is formed by the aponeurosis of the external oblique and transverse abdominal muscle. This layer consists of dense connective tissue and is important as it is the holding layer for closure of the ventral midline incision.[2] It extends from the xiphoid process to the pre-pubic tendon and varies in thickness from ~3mm craniad and increases caudally to ~10mm.[9] It has been demonstrated that it takes ~8 weeks following ventral midline celiotomy for the linea alba to regain its strength and form mature collagen bundles.[15] Fibers of the linea alba run in a perpendicular direction to the suture tension placed on the linea during closure and it is thought that this feature offers a more secure closure.[16] Other advantages of this closure are: the incision may be extended if required, there is minimal hemorrhage and it is easy to perform.[2] When performing a repeat celiotomy, no differences in tensile strength were observed between performing a ventral paramedian closure versus a ventral midline celiotomy, however, the tensile strength of both were significantly lower than control horses.[7] A second paper examining the incisional complications following right sided paramedian celiotomies suggested that there are less complications than with ventral midline, with only 16.8% of horses developing complications.[17]

iii. Incisional Complications and Consequences

Unfortunately, complications associated with the incision are not uncommon following ventral midline celiotomy in the horse. Multiple large studies have been completed investigating these complications and rates vary depending on the location, time and inclusion criteria: 37%[3], 28.6%[8], 26.9%[5], 25.4%[18], 16%[19], 7.4%[20] and 25.4%.[21] When horses require a second laparotomy these rates can double with reports describing incisional complications in 26-88% of these horses.[1,8] These problems include incisional infection, herniation, acute incisional dehiscence, suture sinus formation, swelling, incisional drainage, and abscess formation.[5,8,22-24]

1. Incisional Infection

Incisional infection is the most commonly reported complication and depending on the inclusion criteria rates range from 4-44% following colic surgery.[5,8,22,25] Incisional drainage may be considered normal if it is mild and serous in character and occurring within the first 12-24 hours post-operatively, however after this time period it is considered abnormal with these horses likely developing an incisional infection.[21] Various risk factors have been reported to contribute to the development of incisional infections. These factors may be related to the pre-operative period, intra-operative period, post-operative period or to the individual horse's immune function and nutritional status. During any surgical procedure, there are four sources of bacteria: the air, the patient, the surgeon and the instruments.[26] Infection of a surgical wound is likely from direct inoculation of the patients endogenous flora either from the skin or abdominal viscera during abdominal surgery.[26] When quantitatively measured, it has been demonstrated that when a surgical site is inoculated with greater than 10^5 microorganisms per gram, the risk of developing a surgical infection is greatly increased.[27-29] It is important to note that the presence of foreign material, such as suture material will significantly reduce the dose of bacteria required to cause an infection.[26] However, several factors play a role, such as the immune status of the patient, which may be compromised in a horse requiring emergency colic surgery, and the virulence of the bacteria involved. A recent study cultured bacteria from 97% of the infected abdominal incisions with the three most commonly isolated bacteria being *Escherichia* coli, *Enterococcus* species and *Staphylococcus* species.[21] These bacterial isolates are similar to those that Ingle-Fehr et al. obtained in 1997, *Streptococcus* species, *Staphylococcus* species and *Esherichia* coli.[30] Ingle Fehr et al. also demonstrated that bacterial culture during surgery did not correlate with the development of a surgical site infection.[30] Both of these studies suggest that inoculation likely occurs from endogenous flora or contamination post-surgery. Another study by Rodriguez et al. further supported these findings.[31] During exploratory laparotomies, cultures were taken from enterotomy sites, peritoneal fluid, and resection and anastomoses sites. Ninety six percent of horses had a positive culture, with the most common isolates being *Escherichia* coli, *Streptococcus* species and *Enterococcus* species.[32] Incisional drainage was considered as 'continued drainage' if it lasted longer than 12 hours post operatively or if it occurred more than once. Ten percent of horses had continued drainage, indicating that routine intraabdominal culture did not predict the development of incisional infection.

Certain factors prior to surgery are correlated with an increased risk of developing incisional problems or infection. Horses that are more systemically compromised when they present initially appear to be at a greater risk of developing an incisional complication. These included horses with a total protein (TP) value between 60-89g/dl, reduced or absent gut sounds, packed cell volume (PCV) on admission >48%, a greater bodyweight, and elevated peritoneal fibrinogen.[5,18,21] Intra-operative factors reported to increase the risk of wound complications include intra-peritoneal contamination, dissection of the linea alba prior to closure, small intestinal resection and cecal/large intestine obstruction.[3,5,21] Often, results can have conflicting findings. Isgren et al. reported that a 3-layer closure is protective for the development of incisional infections, however, a randomized controlled study reported no significant difference between the development of infection following a 2 or 3 layer closure.[21,33] This may be explained in part by the latter study having a longer study period therefore detecting incisional complications over a longer time period following surgery.

Often, surgeons elect to cover the incision post-operatively to reduce contamination, however, evidence supporting this is conflicting. In 1989, Kobluk et al. reported that the use of a stent bandage to cover the incision did not result in a statistically significant increase in incisional complications.[8] In contrast, Mair et al. found that applying a stent following surgery significantly increased the risk of developing an incisional complication ($P < 0.0002$), whereas applying an incise drape significantly reduced this rate ($P < 0.0001$) and the use of an abdominal belly band in the immediate post-operative period decreased the rate of incisional infections by 45%.[5,6] Recovery from general anesthesia is also a critical time period as it has been shown that obtaining a high number of bacteria colony forming units (CFU), likely from the floor of the recovery stall, is a risk factor for developing subsequent drainage and infection.[34]

Below is a table that concludes the findings of multiple large studies and highlights risk factors and protective factors for an incisional infection as well as linea alba closure type and suture material used.[3,5,8,18,21,30] One risk factor that is apparent in more than one study is performing an enterotomy.[18,30] In relation to the linea alba closure type and suture type or pattern, certain studies have identified related risk factors. Mair et al determined that dissecting the linea alba prior to closure is a risk factor, with others determining that closure of the linea alba with a near-far-far-near suture pattern was detrimental, as well as the use of polyglactin 910.[5,8,18] With numerous studies performed various risk factors have been identified. To the best of the authors knowledge, there has not been a previous study that has examined different knot combinations for closure of the linea alba or if using knots with a smaller volume or less suture material would decrease the risk of infection. However, the literature advises the surgeon to use a minimally reactive suture with the least amount of suture material.[1,35] This is likely advised as the presence of suture material in the linea alba can cause mechanical irritation. This irritation seems to be directly related to suture size, suture stiffness, knot configuration and volume, with the addition of extra throws on the knot greatly increasing knot volume and tissue reactivity.[36,37] It has been previously demonstrated in large gauge suture that self-locking knots, in particular, the forwarder and Aberdeen knot, have a smaller weight and volume in contrast to the traditional surgeon's knot.[10-13] Based on these findings it would seem logical that using these knots for closure of the linea alba may decrease mechanical irritation and inflammation and may decrease the risk of infection.

Table 1. List of risk factors and protective factors for the development of an incisional infection post-operatively as determined by various studies.

Author	**Year**	**Infection rate**	**Criteria**	**Risk Factors**	**Protective factors**	**Closure**	**Suture**
Kobluk et al.	1987	28.6% 1st surgery 87.5% 2nd surgery	-Serosanguineous fluid -Drainage with suspected infection -Confirmed infection	Closure of the linea alba with near-far-far-near suture pattern		Simple interrupted -59.1% NFFN -7.6% NFFN + simple interrupted -30.3% Horizontal mattress -1.5% Continuous -1.5% Unknown -6.1%	Polyglactin 910 -80.3% Polyester -18.2% Gut -1.5% Unknown - 6%
Philips et al.	1993	28%	Incision suppuration	Cecum/large colon lesions		Simple continuous	Polyglactin910
Ingle Fehr	1997	24-44%	Incisional drainage 24% Positive bacterial culture 44%	-Clean contaminated surgery -Preoperative peripheral left shift		Not reported	Not reported
Honnas	1997	25.4%	Purulent discharge +/- bacteria culture	-Increased fibrinogen in peritoneal fluid pre-operatively		Inverted interrupted cruciate - 56% NFFN -26.1%	Polyglactin 910 - 41% Polydioxanone -33%

				-Performing an enterotomy -Use of polyglactin 910			Polyglycolic Acid -2% Unknown -24%
Mair	2005	29% -25% drainage -4% infection	Drainage - serous/ serosanguinous discharge with local edema and no heat or pain Infection - purulent discharge with swelling, heat & pain	-Intraperitoneal contamination -Intraperitoneal antibiotic administration -Repeat laparotomy -Dissection of linea alba prior to closure -Application of stent bandage	-Intraperitoneal heparin -Administration of antibiotics into the wound -Application of incise drape over incision	Simple continuous	Double stranded polyglactin 910
Isgren	2017	25.4%	-Purulent or serous discharge from the laparotomy incision of >24 h duration that developed during hospitalization	-Greater BW -PCV > 48% on admission -Small intestinal resection -Post operative colic -Winter & summer months	3-layer closure	Not recorded	-Braided lactomer single strand – 12% horses -Braided lactomer on loop – 88%

However, despite all these risk factors, repeat celiotomy appears to pose the greatest threat for development of an incisional infection.[1,6] This has been proposed to occur as a result of the presence of bacteria on the wounded edges being transferred to the incision.[6,16] In addition, the tissue is friable and edematous due to the phase of wound healing following recent surgery, which reduces the natural barrier against infection.[6,16]

2. Incisional Hernia and Dehiscence

Incisional herniation can occur relatively frequently, affecting up to 16% of horses undergoing celiotomy, resulting in significant patient morbidity.[8,23,25,30] These hernias typically development within four months of the surgery, with reports of occurrence from 2-12 weeks post-operatively.[23] Incisional herniation is suggested to occur due to complications of the celiotomy closure, such as protrusion of the omentum between sutures, partial tearing of the suture material through the tissue, suture breakage, knot slippage, excessive incisional edema, previous celiotomy, post-operative pain, patient weight >300kg, and incisional infection/drainage.[6,8,23,38-40] Horses are reported to be 12 times more likely to develop an incisional hernia following a second celiotomy.[23] Some researchers report that incisional infection preceded herniation in only a small number of cases (1 out of 4), suggesting other factors, such as uncontrolled exercise post-operatively, early suture failure, violent recovery or poor linea alba reconstruction may play a role.[8] This is in contrast to a study by Ingle-Fehr et al. that reported the odds of developing an incisional hernia were 62.5 times higher in horses that had incisional drainage.[30] Repair of hernias involve a second surgery to revise the incision and depending on how large the hernia is, it may be closed primarily using sutures or the placement of a mesh implant may be required.[23] This surgery should be delayed for approximately 3-4 months after the initial surgery to allow inflammation and infection to resolve, and the development of a mature hernial ring for repair.[16] Although the prognosis is generally good, there is increased morbidity and the delay of return to work which may be up to a year post-operatively.[23]

Complications of the closure can also contribute to acute incisional dehiscence post-celiotomy, which fortunately occurs with less frequency (3-5%), but causes significant patient morbidity and mortality.[8,16,23,25] Factors reported to contribute to acute dehiscence include absorbable sutures weakening at a time when the strength of the wound is dependent on them, increased abdominal pressure from a violent recovery from general anesthesia resulting in failure of the suture material at the knot, knot slippage or unravelling, and sutures tearing through tissue as a result of improper placement.[8] It is likely that the greatest cause of acute dehiscence is strain placed on the suture material, this can occur either during the recovery from general anesthesia period or post operatively as a result of continuing abdominal pain.[16] The presence of severe infection that results in delayed healing may cause dehiscence following tissue necrosis, however, infection is not a necessary prerequisite for dehiscence.[8,16,25] It has been reported that there is a trend for wound strength to decrease 5-10 days post operatively, therefore suggesting that this may be a time period where the occurrence of dehiscence is more likely.[41] When delayed dehiscence occurs, it is usually preceded by a large amount of peritoneal fluid drainage and gap formation in the linea alba.[16,42] This puts an emphasis on the need for strong suture and surgical knots in the immediate post-operative period as these are what is primarily holding the incision together.

b. Principles of Suture Materials, Patterns and Knots used in Large Animal Surgery

For any surgeon performing a celiotomy, one must consider what suture material to use. Adequate strength of the suture material is of paramount importance as it is solely responsible for maintaining apposition of the linea alba in the immediate post-operative period (Figure 1). In addition, many other characteristics must be considered such as the degradation of the suture material and the loss of strength over time, the security of knots completed with a certain material, and if the use of a certain material may increase or decrease the risk of developing a surgical site infection. Every surgeon should be well versed in the characteristics of the various suture materials and the disadvantages and advantages of the certain types. Below is a review of the principles of suture material, patterns and knots used in large animal surgery.

Figure 1. Least squared mean (± SEM) tensile strength of healing linea alba in horses at selected times after ventral median celiotomy, taken from Chism et al. 2000

	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*

* Significantly different ($P < .05$) than the control value at that location.

i. Suture Material

There are many ways to classify suture material. It may be categorized according to its degradation behavior, absorbable or non-absorbable. Another classification is its' composition, it may be composed of natural materials such as chromic gut, however, the majority of suture currently utilized is synthetic. Finally, suture can be divided into its' structural properties, it may either be monofilament or multifilament.[35] The type of suture utilized for linea alba closure is often dictated by the surgeon's preference and availability within the surgeon's geographical location.[16] There is a wealth of literature available comparing various suture structural and mechanical properties to aid the surgeon in this decision. It is important to note that many of these studies are performed *in vitro* and do not take into account the cyclical strains and biological effect of the tissues involved and therefore these studies must be interpreted accordingly.[6]

ii. Suture Characteristics

There are several other properties that one must consider when selecting a suture material. Flexibility is an important characteristic that allows for easy suture handling and is important, both for the successful ligating of vessels, and the completion of a continuous suture line.[35] The flexibility of a suture material is a reflection of its' torsional stiffness and diameter, with the flexibility increasing as the diameter decreases.[43]

The elasticity of a suture is another important contribute, as it allows the suture to undergo elastic deformation, followed by returning to its original length without stretching or losing tensile strength.[35] An inelastic suture may be detrimental to wound healing as it may not allow for expansion with edema and inflammation followed by a return to its original structure when the swelling subsides.

Memory is another essential feature of suture to consider. Memory is defined as the ability of a suture to return to its original shape after deformation, such as tying.[35] For example, sutures with a high degree of memory, such as monofilament material, are more difficult to handle due to their stiffness.

Briefly, the co-efficient of friction is a measure of the slipperiness of the suture material.[35] It, along with the surface characteristics determine the tissue drag of the suture. More importantly, it affects the likelihood of a knot to loosen after it is tied. Multifilament suture material has a higher coefficient of friction and therefore greater knot security.[44,45]

iii. Absorbable versus Non-Absorbable Suture Material

As previously stated, it takes approximately 8 weeks following celiotomy for the linea alba to regain similar tensile strength and collagen formation to that of prior to surgery.[15] They found that a significant increase in tensile strength was observed between 2 and 4 weeks.[15] This finding places an emphasis on the importance of the suture and knot combination tensile strength in the first 2 weeks post operatively, and shows that it does not need to retain its' tensile strength after 8 weeks. The resorption and loss of tensile strength of absorbable suture varies, with some losing complete tensile strength within 21 days, however, most undergo degradation and loss of tensile strength within 60 days.[35,46]

Degradation of natural materials occurs by proteolytic enzymes produced by inflammatory cells. When chromic gut is used, the process of degradation can be accelerated in an unpredictable fashion in the presence of infection, due to increased levels of inflammatory cells in the area. This can be complete within 7 days.[42] In both humans and horses, this suture material has demonstrated a higher incidence of wound dehiscence.[23] As a result, non-synthetic absorbable sutures are not currently used in equine celiotomy closure and have been replaced by synthetic materials.[16]

In comparison, the newer, synthetic, absorbable materials are broken down by non-enzymatic hydrolysis of ester bonds that does not involve the inflammatory cascade.[35] Following hydrolysis, phagocytosis of the suture may occur depending on particle size.[47] Synthetic absorbable suture material is typically used for closure of the linea alba during celiotomies with both monofilament and multifilament available. Many of these suture materials have been reported for use in ventral midline closures, such as polydioxanone, polyglyconate, polyglycolic acid, braided lactomer and polyglactin 910.[4,6,9,48,49] These various suture types offer certain advantages and disadvantages that relate to their monofilament or multifilament nature as outlined below. Polyglactin 910 is reported as the suture of choice for closing the linea alba when completing ventral midline celiotomies, unless an excessive amount of contamination occurred or there is an infection present.[50]

Table 2. Structure, absorption times and tensile strength of suture material commonly used in the linea alba during celiotomy closure[35]

Suture Type	Structure	Absorption	Tensile Strength
Polydioxanone	Monofilament	180 days	Reduction 25% at day 14, 30% at day 28, 50% at day 42
Polyglyconate	Monofilament	180 days	Reduction 25% at day 14, 30% at day 28, 50% at day 42
Polyglycolic acid	Braided multifilament	60-90 days	Reduction 35% at day 14, 65% at day 21
Braided lactomer	Braided multifilament	56-70 days	140% of USP requirements at day 0, 80% at day 14, 30% at day 21
Polyglactin 910	Braided multifilament	56-70 days	Reduction by 25% at day 14, 50% at day 21, 100% at day 35

Synthetic non-absorbable suture material is infrequently used for celiotomy linea alba closure as it has been shown to increase the risk of suture sinus and abscess formation and is therefore no longer recommended.[6,16,51] A report by Trostle et al. describes the development of suture sinus formation in 3 horses following the use of polypropylene suture, a synthetic non-absorbable suture material.[51] It was believed that the mechanical properties, rather than the chemical properties of the suture material likely caused tissue trauma and irritation resulting in the sinus formation. It was therefore advised that if polypropylene is used, that the amount should be limited, especially for knot formation to decrease the risk of suture sinus formation. As a result, this suture material is not commonly used for ventral midline celiotomy closure.

Stainless steel, a monofilament non-absorbable suture has demonstrated high strength and knot security and as it is a monofilament, does not wick bacteria.[52] Although it is not routinely used for celiotomy closure, its use is suited in infected areas and secondary closure of dehisced incisions.[53] Disadvantages of this suture material include: poor handling qualities, metal fatigue that may result in breakage, projection of wire ends through the skin and suture sinus tracts have been reported years after placement.[16,42]

iv. Monofilament versus Multifilament Suture Material

Monofilament synthetic absorbable suture material such as polydioxanone or polyglyconate may be used for closure of the equine linea alba. Monofilament suture is generally manufactured by a process involving melt extrusion that results in the formation of an outer and inner core as the material cools.[54] It is well recognized that monofilament may be damaged during rough handling that can occur during celiotomy closure, such as bending, twisting or grasping with hemostats. This handling results in damage to the outer core, which in turn reduces the cross sectional area altering the cross linking of fibers and therefore decreasing the tensile strength of the suture.[16]

This important point must be considered when one elects to use monofilament suture to close the linea alba. Other disadvantages include the higher bending stiffness, greater memory, and lower coefficient of friction. These properties result in poor handling and lower knot security.[35] On the positive side, monofilament suture causes less tissue drag, less risk of infection and reduced tissue reaction in comparison to multifilament suture.[35]

Polyglactin 910 is the most commonly used multifilament suture for celiotomy closure.[9,49] Multifilament suture is made from multiple filaments twisted or braided together.[35] This results in good handling properties and superior knot security in comparison to monofilament.[35] However, there are some disadvantages that must be noted. Capillarity is the process where fluid and bacteria can move into the interstices of a multifilament suture and set up a persistent infection. This is thought to occur, as the body's white blood cells are too large to enter these crevices to combat the infection.[9,35] Polyglactin 910 was associated with a higher risk of incisional infection in a retrospective study using univariable and multivariable analyses, in comparison to polydioxanone and polyglycolic acid, however, the number of horses examined was small.[18] This higher rate of infection was believed to occur as a result of contamination from enterotomy sites, allowing bacteria to establish an infection in the suture material.[18] Despite these findings, polyglactin 910 is reported as the suture of choice for celiotomy closure unless extreme contamination has occurred.[50] Another disadvantage of multifilament is the increased tissue drag, however to rectify this, multifilament may be coated with a lubricant to decrease both tissue drag and capillarity.[35] In comparison to monofilament suture, advantages of multifilament include: it does not stretch or elongate, has little memory and is largely unaffected by rough handling.[16]

vi. Coated Suture Material

Both monofilament and multifilament suture may be coated to improve their physical properties. This is particularly applicable to multifilament suture where coating can decrease capillarity, reduce the coefficient of friction and therefore also decrease tissue drag.[35] It is possible that the surface coating may become damaged during the suturing process therefore resulting in the previous discussed disadvantages of multifilament suture material. Although coating can improve handling properties one disadvantage is that it reduces knot security therefore resulting in the requirement of additional knots.[16,35] Coating agents typically used include lubricants and antimicrobials.

Lubricants commonly used for the various multifilament suture include polyglactin and calcium stearate, polycaprolate, glycolide copolymer, polyamide, polybutilate, silicone, polyethelene and polyester.[35] However, it is important to remember that coating reduces knot security, with the recommendations of adding an extra throw to the knot if the suture is coated.[16,35]

As previously mentioned multiple variables have been associated with the development of a surgical site infection following ventral midline celiotomy with few showing a protective factor. It was postulated that coating suture with antimicrobial substances may decrease the risk of surgical site infection. Triclosan is a broad-spectrum biocide with its mode of action aimed at the Fab I gene where it blocks bacterial fatty acid synthesis.[55]

In vitro studies, animal model *in vivo* studies, and human clinical studies have all demonstrated a potential benefit of the addition of an antibacterial substance-Triclosan to suture material for the prevention of infection.[55-59] In one *in vitro* study, sterile polyglactin 910 sutures coated with Triclosan, were inoculated with methicillin resistant *Staphylococcus* aureus and *Streptococcus* epidermidis and cultured in media for 24 hours. All sutures demonstrated a zone of inhibition around the bacteria.[56] These zones of inhibition were still present following 10 passes through fascia and subcutaneous tissue.[56] Using the same triclosan coated suture, a guinea pig model demonstrated a significant inhibition of *Staphylococcus* aureus after inoculation between control and treated groups ($P<0.05$).[57]For this model, the suture material was implanted subcutaneously, followed by direct challenge of *Staphylococcus* aureus through an indwelling catheter. Following this, Marco et al. found that this antibacterial suture decreased the number of positive cultures following surgery in an animal model of severe orthopedic intraoperative contamination by 66.6%.[58] In a randomized, human prospective study wound healing characteristics were similar between triclosan treatment group and control although the triclosan treatment group reported less pain immediately post operatively.[59] A second human study examining incisional infections following cardiothoracic surgery reported 24 patients from a group of 103 that had conventional sutures developed incisional infections, 10 superficial and 24 deep. This was in comparison to the treatment group closed with triclosan coated sutures (n=376) where no incisional infections developed.[55]

Despite these positive results, a randomized, prospective study of 100 horses following ventral midline celiotomy did not support these findings.[60] The control group was treated with subcutaneous conventional 2-0 United States Pharmacopoeia (USP) polyglactin 910 sutures and the treatment group was closed with triclosan coated 2-0 USP polyglactin 910. It was found that antibacterial coated suture did not decrease the likelihood of developing an incisional infection.[60]

The reason for this lack of correlation to the previous mentioned studies is not immediately apparent. It is possible that different bacteria were present in the wound or that the bacteria were present in too large a quantity. In addition, Bischofberger et al. found a trend towards an increase in incisional edema in the treatment group leaving a question regarding the clinical use of antibacterial-coated suture in ventral midline celitomy closures. Other antibacterial substances to consider for suture coating include silver doped bioactive glass. In vitro studies have demonstrated an inhibitive effect on *Staphylococcus* epidermis to suture when coating with this substance, however, to the best of the author's knowledge, this has not been examined in horses.[61]

vii. Suture Size

Another important characteristic to consider when choosing suture material is the suture size. There are two systems to describe suture sizes. Firstly, the United States Pharmacopoeia system (USP) basing the sizes on cross sectional area where 0 is the baseline, smaller sizes add 0's, i.e. 2-0 is the same as 00, and increasing size from baseline is denoted 1,2,3 and so on. The second system is the European Pharmacopoeia that uses a metric system, where a number corresponds to 1/10 of the suture diameter in millimeters (mm), i.e. size 1 metric suture has a diameter of 0.1 mm.[35] The size of the suture used for celiotomy closure is dictated by the surgeon's preference, the commercial availability depending on geographical location and the size of the horse. USP 3 polyglactin 910 is the largest suture available in the United States for celiotomy closure, however larger suture, up to USP 7, is often used outside the country.[6,49]

Basic surgical biomechanical principals state that the selected suture should be as strong as the surrounding tissue, however, excessively large or oversized suture material may weaken the wound repair by causing excessive tissue reaction.[35] In 1994, Trostle et al. determined there was no single suture material routinely used for closure that is initially as strong as the linea alba.[9] For this study a suture loop was tied through a section of linea alba and distracted to failure in a universal testing machine. Suture material tested included USP 5 polyester, USP 3 polyglactin 910, USP 2 polyglycolic acid, USP 2 polydioxanone, USP 2 polypropylene, USP 1 polyglyconate and USP 2 nylon. All suture loops failed before fascial failure with 93% failing at the knot demonstrating that the suture was not as strong as the tissue in which it was placed.[9] However, Anderson et al. demonstrated that USP 7 polydioxanone was as strong as the surrounding tissue in an *ex vivo* model.[49] Although, the testing method greatly varied from that used by Trostle et al., all 7 constructs tested with USP 7 polydioxanone failed adjacent to the suture along the abdominal wall, in comparison to the USP 2 polyglactin 910, where suture was the main mode of failure.[49] One disadvantage with using USP 7 polydioxanone is the placement of a large suture material in the surgical incision which may result in excessive tissue reaction, therefore delaying healing.[35]

viii. Suture Breaking Strength

The task of the surgeon when completing a ventral midline celiotomy can loosely be compared to an engineer. For any task undertaken, an engineer requires knowledge of the stresses and strains the structure will be placed under and the materials needed to withhold these pressures. As a surgeon, there are many variables to consider when closing an abdomen. Unfortunately, we are unaware of the intra-abdominal pressures in the immediate post-operative period, exerted during recovery from general anesthesia, although these are likely the highest pressures experienced. However, as a surgeon we have the responsibility of knowing which suture material is appropriate and is less likely to fail under these pressures.

There are many ways to test the strength of a suture that will be discussed in detail. First, the most basic way of testing a suture is by determining the tensile strength (TS) of that suture material. This is defined as the force that the untied suture can withstand before failure when the force is applied in the direction of its length.[35] This is accomplished by placing a strand of suture between clamps in a universal testing machine and distracting it until failure. This is commonly utilized worldwide to test polymers and fabrics in engineering labs and is likely important in the initial testing of suture material. However, this test is rarely performed in recent studies.[10-13] When testing a suture material with application to surgery it is best to perform testing under conditions which closely approximate an *in vivo* scenario.[62] It is unlikely that suture material is applied clinically without tying two strands together with a knot or placing a knot within the suture line. This is an important distinction as the addition of a knot to the suture greatly alters the mechanical properties and has been shown to decrease the tensile strength by up to 35%.[63] When testing with the addition of a knot some studies refer to this failure as the breaking strength.[64,65] However, when the suture and knot combination are tested in isolation it is more accurate to refer to this failure as the knot holding capacity (KHC). The KHC is defined as the maximum load to failure when tension is applied to the knotted suture material.[35]

ix. Effect of the Knot on Suture

As previously mentioned, the addition of a knot to a suture strand can greatly reduce the tensile strength of the suture. A more standardized way of comparing the KHC for knots and suture material is the relative knot security (RKS).[35] This is expressed by the formula RKS (%) = (KHC/TS) x 100 and is the knot holding capacity expressed as a percentage of the unknotted suture material. Therefore, a RKS of 100% would suggest that the addition of the knot to the suture strand has not resulted in a decrease in strength, however, this is not often the case with the RKS of surgeon's and square knots tested *in vitro* reducing the RKS by > 40%.[13] When examining failure of square and surgeon's knot, 75.3% of these failed by breaking at the junction of the knot and the standing end of the suture with 24.7% of these failing by unravelling.[11] This likely occurs as the increase of energy is directly translated to the knot/suture interface, resulting in failure occurring at this point.[66]

1. Knot Security

Knot tying is an essential part of surgery, and as it is evident how greatly the addition of a knot to a suture strand can greatly alter the mechanical properties of the suture material, it is important to ensure a secure knot is tied. Two factors to consider when tying a knot are loop security and knot security. Loop security is the ability to maintain a tight suture loop as the tissues are apposed during surgery.[35] Poor loop security would result in lack of tissue apposition, which would be detrimental for wound healing. Loop security is not often assessed during *in vitro* suture testing. Rosin et al. defines a secure knot as one that breaks rather than slips or unravels when tested to failure under tension.[67] Kummerle defines knot security as *"the effectiveness of the knot at resisting slippage when a load is applied."* To ensure this, a suture tag length of greater than 3mm is recommended for knot integrity and to prevent slippage.[35] However, when square and surgeon's knot were tested in a recent study 24.7% of these failed by slippage and unravelling of the knot which raises serious questions regarding the security of these knots.[11] In contrast, self-locking knots, which are less commonly used in veterinary medicine, are unlikely to fail as a result of slippage due to their self-locking nature.[12,13] In addition to the suture end length and type of knot, other factors reported to affect knot security are the suture material, knot configuration, suture diameter, number of throws and exposure to biological media.[10-13,35,68,69] These factors will be discussed in the following sections.

2. Knot Type

Various knots have been described, often originating from knots utilized in the fishing industry. Knot types commonly used for open equine surgery include a square and surgeon's knot (Figure 2.1 and 2.2). A knot is constructed by combining two suture ends and laying at least two throws on top of each other and tightening them. For the square knot the direction of the throws is reversed each time until the required number of throws are placed.[35] If the direction is not reversed, this results in the formation of a "granny knot", which is prone to slippage (Figure 2.3).[35] As previously mentioned slippage is undesirable unless under certain conditions such as the need for sliding a knot into a deep or confined place. Loop security is an important aspect of knot completion and if one is unable to maintain tissue apposition with a square knot then a surgeon's knot should be utilized.[35] A surgeon's knot is completed by an additional turn placed on the first throw. As this adds extra suture material to a wound it should only be used under necessary conditions where tension cannot be maintained with a square knot.

Figure 2.1 A surgeon's knot

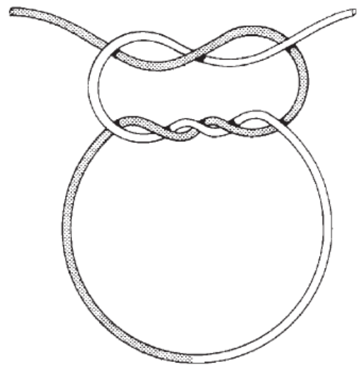


Figure 2.2 A square knot

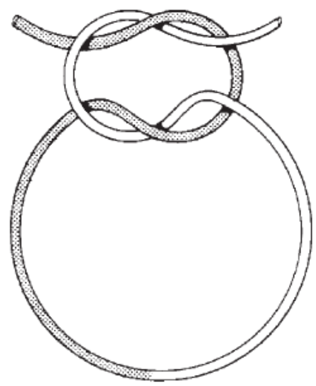
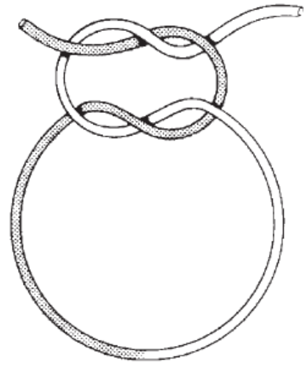


Figure 2.3 A granny knot



A second type of knot that should be considered for use in equine surgery is the self-locking knot. Certain advantages of self-locking knots over traditional surgical knots have been described and include increased knot holding capacity, increased knot security and a smaller weight and volume.[10,12,13,66] Various self-locking knots exist, and a few have been described for use in human and veterinary surgery.[10,12,13,66,70,71] In particular, properties that have been investigated include knot efficiency and slippage, KHC, RKS, weight and volume. Self-locking knots were originally suggested for use as alternatives for conventional knots when used in a continuous suture line.[66] In contrast to traditional knots, self-locking knots do not need to be large and complex to offer a reliable knot.[66]

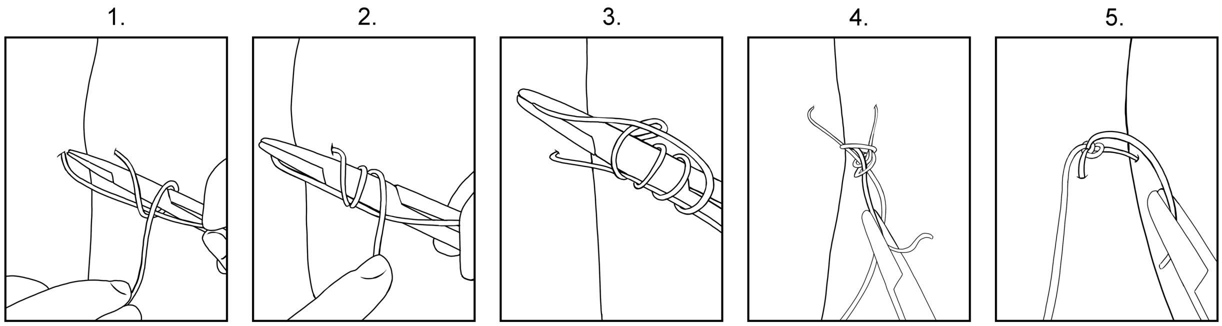
In 1994, Israelsson et al. assessed the physical properties of three self-locking knots in comparison to traditional start and end knots.[66] The self-locking knots in question were the modified half-blood knot, the chain stitch and the original half-blood knot. Three different suture materials were used: braided polyglactin, polydioxanone and nylon, in three different sizes: USP 1, 2.0, and 4.0 with all sutures tested dry. Performance and volume were similar for the three types of self-locking knots. The strength of the self-locking knots were significantly greater than the conventional knots apart from USP 4.0 nylon and the volume of the self-locking knots were significantly smaller with the exception of USP 4.0 nylon.[66] In conclusion, they found that the traditional knots caused an ~40% decrease in strength of the suture materials in contrast to the self-locking knots which only decreased the suture strength by an average of 10%.[66]

In human surgery, two communications to the editor have described the use of a sliding self-locking knot that appears to be the same as the modified half-blood knot described by Israelsson

e. al.[70,71] Both of these reports describe the use of this knot as a start knot in a continuous suture line in bariatric surgery for the reinforcement of laproscopic sleeve gastrectomy. Several advantages of the use of this sliding self-locking knot are proposed. Its use places a small amount of foreign material in a delicate part of the human stomach; it is an easy maneuver to perform and results in a secure knot. This is a result of its own auto-locking system in that the more you pull on the free end the more the knot becomes locked. [72]

Until recently, the use of this sliding self-locking knot was not documented in veterinary surgery. In 2016, Gillen et al. describes the use of this sliding self-locking knot, 'the forwarder knot' in large gauge suture and compared it to the square and surgeon's knot when used as a start knot in a continuous suture pattern.[13] These knots were tested with dry suture material using USP 2 and USP 3 polyglactin 910 and USP 2 polydioxanone. The forwarder knot was completed with 3-6 throws and the square and surgeon's knot with 4-8 throws. The results concluded that the forwarder knot was superior to the square or surgeon's knot when used as a start knot in a continuous suture line. The strongest forwarder knot was one completed with 4 throws with USP 3 polyglactin, this knot had an RKS of 84.5% which was significantly higher than the RKS of any square or surgeon's knot (RKS of the highest was 58.8%). In addition, the forwarder knot had a significantly smaller weight and volume in comparison to the surgeon or square knots completed with the same number of throws. Finally, it was demonstrated that the forwarder knot offered better knot security as none of the forwarder knots slipped or unraveled, in contrast to the square and surgeon's knot where between 30-90% of these completed with 4 throws unraveled.[13] The forwarder knot is completed using needle drivers and a free hand as depicted in Figure 2.4.

Figure 2.4 Formation of a forwarder knot

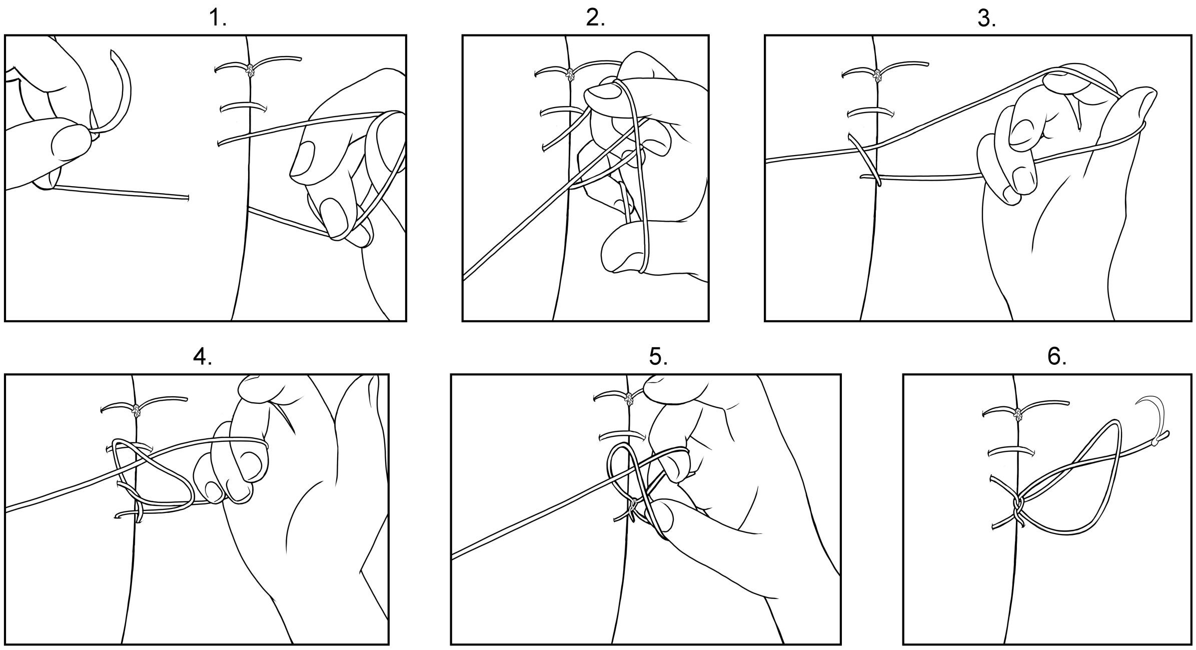


1) A bite is taken through the tissue from left to right. The free end is in the right hand and the working end is in the left hand. The working end is turned around the needle drivers and the free end to complete one throw. 2) The working end is turned around the needle drivers and free end again, each time representing a throw. 3) The working end is grasped by the needle drivers through the placed throws. 4) The needle drivers are withdrawn pulling the working in through the looped throws. 5) Both the free end and working end are pulled in opposite direction tightening and locking the knot.

A second self-locking knot that is gaining popularity in veterinary surgery is the Aberdeen knot.[10,12,68,69] The Aberdeen knot was originally described in 1930 and was named after Sir James Learmonth, a Professor of Surgery, at Aberdeen University, who discovered that it used less thread than a conventional square knot.[68] It has been also described as a 'Chain-Stitch' knot and a 'Loop Knot'.[66,68] The Aberdeen knot is employed as an end knot for the completion of a continuous suture line. This knot was one of the self-locking knots described by Isrealsson et al. that outperformed the conventional end knot in both strength and size.[66] Similar results were demonstrated by Shaw et al.[73] In 2010, Schaaf et al. assessed the Aberdeen knot using USP 2.0 polydioxanone for use in small animal surgery.[69] Again, it was found that the Aberdeen knot outperformed the square end knot with a significantly higher mean RKS of 81.08%, in contrast to the square end knot of 59.69%.[69] In addition, they found the Aberdeen knot to have a significantly smaller volume by 22-70%.

Recent studies from our laboratory show promising results for the use of the Aberdeen knot in large gauge suture such as that commonly used for closure of the equine abdomen. The Aberdeen knot has been tested with dry suture and following exposure to biological media.[10,12] Gillen et al. compared the strength and size of an Aberdeen, square and surgeon's knot with USP 2 and USP 3 polyglactin 910 and USP 2 polydioxanone. The Aberdeen knot was tested after completion with throws 3-6 with both 1 and 2 turns, and the square and surgeon's knots tested with throws 4-8. The Aberdeen knot had a significantly higher KHC and RKS than the square and surgeon's knot combination for all suture types and throw combination except the Aberdeen knot completed with 3 throws and 1 turn using USP 2 polydioxanone. In addition, the Aberdeen knot was significantly smaller by 18-47%.[12] Following this, the Aberdeen, square, and surgeon's knot were tested in a similar manner but following exposure to biological media commonly encountered during equine abdominal surgery. These included equine serum, equine fat, isotonic solution and carboxymethocellulose. Again, the Aberdeen knot demonstrated a significantly higher KHC and a smaller weight and volume compared to the square and surgeon's knot.[10] In contrast to another study examining knot security following exposure to a fat medium that found an additional throw was required when using 2-0 polydioxanone, Coleridge et al. found that exposure to biological media increased the KHC of square, surgeon's, and Aberdeen knots.[10] The Aberdeen knot is completed using both hands as depicted in Figure 2.5.

Figure 2.5 Completion of an Aberdeen knot



1) The final bite in the continuous suture line is taken from right to left leaving a large loop. The working end with the needle attached is in the left hand. 2) A second loop is created in the working end and passed through the first loop while maintaining tension. 3) The second loop is created and equals one throw. 4 and 5) Step 2 and 3 are repeated to create required number of throws. 6) The working end is pulled through the final loop and tightened to lock the knot.

<u>3. Knot Size</u>

Another important aspect of the knot that affects knot security is the knot size. This is influenced both by the size or diameter of the suture material and the number of throws used to complete the knot. Increasing the gauge of suture material has been reported to increase the strength or KHC of the knot construct and therefore increase knot security. [69,74]

Gillen et al. found when testing the KHC of square and surgeon's knots using large gauge suture USP 2 and USP 3, they offered a higher KHC than that of smaller gauge suture material USP 2-0 previously tested. [69,73] However, it was interesting to note than when the RKS, which is a standardized way of comparing suture strength across different material and suture gauges, was compared, that it was in fact higher for the smaller gauge suture as tested previously by Muffly et al., Roisin et al., and Schaaf et al.[67,69,74] This was hypothesized to occur because of an inability to tighten and cinch down on large gauge suture in comparison to a smaller gauge one, therefore resulting in an asymmetrical knot.[11] The formation of an asymmetrical knot results in increased tension on one end of the suture therefore increasing the risk of knot slippage or unravelling.[6,75]

Knot asymmetry is an important point to note and is reported to be the reason that the KHC of the end knot of a continuous suture line is lower than that of a start knot, therefore requiring an additional throw for both square and surgeon's knots.[11,73,74] This is reported to occur when forming an end knot in a continuous suture line with a square or surgeon's knot, as the loop is incorporated into the knot, resulting in asymmetry in the knot, which can be further exacerbated if the loop is not grasped exactly in the center when tightening the knot.[11,74]

The number of throws used to complete a knot is the second component that contributes to increased knot size. The characteristics of suture material that affect knot security are memory and coefficient of friction. The addition of throws to a knot are required to prevent the knot from slipping and unraveling under tension. Suture with a high memory and a low coefficient of friction is more likely to unravel or return to its original shape after tying. The number of throws required to form a secure knot is dictated by the suture material, suture gauge and knot type and these optimum throw numbers have been established for certain knots, suture materials and suture gauges.[67] The ideal surgical knot would be one that has a high KHC, a high RKS with a low weight and volume. Determining the optimum number of throws required for a secure knot involves establishing a balance between these factors.[65,66] Increasing the number of throws beyond that which is required is detrimental as an increased amount of foreign material has been shown to cause an increase in inflammation within a wound which may adversely affect healing.[37,51] This topic will be further discussed along with wound healing in a subsequent section.

In 1976, Tera et al. examined the tensile strength of 12 different knots using different suture material in size USP 3-0. A combination of square, granny and surgeon's knots were examined using a variety of different throws.[76] Irrespective of the suture material used, it was found that the more turns and throws used to make the knot, the higher the knot holding capacity and the greater the relative knot security. The knot holding capacity almost doubled by increasing throw number from 2 to 6. In this study ear tags were cut to a few millimeters and defined as failure if slipped greater than 2mm. In total 175 out of 720 knots failed by complete slippage.

Rosin et al. examined the number of throws required for small gauge suture USP 2-0 to form a secure knot that fails by breaking rather than slipping under tension. For all knots tested the ear tags were cut at 3mm. It was determined that for a square start knot completed with polyglycolic acid, polypropylene or polyglactin, 3 throws were required, 4 throws for chromic gut, and 5 for polydioxanone. It was noted that the surgeon's knot was as secure as the square knot and for the end knot 1-3 extra throws were required to make the end knot secure.[67] This is likely explained by the asymmetric knot loop as previously discussed. The optimum number of throws has also been established for large gauge suture. Gillen et al. examined the KHC, RKS, weight, and volume of surgeon's start, square start, surgeon's end, and square end knots completed with USP

2 and USP 3 polyglactin 910 and USP 2 polydioxanone. No significant differences were found for RKS and KHC between surgeon's and square knots. Based on the findings it was recommend to place 6 throws on the start knot and 7 on the end knot.[11] This was recommended as the KHC did not increase significantly with increasing throws beyond these and when less than 6 throws were added to the start knot 25% failed by unraveling. [11] When 7 throws were applied to the end knot 0.04% failed by unravelling in comparison to 62.2% tied with less than 7 throws.

As previously mentioned, self-locking knots offer certain advantages over conventional knots when used in a continuous suture line. These knots have also been assessed to determine the optimum number of throws required to form a secure knot.[12,13,66] Stott et al. examined the security and average breaking force of an Aberdeen knot completed with USP 0 polydioxanone and determined that one completed with three throws and two turns was the strongest knot and unlikely to slip.[77] Both the forwarder and Aberdeen knots have been examined in large gauge suture commonly used in equine abdominal surgery to determine the optimum throw number for knot security. The strongest forwarder knot was completed with polyglactin 910 and 4 throws, however no significant increase in KHC or RKS was noted in either USP 2 polyglactin 910, USP 3 polyglactin 910 or USP 2 polydioxanone with an increase in throw number from 3 throws to 6 throws. This suggests that 3 throws are adequate for completing a forwarder knot in large gauge suture material. In addition, due to the self-locking nature of the knot, none of the forwarder knots failed by unraveling.[13] Gillen et al. also examined the RKS and KHC of Aberdeen knots and found no significant increase in KHC or RKS between 3 and 6 throws when using USP 2 or USP 3 polyglactin 910. However, it was noted that the RKS and KHC was significantly lower when using USP 2 polydioxanone.[12] Based on these findings it was recommended to complete an

Aberdeen knot with 4 throws and 1 turn when using large gauge suture material. Regarding knot security, none of the Aberdeen knots were observed to fail by unraveling.

4. Suture Material

It has been shown that different suture material type affects knot security. Polyglactin 910 has been shown to produce a consistently higher KHC in contrast to polydioxanone, regardless of the suture gauge.[10,11,78,79] This is likely explained by the fact that polyglactin 910 is a multifilament braided suture in contrast to polydioxanone which is a monofilament. Mechanical properties of the braided suture material that explain these differences are that the irregular surface formed by the braided material increases the coefficient of friction, which when tied in a knot, will increase the knot security.[35,80,81] In contrast, when polydioxanone, a monofilament suture material, is placed under tension it demonstrates plastic deformation or stretching which may lead to knot distortion and failure.[75,80] This plastic deformation was observed with slow motion video to occur prior to failure at the knot-suture junction with large gauge suture, which may have resulted in weaker knots.[11] When examining specific suture materials, differences in strength and security do exist. Trostle et al. demonstrated that USP 5 polyester had a higher breaking strength than all other suture material tested, however, it also had the largest diameter.[9] It was interesting to note that USP 3 polyglactin 910 and USP 2 polyglycolic acid had similar strength, although the diameter was different. Both of these were significantly stronger than USP 2 polydioxanone and USP 2 polypropylene, with USP 2 nylon being the weakest suture material tested.[9]

x. Effect of Fluid Media on Suture Material and Knot Efficacy

As previously mentioned, knot security is influenced by both knot type and knot size/suture size. Another important factor to consider is the exposure of the knot to physiological conditions encountered during surgery. Prior to usage all materials must undergo testing. For suture, this is typically performed *in vitro* following removal from the suture package and tested in universal materials testing machines. However, suture is unlikely to remain dry when it is placed in the wound for closure.[68] This occurs either during the suturing process or in the post-operative period.[82] In the equine colic patient fluid or biological media typically encountered includes equine serum, equine fat, isotonic solution, and carboxymethocellulose.[35,78,83]

It is well known that the coefficient of friction is an important determinant of knot security.[35,44,82] The frictional force of a knot depends on the coefficient of friction, the number of throws, the angle of these throws, and the forces causing the throws to cinch down on themselves. If the total frictional force is high, the knot may remain firmly in place, however, if it is low, it may slide resulting in failure.[44] It has been demonstrated that the coefficient of friction has a linear relationship with tensile strength and relative knot security.[84] It has also been demonstrated that the effect of coating suture material or exposing them to lubricants or various biological media affects the coefficient of friction and therefore knot security.[10,44,68,69,82,83,85] The results from these studies have been varied with some researchers observing that knot slippage or failure occurred at lower forces or a decrease in the coefficient of friction occurred in suture exposed to saline or biological conditions in comparison to dry suture.[44,85,86] In contrast, other researchers found that exposure to fluid media, saline and glycerin, resulted in greater frictional forces, with suture exposed to glycerin having the highest values.[82]

To explain these findings, Gupta proposed a theory in 1985.[82] This theory stated that when lubrication is applied to suture material it can lead to two different states: boundary and hydrodynamic states. The boundary state occurs when a very thin layer or monolayer of lubricant is applied to the suture material, leading to less points of contact between the suture strands within the knot, resulting in a decrease in the coefficient of friction. In contrast to this, the hydrodynamic state occurs when a thick film of lubricant occurs on the surface of the suture material so that the suture surfaces are generally not in contact. When this occurs, the frictional force is determined by the resistance of the lubricant to shear. In conclusion, the greater the viscosity of the lubricant, the greater the frictional force. Gupta hypothesized that the increase in knot slippage observed when tied in fluid environments (lubricated) was predominantly a result of the boundary state within the knot. This was thought to occur as the knot is placed under tension it expels the excess fluid resulting in fewer lubricant molecules present, therefore existing in a boundary state. [82] In summary, the findings of Gupta and other earlier reports suggest that exposure of suture to lubrication may increase or decrease the friction and is likely dependent on the nature of the application, nature of the lubricant, and knot construction.[44,82,86]

It has been demonstrated that exposure to various fluid media have an immediate effect on the frictional properties of suture, affecting knot strength and knot security. A second important aspect is to determine if these various fluid media affect the suture in the long-term period such as causing the suture to degrade or become weaker over time, which may be detrimental to wound healing conditions or wound strength in the post-operative period. As a result, studies examining the effect of fluid media on suture properties are typically divided into studies evaluating the effects of short-term exposure and long-term exposure to fluid media.

1. Short Term Media Exposure

The majority of past studies fall into this category. Most of these studies have examined small gauge suture following exposure to saline, plasma or fat without having a dry or unexposed suture group.[67-69,87] More recently, there are reports examining large gauge suture material commonly used in equine surgery comparing these findings to a dry control.[10] It is important to compare findings to a dry control to determine the effect of media on a particular suture as most *in vitro* suture testing is performed dry. Rosin et al. examined the number of throws required to make a secure square knot for various small gauge (USP 2-0) suture materials following incubation in canine serum for 24 hours.[67] Throw numbers were determined for the various suture to form a secure knot, however, the failure strength of these knots or a dry comparison was not examined. Rosin et al. only examined conventional square knots, whereas others have examined self-locking knots and their behavior following exposure to fluid media.[10,68,69] Schaaf et al. examined the Aberdeen knot tied with USP 2-0 polydioxanone.[68] It was found that the minimum secure configuration in fat and plasma was 3 throws and 1 turn but there was more suture tag slippage in fat compared to plasma, showing that fat covering decreases the knot security significantly in comparison to plasma. In addition, it was concluded that an Aberdeen knot tied with 4 throws and 1 turn slipped significantly less than 3 throws and 1 turn in plasma. Following this, Schaaf went on to compare a secure Aberdeen knot exposed to fat and plasma to square start and end knots.[69] Again it was polydioxanone USP 2-0 that was tested and it was interesting to note that an extra throw was required to form a secure square start knot in fat compared to plasma. Unfortunately, a dry comparison was not assessed in either of these studies.[67,69]

More recently, Coleridge et al. examined the effects of fluid media on square, surgeon's and Aberdeen knots completed with two different large gauge suture materials, USP 3 polyglactin 910 and USP 2 polydioxanone.[10] In addition to a dry control group, suture was exposed to equine fat, equine serum, saline, and carboxymethocellulose. No significant differences in KHC, weight or volume were observed between different media ($P > 0.08$). In contrast to other studies, it was found that Aberdeen knots completed with either suture material exposed to fluid media had a significantly higher KHC than those dry ($P < 0.001$). Surgeon's knots tied with 5 and 6 throws and square knots tied with 5 throws with polyglactin 910 had significantly higher KHCs than their dry counterparts. These findings were thought to occur as exposure to fluid media allowed the load to be distributed over a larger area, therefore decreasing stress accumulations at focal points that would typically lead to failure.[10,12]

2. Long Term Media Exposure

The first description of suture material testing following exposure of suture to biological media was in 1973 when Herrmann et al. evaluated the tensile strength of sterile suture loops placed within the subcutaneous tissue of both rats and rabbits after varying time periods.[88] No change in tensile strength of both braided polyester and polyglycolic acid was observed after 1 week *in vivo*. The knot type tested in this study was a square knot consisting of 3 throws. Following this, Tera et al. performed a larger study examining the tensile strength of 12 different types of surgical knots constructed with various suture materials implanted into the subcutaneous tissue in rabbits after 1 week.[89] All suture materials were USP 3-0 and in addition to testing these knots after *in vivo* exposure, they also compared the results to that of a previous study where dry suture was tested.[76] The knots tested were a variation of square, surgeon's and granny knots with different numbers of throws designating them as 'simple' and 'complex'. The main finding from this study was that a greater proportion of knots slipped *in vivo* (72%) in contrast to almost half of that *in vitro* (35%). Slippage was very common in the 'simple knots' in comparison to the 'complex knots' where it occurred with less frequency. It was noted that there was a greater standard of deviation in the *in vivo* tested knots with variable results from different animals suggesting that *in vivo* conditions may fluctuate between animals. Seven of the 11 suture materials demonstrated a loss in knot-holding-power *in vivo*, these included monofilament steel, chromic and plain catgut, polyester and braided polyester, polyethylene and polypropylene. Polyglycolic acid tensile strength remained unchanged after 1 week *in vivo*, further supporting similar findings reported by Herrmann et al.[88,89]

It is well known that suture materials are often exposed to a variety of bodily fluids during surgery. As a surgeon, it is important to determine if these fluids may affect the tensile strength of suture material in a negative way. The pH of bodily fluids can change under pathologic conditions and it is important to recognize that this change in pH may alter the biomechanical properties of suture material. Chu et al. demonstrated that absorbable sutures were more sensitive to pH than non-absorbable ones and that strong alkaline conditions adversely affect the tensile strength of suture when compared to physiological and acidic pHs.[90] Of the absorbable sutures tested, polyglycolic acid and polyglactin 910 showed better strength retention than plain catgut. Both of these sutures retained >90% of strength after 7 days of immersion in acidic and neutral pHs, with polyglactin 910 showing the best strength retention in alkaline pH. In addition, polyglactin 910 demonstrated the smallest variation in elongation with pH change.[90]

In 1992 Campbell et al. reported the first study to examine the mechanical properties of large gauge suture material following implantation in the horse.[64] Properties examined were maximum breaking strength, energy absorption, and percentage elongation to breaking point. Suture materials examined were USP 5 braided polyester, USP 2 polydioxanone, USP 2 polyglycolic acid and USP 2 monofilament nylon. However, polyglactin 910, a large gauge suture that is now commonly used to close the linea alba was not included for assessment. The 'loop test' (tying a knot and distracting from the inner loop) was used to assess the knot parameters and a jig was used to tie the knots prior to implantation to ensure standardization. Three hundred knots were tied (75 of each material) and 60 of each material were implanted subcutaneously in the left flank and chest wall for 7, 14 and 28 days with 15 remaining dry for *in vitro* testing. It was found that 93.6% failed at the knot, and that polyglycolic acid was significantly stronger throughout the *in vitro* study. Braided polyester had the greatest maximum breaking strength throughout the *in vivo* testing and monofilament nylon was significantly weaker and had the greatest percentage elongation except on day 28. Although *in vitro* and *ex vivo* parameters were examined, these were not compared for statistical significance. Examining the figures, it appears that polydioxanone had a higher breaking strength at 7 and 14 days *in vivo* compared to *in vitro* but not at 28 days. Although braided polyester had a higher overall maximum breaking strength, the authors warned about the risk of suture sinus formation and concluded that polydioxanone had better overall mechanical performance even though polyglycolic acid was more commonly used during that time period.[64]

More recently another research group examined the effect of elasticity and breaking strength of three large gauge USP 2 suture materials glycolide/lactide copolymer, polyglactin 910 and polydioxanone following incubation in phosphate buffered saline, equine serum, inflamed equine peritoneal fluid and equine urine for 7, 14 or 28 days.[91] This study examined the unknotted suture material and determined that incubation time had a significant effect on yield strength, percentage elongation and young's modulus in all media ($P<0.0001$). Suture type significantly affected changes in these parameters with glycolide/lactide copolymer showing the most rapid degradation and polydioxanone showing the least variation across media and incubation period.[91] The same research group went on to examine the effect of incubation of these same suture materials in phosphate buffered saline and inflamed equine peritoneal fluid on knot security.[83] The 'loop test' was utilized and knots completed were a square knot with 4 throws, a surgeon's knots with 5 throws and a triple knot with 7 throws. All loops failed at the knot with 9.8% failing by slipping. Significant differences in force to failure was seen for polydioxanone between the square and surgeon/triple knot. Polyglactin 910 showed a significant difference in force to failure between the square and triple knot, with the triple knot having a higher failure load. Incubation in phosphate buffered saline resulted in an increased force to failure for polydioxanone, and a decrease for glycolide/lactide and polyglactin 910. Incubation in inflamed peritoneal fluid caused a rapid decrease in force required to failure for glycolide/lactide and polyglactin 910 but not polydioxanone.[92] It was concluded that the mechanical properties of suture materials and exposure to various media affect knot security and for polydioxanone a square knot is insufficient to complete a secure knot.

xi. Suture Patterns

Another task for the surgeon prior to finishing surgery is to determine the optimum suture pattern to appose the tissue involved. Multiple factors must be considered, such as the strength that various patterns offer, how these patterns may interfere with wound healing and local blood flow, and finally if the tissue in question is under tension and if so how it should be properly apposed. One must refer to the basic surgical biomechanical principles.[35] These state that the selected suture should be as strong as the normal tissue through which it is placed and the placement of oversized suture or excessive amounts of foreign material within the wound may cause excessive tissue reaction which may be detrimental to wound healing.[35] It is often not possible to satisfy all of these principles, however, multiple scientific reports document certain pros and cons to different closure techniques that should be adhered to in an attempt to optimize wound healing.[4,9,35]

1. Continuous versus Interrupted Suture Patterns

Suture patterns can be broadly classified as interrupted or continuous, with each pattern having certain advantages and disadvantages (Figure 3.1 and 3.2). It is believed that simple interrupted suture patterns offer a more secure closure because if one of the sutures fails it does not compromise the entire suture line.[35] Contrary to this statement, it has been demonstrated that a simple continuous suture line offers a stronger closure than an inverted interrupted cruciate one when completed with USP 3 polyglactin 910 in an equine *ex vivo* cadaver celiotomy model and compared to a simple interrupted suture pattern using an in vitro model.[4,41] Interrupted patterns are also thought to be advantageous as they allow for reconstruction of irregular wound edges and therefore precise adjustment for tension on the wound at each placement point.[35] In addition, they do not form a purse string effect when tightened in hollow viscera as may occur with continuous patterns and it is thought they have less interference with the blood supply at the wound margin. Finally, in the case of a draining wound or infection post-operatively they allow for part of the suture line to be reopened without compromising the entire incision.[35]

Figure 3.1 A simple interrupted suture pattern [35]

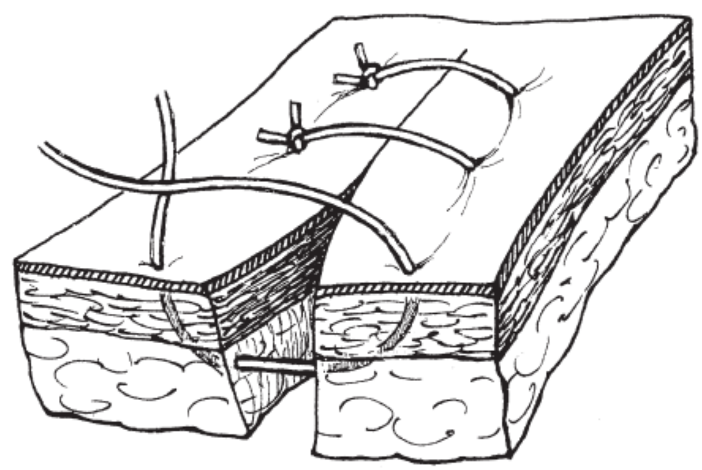
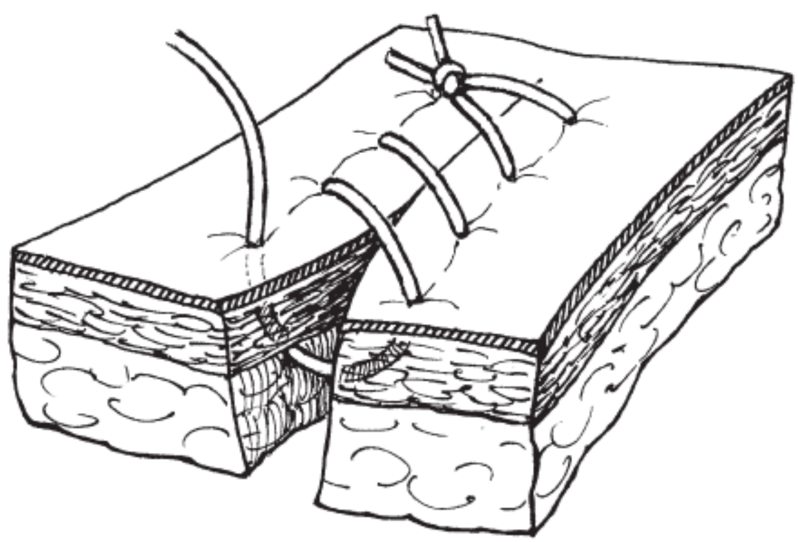


Figure 3.2 A simple continuous suture pattern [35]



In contrast, a continuous suture line offers certain advantages over an interrupted one. As fewer knots are placed in the incision this results in an overall smaller volume of suture material placed in the wound.[35] Regardless of the suture material type all suture placed in the wound is considered a foreign body to the tissue and results in a certain level of mechanical irritation which leads to a certain degree of tissue reaction. It is thought that an excessive level of tissue reaction may play a role in the formation of a suture sinus.[51] It has also been stated that the amount of tissue reaction caused by the mechanical irritation is directly related to suture size, suture stiffness, knot configuration, and knot volume.[36,37,51] In this regard, it would seem that a continuous suture line with fewer knots would be preferable. A second advantage of continuous suture lines is that they are completed quicker therefore decreasing surgical times.[35] This is important both from an anesthetic point, in addition to a surgical one. It has been demonstrated that increased surgical times can result in decreased survival rates for horses along with increased risk of incisional complications.[1,19,93] Continuous suture lines offer even distribution of tension resulting in a better holding power against stress as demonstrated by Magee et al.[4] Finally it allows for a tighter seal of certain tissues such as skin and hollow viscera.[35]

A further characterization of suture patterns is the way they appose the tissue edges. They can be appositional, where the two tissue edges are evenly approximated. They may be inverting or everting that have their relevant uses for different tissues. In the case of the equine linea alba the goal is to evenly appose the tissue edges.[35]

2. Tension Relieving Sutures

Tension relieving sutures will be briefly mentioned as they were historically used for closure of the equine linea alba.[8] The goal of tension relieving sutures is to redistribute the tension across the wound edges allowing for closure and avoiding any strangulation or necrosis of the tissues. The ability of tension relieving sutures to allow for this is based on the number of suture segments that are parallel to the lines of tension.[94] Tension relieving sutures are often preplaced away from the wound edge, which is apposed, followed by tightening of the sutures.[35] Commonly used tension relieving sutures include horizontal and vertical mattress and a combination of near and far placed sutures. The use of a near-far-far-near suture pattern has been described for celiotomy closure in the horse and has been associated with the development of incisional infections.[8] Kobluk et al. examined the risk of developing incisional complications in 78 cases from 1983 to 1985.[8] Factors examined included peritoneal fluid presence, enterotomy or resection, suture material and pattern, type of skin closure and use of a stent on the incidence of incisional complications and found that the only significant risk factor was the use of a near-far-far-near suture pattern ($P < 0.05$).[8] This was thought to occur as a result of undermining tissue that was necessary to place the sutures and therefore creating dead space increasing the risk of infection. At the time, placing near-far-far-near sutures was reported to increase the strength of the linea alba closure by 50-100% in contrast to simple interrupted, however, based on Kobluk's findings, it is disadvantageous to use this suture pattern due to increased risk of surgical site infection.[8,95] On the other hand, a second large study examining risks for incisional infection following celiotomy did not find that suture pattern had an influence on the development of a post-operative incisional infection.[18] The near-far-far-near pattern was used in 26% of horses, however, in this study the tissues were not undermined which was believed to be the reason that the infection rate was reduced. Nonetheless, this suture pattern is not currently routinely used to close equine celiotomies in the US.[4,6,96]

3. Optimum Suture Pattern

Magee et al. demonstrated that a simple continuous suture pattern offered a stronger closure in comparison to an inverted, interrupted, cruciate pattern.[4] In this study, an inflatable bladder was placed in equine cadavers through a celiotomy incision which was subsequently closed and the bladder inflated until failure. The continuous pattern had a significantly higher bursting pressure than the interrupted pattern ($P = 0.01$). In addition, significantly less suture material was used with the continuous pattern, which may be advantageous to healing ($P = 0.0002$).[4,35,37,51] However, it was interesting to note that they did not find a significant difference in closure time between the two groups. [4] An alternative to completing a simple continuous suture line without the use of a starting knot has been described.[48] This involved the use of USP 2 braided lactomer 9-1 as the suture, where a swaged needle was passed through a loop instead a creating a start knot in the continuous suture line. The bursting pressures achieved (420-479mmHg) were comparable to that of a simple continuous suture line (433.5 ± 39.4mmHg).[4,48] Proposed advantages of this method included a quicker surgical time and a reduction in the number of knots within the wound, however one must question the extra amount of foreign material involved in a double stranded suture line and a four-stranded knot.

Addressing the various findings, a simple continuous suture pattern using a synthetic absorbable suture material is the most commonly used suture pattern to close equine celiotomy closures.[6,96]

This is likely due to some of the aforementioned advantages such that it offers an even distribution of tension and therefore a more secure closure.[4,6,35]

xii. Tissue Bite Size

As mentioned, various factors affect the strength and outcome following ventral midline celiotomy in the horse. From a mechanical point of view, suture pattern, suture material and knot configuration are important aspects to consider when wishing to optimize strength and wound healing. Another important mechanical factor to consider for closure of the equine abdomen is the tissue bite size from the wound edge and the tissue bite size interval. This has been studied in humans with a recommended tissue bite size of 12-15mm.[64] Suture interval, amongst other factors, will dictate the amount of suture material required. In human surgery, one parameter that has been examined is the suture length to wound length ratio (SL:WL). This incorporates the length of the suture used for the entire incision in comparison to the length of the incision, to provide recommendations on adequate suture length. The optimum (SL:WL) of > 4:1 is advised for celiotomy closure.[38,97] This ratio is recommended to provide adequate reserve suture in relation to the length of the wound to allow incisional lengthening during times of abdominal distension.[38] This ratio results in a tissue bite interval equal to the tissue bite size.[98]

In horses both the optimum tissue bite size and interval have been investigated, however, the optimum SL:WL ratio has not been established.[9,48] In 1994 Trostle et al. tested sections of linea alba placed in clamps in a universal testing machine with one suture loop placed through the tissue at different distances from the fascia edge.[99] The tested distances were 6, 9, 12, 15,18, and 21 mm from the tissue edge. It was found that both the tissue bite size and linea alba thickness accounted for the variability in breaking strength with the bite size accounting for 44% and the tissue thickness for 24% of the variability observed. The tissue bite size did significantly affect the breaking strength and this was seen in a logarithmic fashion with breaking strength increasing gradually up to 15mm. After 15mm there was not a significant increase in strength observed.[99] In laboratory animal models it is suggested that there is a transition zone between the linea alba and the rectus abdominus muscle and when sutures are placed lateral to this transition zone there is a twofold increase in breaking strength, when compared to placement within the linea alba.[98,100] The authors of the equine study suggest that the region within of 12-15mm of the center of the linea alba likely corresponds to this transition zone in horses, and based on the increased strength of sutures placed 15mm from the linea alba edge, this is an optimum distance for bite placement.[9]

More recently Hassan et al. examined the bursting pressure in horse cadavers between different tissue bites intervals of 10mm and 15mm while using a tissue bite size of 15mm.[48] This was using the same *ex vivo* model as originally described by Magee et al.[4] They also assessed SL:WL which was calculated by dividing the total suture length used to close each incision to the total length of the incision. They did not find any significant differences between closure time, total amount of suture used, SL:WL, or bursting pressure.[48] For this study they used USP 2 braided lactomer 910 and they found that failure of the fascia was the main mode of failure, that this occurred more frequently with the 15mm interval (86%) in comparison to 57% for the 10mm interval, however, this was not significantly different. Although not significantly different, closure time and suture length was less for 15mm x 15mm intervals and the authors suggest these may be potential advantages of using these intervals for clinical cases.

c. Methods of Evaluating the Mechanical Properties of Suture Materials and Knots

Various methods have been described to test various components of the celiotomy closure. This includes *in vitro* methods, *ex vivo* and *in vivo*. *In vitro* methods are most commonly used and typically isolate the suture material for testing. Materials testing has been performed for over a century determining the strength and elasticity of various materials.[101,102] The tensile strength of suture material is the force required to cause failure when force is applied to the untied suture in opposite directions.[35] This is an important physical property to determine for suture material. However, in the initial descriptions of suture testing it was determined that the coefficient of friction was an important parameter to consider as it plays a key role in determining knot security.[44,82,84] Different techniques have been described to assess this throughout the last four decades. Initially, testing involved twisting suture material around itself at various angles. The coefficient of friction was then calculated using a formula where the coefficient of friction is equal to the natural logarithm of the ratio of the two forces, divided by the angle of suture contact in radians (Figure 4.1).[84] At the time it was thought this was a more accurate way of testing knot security as it eliminated the human element.[84] In addition, it was concluded that the coefficient of friction was independent of suture size but had a characteristic linear relationship with the tensile strength and relative knot security.[44] This demonstrated how important knot security is, and how much the addition of a knot to a suture line alters its mechanical properties.

Figure 4.1

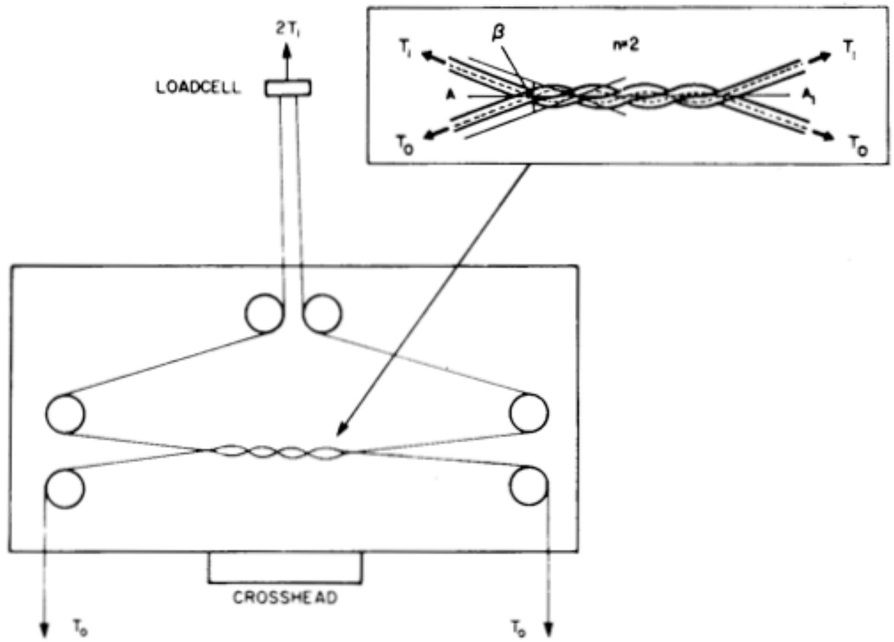


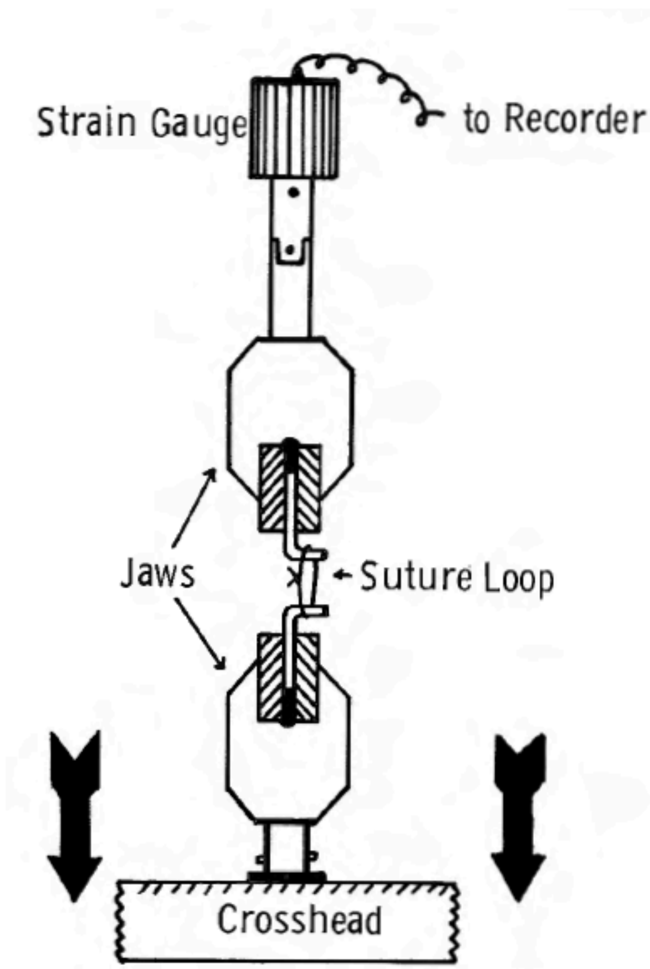
Diagram from Gupta et al. to depict the apparatus used to perform the twist test, load values obtained were used to calculate the coefficient of friction.[44]

i. In Vitro Testing

To test the strength of various suture materials a standardized method of testing is important to establish. It was recognized early that the addition of the knot to a strand of suture significantly alters its strength and in addition, it is unlikely for suture to be used *in vivo* without completing a knot. As a result, most suture testing incorporates the knot during the testing process. *In vitro* testing can be divided into testing a single cycle to failure or cyclical testing. The majority of suture testing tests a single cycle to failure and this will be discussed in more detail in the following section.

Generally there are two ways to test a knotted suture strand, the ‘loop test’ and the ‘single strand test’.[84,103] Early reports describe the technique used by the United States Pharmacopoeia (USP) as tying a suture strand around a piece of rubber and distracting the ends of the suture.[84] In the early 1970’s Herrmann et al. tested various suture material using the ‘loop’ technique.[84] Knots of suture material were tied over a rod and transferred over the ends of two right angle rods that were gripped in a tensiometer (Figure 4.2). The jaws were distracted at 1cm/minute until failure.

Figure 4.2



Schematic depiction of the experimental design for performing the ‘loop test’ as originally described by Hermann et al.[84]

This method differed from the one described by the USP but was believed to more accurately mimic an *in vivo* scenario and in addition allowed for estimation of the knot security. It was found that the values obtained by this method were exactly twice those described by the USP method.[84] This was attributed to the fact that the loop technique distributes the stress on two segments of suture material.[84] Later in the same decade Holmlund et al. compared the two methods; the loop and single strand.[103] It was concluded that factors other than the knot properties influenced the values obtained with the loop technique whereas with the single strand a more elective estimation of the knot properties were allowed.[103] These testing techniques allowed a standard testing for comparison between studies and formed the basis of suture testing. These techniques are still used today and modifications of them have been established to take into account both the KHC and knot security.

In 1989, Rosin et al. describes a modification of the single strand test.[67] This group were primarily interested in answering questions regarding knot security. Various suture materials were examined with square, granny and surgeon's knots used to start and end a continuous suture line. Instead of using the single strand method where suture tag ends were distracted, the knots were completed with the ear tag cut to 3mm and the other tag that would be placed in the tissue was left long. The loop was placed over a cylinder and the long end placed in grips with a constant load applied (Figure 4.3).

Figure 4.3

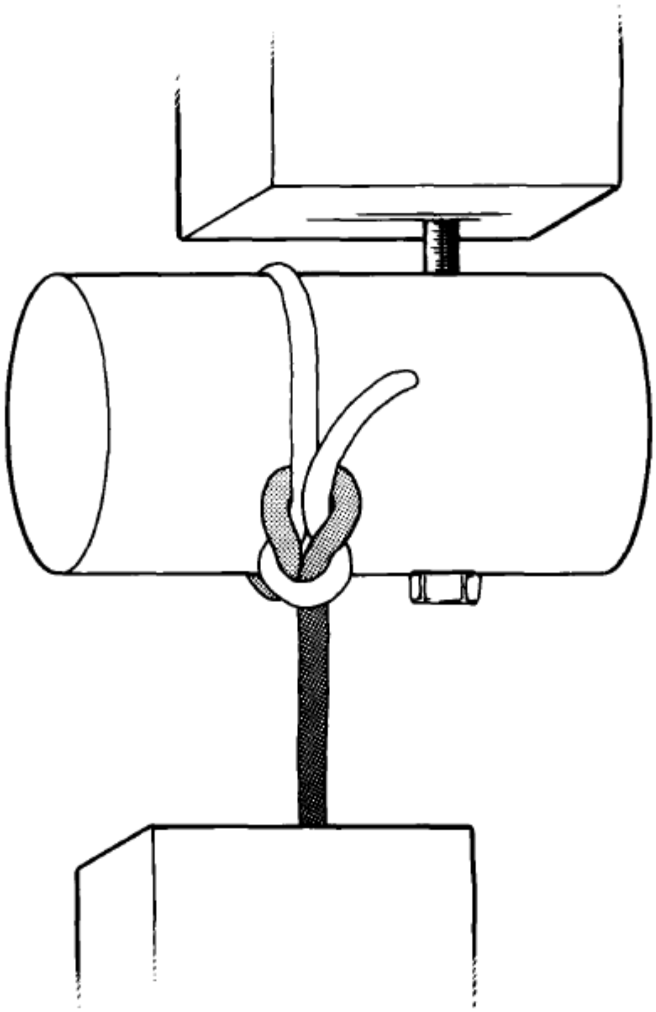
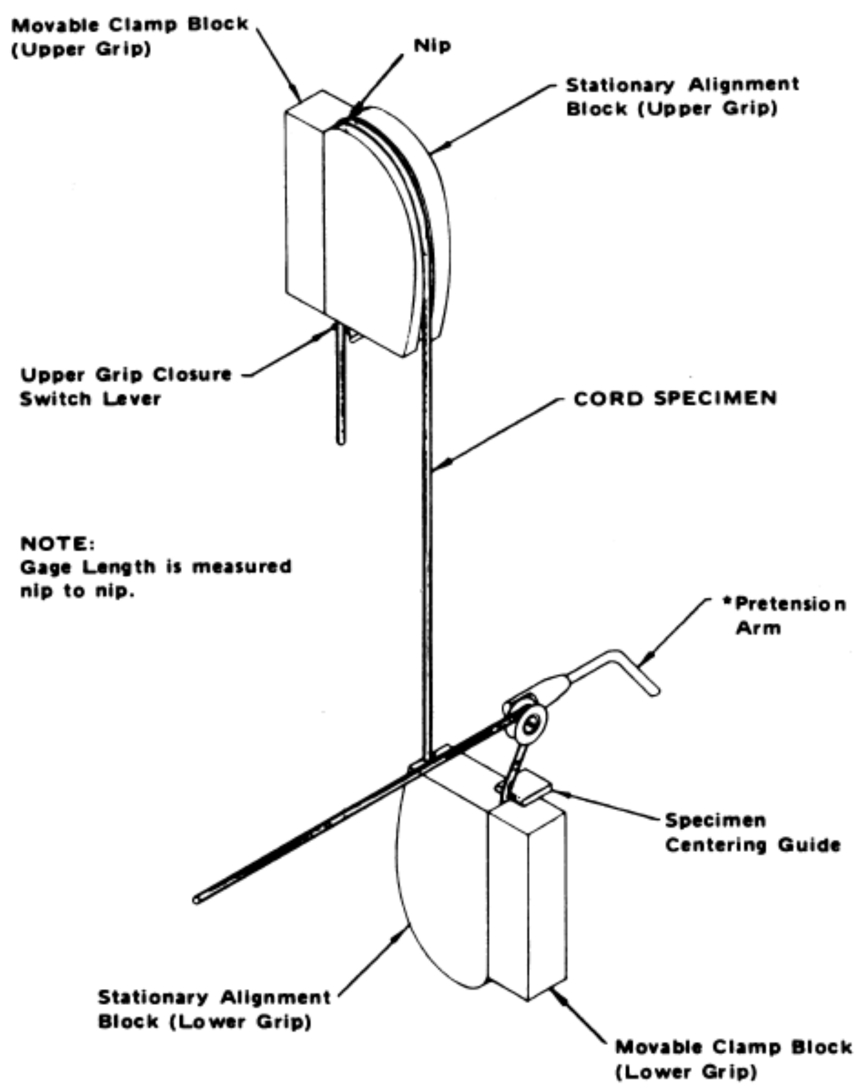


Diagram to depict the testing apparatus used by Roisin et al., to perform a modification of 'the single strand' method for testing knot security and strength.[67] The knot loop is placed around the cylinder that is held in the by the upper grip of the testing machine. The ear is cut to 3mm and the long end is placed in the clamps of the lower grip in the materials testing machine.

This type of testing is commonly used today.[10-13,68,69] However, one of the problems potentially encountered with this method involves the placement of the suture strand in the clamp. This area can be the concentration of stress accumulation which may result in failure at this location and therefore giving false or inaccurate results. Chu et al. posed a solution to this problem with the use of a yarn grip.[104] This grip was specifically designed for use with fibrous materials. In contrast to conventional clamps that were previously used, the yarn grip does not pinch the sample, instead specifically shaped mandrels distribute the force over a length of the sample avoiding braking at the clamp points (Figure 4.4).[104]

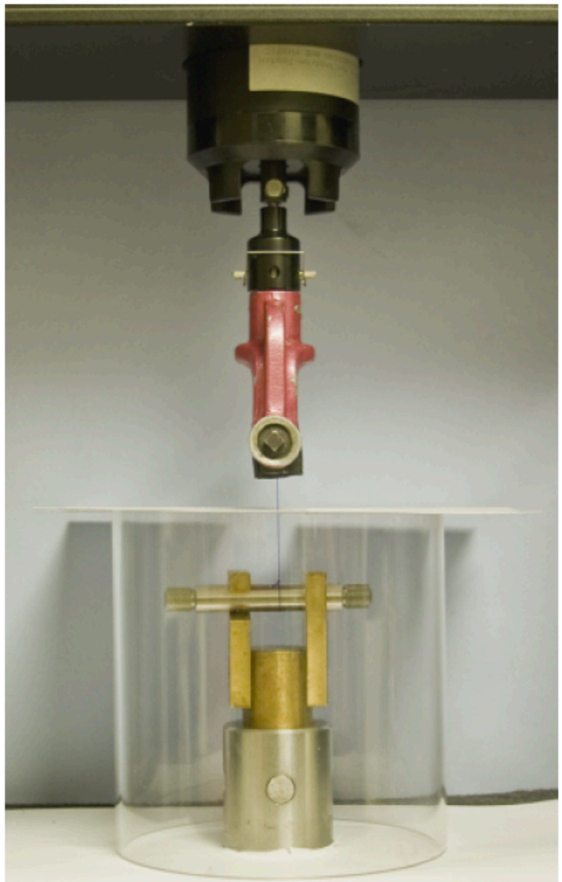
Figure 4.4



A schematic depiction of the specimen loading apparatus and the yarn grip as originally described by Chu et al.[104] The cord specimen is placed over the specially shaped mandrel of the yarn grip that distributes the force gradually over a length and avoids clamping the specimen creating stress accumulation in a focal point.

This group also compared the stress-strain curves achieved by the yarn grip and compared them to conventional grips. These were consistently different, especially at the plastic deformation region of the curve. It was concluded that using the yarn grip more accurately represents the properties of suture materials as a result of elimination of grip-induced failure. In addition, this report is one of the earlier ones to use Instron® technology that is now commonly used.[104] Prior to this the use of a dynamometer consisting of a strain gauge and various load cells has been described.[76] Others have made an attempt to overcome the focus of stress accumulation with suture clamping without using a yarn grip. Schaaf et al. glued the long end of the suture strand to rubber pads that were then placed in the machine clamps when testing surgeon's, square and Aberdeen knot using a 'single strand' technique (Figure 4.5).[69]

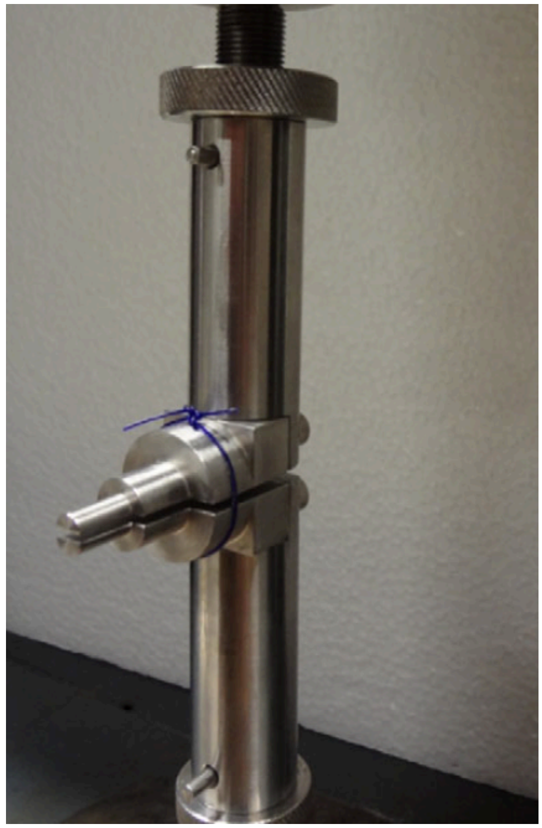
Figure 4.5



Photographic image of the apparatus used by Schaaf et al.[69] The knot is tied around the metal rod that is fixed to the lower grip of the materials testing machine. The long end of the suture strand is glued between rubber pads that are clamped in the upper grip of the materials testing machine. A catching system is placed around it to capture the knot for further investigation.

Recent literature describes variations of the ‘loop’ and ‘single strand’ technique for assessing knot strength and security. In 2015, Sanders et al. investigated the effect of knotting technique, suture material, and incubation periods in saline and inflamed peritoneal fluid, on knot security.[83] Knots were tied over a 20mm plastic mount, followed by incubation. For the mechanical testing a 20mm diameter split circular jaw was used after mounting on a materials testing machine (Zwick Z005, Roell, Ulm, Germany) (Figure 4.6).

Figure 4.6

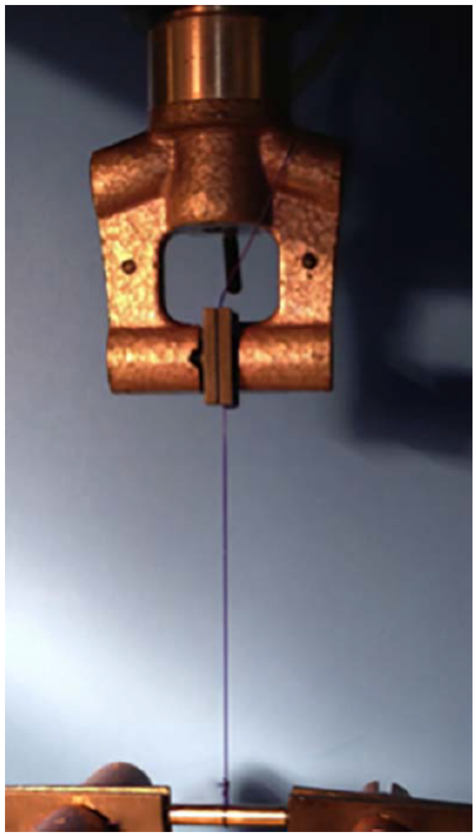


Photographic image of the apparatus used by Saunders et al. to test knot security and strength using the 'loop technique'.[92] Suture loops were mounted on a custom made 20mm diameter split circular jaw in the materials testing machine.

This distracted the loops, similar to the 'loop' technique previously described.[84] In 2016 and 2017, Gillen et al. evaluated the KHC, RKS of surgeon's, square, Aberdeen, and forwarder knots completed with large gauge polyglactin 910 or polydioxanone.[11-13] For this testing, a universal testing machine (Series 5565, Instron®, Norwood, MA) was used. The knot was tied over a fixed metal rod that was clamped in a fixed grip on the lower end of the machine, the suture tag was cut to 3mm in length and the long end was clamped on the upper grip of the machine that was attached to a load cell (Figure 4.7). These clamps were distracted at 20mm/min until failure.

Using the same set-up Coleridge et al. went on to examine the effect of biological media on the KHC on surgeon's, square, and Aberdeen knot completed with large gauge suture and either polydioxanone or polyglactin 910.[10]

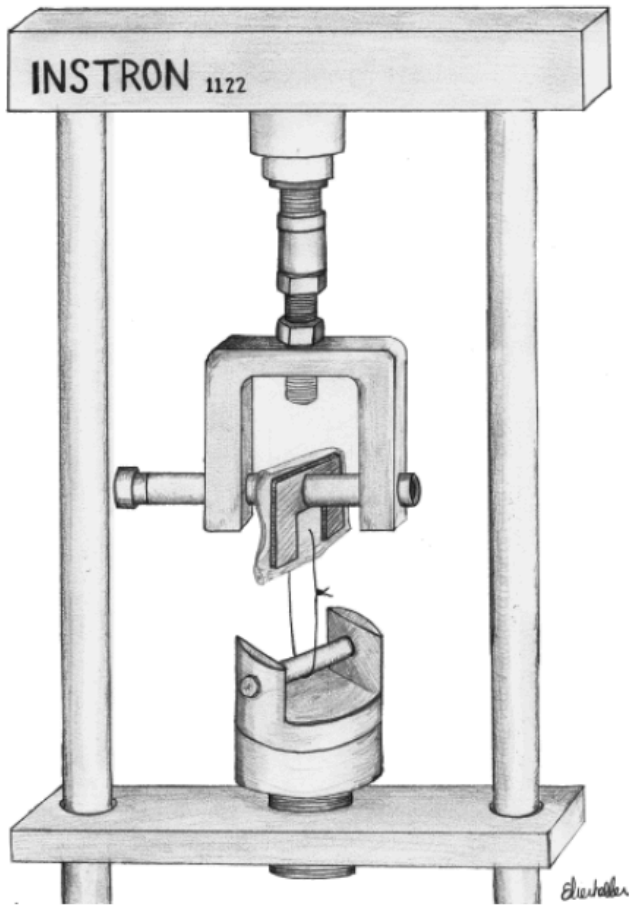
Figure 4.7



Photographic image of the apparatus used by Gillen et al. to test knot security and strength using the 'modified single strand' technique.[11-13] The knot is tied over the rod which is clamped in the lower grip of the testing machine. The free end of the suture is clamped in clamps attached to a load cell.

To incorporate linea alba that may simulate *in vivo* conditions more accurately, Trostle et.al. describes the use of sections of linea alba along with a loop of incorporated suture material to determine the optimum tissue bite size for closure of the equine abdomen.[9] For this study abdominal walls were harvested from horses free of abdominal disease, soaked in saline and frozen at -70°C. Frozen sections were bisected with a band saw and left to defrost. The thickness of the tissue was recorded and it was placed in a U-shaped, flat jawed, gripping device that was attached to a moveable component of a testing machine (Instron 1350 Universal Testing Machine, Instron Corp, Canton, MA). Various bites sizes were taken in different tissue sections and tied around a peg on a fixed portion of the testing machine forming a loop. These were distracted at 100mm/min until failure. The results of this have been previously discussed in relevant sections. In 2005, Fierheller et al. used the same experimental design to test the breaking strength and stiffness of polydioxanone and polyglactin 910 as these sutures had not previously been tested with the incorporation of the equine linea alba (Figure 4.8).[65] In contrast to the findings of Trostle et al. they found that linea alba thickness or abdominal wall position did not influence the breaking strength of their constructs. It was also determined that suture material is more likely to fail than the linea alba and that not surprisingly larger suture materials had higher breaking strengths.[9,65]

Figure 4.8



Schematic depiction of the Testing apparatus used by Fierheller et al. to test knot strength after placement in linea alba.[65] The specimen is attached to a U-shaped gripping component of the materials testing machine. A suture loop is placed through the specimen and placed around a rod attached to the lower grips, both grips are distracted.

ii. Ex Vivo Testing

1. Human and Other Species

It was realized in the early 20th century how important a secure abdominal closure was for human surgery. To decrease patient morbidity and mortality, the abdominal wound edges need to be held in apposition against the forces of disruption until healing is complete.[105] These forces include tension of the musculo-aponeurotic layers of the abdominal wall after muscle contractions or distension from ileus. In an attempt to characterize these pressures to ensure abdominal wall closures were strong enough to withhold these forces, *ex vivo* and *in vivo* projects were designed. In 1948, Haxton endeavored to determine these pressures placed on the abdominal wall.[106] Balloons were passed through inguinal hernias in patients and connected to a manometer. Patients were asked to strain as hard as possible and pressures of 60-80mm of mercury (mmHg) were recorded. In 1948, Drye used a similar experimental design and recorded pressures of 60mmHg after retching and up to 112mm after coughing.[107] Based on these findings, it was concluded that the tension in the abdominal wall necessary to create these pressures must be very high and in the early post-operative period the sutures must be very strong to withhold these pressures and prevent dehiscence.[105]

The next step in this line of experiments was to determine how strong a suture material needs to be to avoid breaking. An *ex vivo* human cadaver study was designed to assess this.[105] A rubber bag was placed in the peritoneal cavity of human cadavers either through a median or paramedian incision with the tubing exciting through separate stab incisions. The aponeurotic layer was sutured and the bag was inflated using an oxygen cylinder. The pressures required to cause wound disruption were recorded. It was found that smaller gauge suture materials failed at lower pressures, larger gauge sutures were more likely to cut through the tissue than break, paramedian wounds failed at a lower bursting pressure that median, tension sutures gave little added support, and that sutures pulled very tightly cut out at lower bursting pressures.[105] It was believed that this type of testing method more accurately reproduced the normal disrupting pressures in the abdominal wound and allowed for comparison between suture materials and methods. This is the first description in the literature of using an inflatable bladder to test the abdominal bursting strength in cadaver tissue and following this description, several others have utilized a similar experimental design.[4,48,49,98,108-111]

In 1984, Poole et al. describes a similar technique using rat cadavers.[108] Ventral midline incisions were closed with 4-0 dacron suture in either a simple interrupted suture pattern, interrupted figure-of-eight or continuous suture pattern. Half were tied loosely, and half tied tightly. Seven days later the rats were euthanized, and a condom was inserted through a rectal perforation, followed by a strong cord tied around the lower abdomen to prevent inguinal herniation. The condom was slowly filled with water and intraperitoneal pressure recorded with a strain gauge transducer attached to a Hewlett-Packard recorder for continuous recording. The bursting pressure was measured in millimeters of mercury (mmHg) and the site of rupture recorded. The continuous suture pattern was associated with the highest bursting pressure. They observed that 68% failed at the cranial third of the incision, 8.6% ruptured at a remote site to the suture line and no abdomens failed by suture breakage.[108]

Rodeheaver et al. used a similar rat cadaver experimental design to test the bursting strength of 3 different sutures, a monofilament polybutester (Novafil™), monofilament nylon (Dermalon™) and a braided polyglycolic acid (Dexon "S"™), 4 different suture sizes, USP 3-0 to 6-0 and 4 different bite widths of 1mm, 2.5mm, 5mm and 10mm.[109] Instead of using a condom inserted through the rectum, a polyvinylchloride cannula was inserted through a stab incision in the vagina and passed into the peritoneal cavity of the rat cadaver. A latex balloon was fixed to the cannula and placed under the abdominal incision. Abdominal pressures were increased by inflating the balloon with water at fixed rate of 0.5ml/second. The pressure was monitored by a Gould pressure transducer that was connected to a calibrated recorder. In addition, the volume expansion of the balloon was recorded by measuring the recorder speed of 1mm=0.5ml of water, the animal was placed on a scale and the increase in weight continuously monitored. Unsurprisingly, smaller gauge suture material failed at a lower pressure. Suture bite width significantly affected security of the abdominal closures, 1mm bites did not pass through the rectus and these failed by cut-through. By increasing tissue bite width, significant increases in bursting pressures were observed ($P<0.001$) until 5mm, after which the pressures remained constant. It was determined that polybutester may be a more advantageous suture to use for abdominal closure as it allowed for a significantly higher volume to be obtained within the abdomen. This was attributed to its unique ability to elongate under small loads.

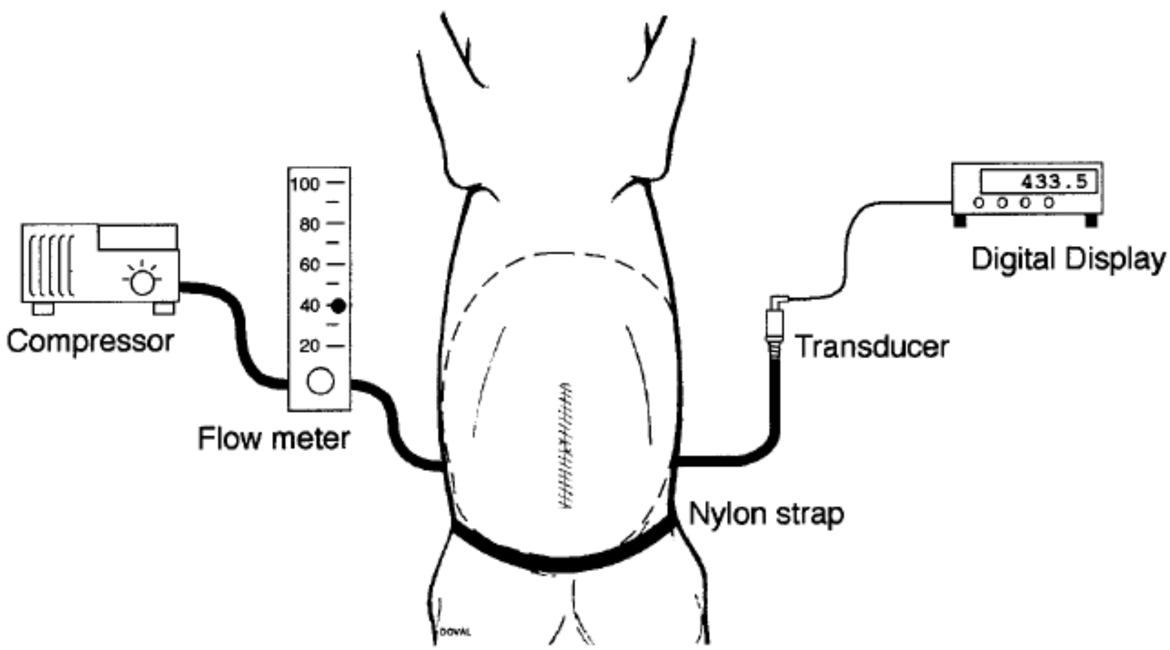
Seid et al. used the rat cadaver experimental design described by Poole et al. and determined that continuous suture patterns had a significantly higher wound strength ($P<0.05$) and required significantly shorter closure times ($P<0.000001$) than interrupted en bloc closure.[110] Using a similar design, Sanders et al. examined the effect of tissue bite size and tightness of tying on the abdominal bursting strength in rats.[98] It was determined that wounds closed with large tissue bites and loosely tied sutures were the strongest with the weakest being the opposite, small bites tied tightly.[98] For the closure, the sutures were pulled as tight as possible for tightly tied suture group and for the loosely tied suture group, these were tightened to the point where the fascial edges touched. Further, researchers used the design described by Poole et al. to compare polydioxanone, polypropylene, and Teflon®-coated braided Dacron® suture materials in a rat model.[111] Wounds were tested at 1, 2 and 6 months post-operatively. Only significant differences were noted at 2 months where Dacron® was significantly stronger. However, as this can induce a greater tissue reaction, the authors concluded that it offered no real advantage unless used in patients with poor wound healing.[111]

2. Equine Species

Regarding abdominal closure, many of the same concerns are raised by equine surgeons when considering the equine abdomen. These concerns are often greater in magnitude due to the size and weight of a horse, in contrast to a human, and the quadrupedal way in which horses move. This results in a large amount of weight, tension and pressure placed directly over the abdominal incision immediately post-operatively. The equine gastrointestinal tract is estimated to hold approximately 221 liters of contents, with the highest amount of strain placed on the linea alba during anesthetic recovery.[112,113] Similar to humans, it is not uncommon for horses to experience ileus and distension of the small intestine following colic surgery which may increase intra-abdominal pressure.[114] However, in contrast to human surgery and likely more significant is the intra-abdominal pressured experienced in the period during recovery from general anesthesia.[113] This occurs as the horse moves from a lateral recumbent position to standing, often in a brief period of time, with the weight of the gastrointestinal contents placing pressure on the ventral midline with the resultant strain experienced on the linea alba. Some of the suture related findings from rat models may be extrapolated to equine surgery, however, it is important to determine the abdominal bursting strength in the horse and to utilize the best suture materials and closure methods to improve equine abdominal closures.

In 1999, Magee et al. was the first to describe an *ex vivo* equine cadaver model to determine abdominal bursting strength similar to those previously described in rats.[4] They investigated the bursting strength of a simple continuous suture line compared to an inverted cruciate pattern. A 40-centimeter (cm) skin incision was made followed by a 25cm incision through the linea alba. A 200-liter (L) inflatable bladder was placed through the incision into the abdominal cavity with an ingress and egress tubing that exited through stab incisions made between the 14th and 15th rib spaces on either side of the abdomen. The ingress tubing was connected to an air compressor and flow meter and was inflated at 40L/min. The egress tubing was connected to a pressure transducer that measured the pressure in mmHg (Figure 4.9).

Figure 4.9



Schematic diagram of the experimental apparatus *ex vivo* equine cadaver model to test intra-abdominal bursting pressures, as originally described by Magee et al.[4] The dotted line represents the inflatable bladder within the abdomen.

The suture material used was USP 3 polyglactin 910 and it was determined that a continuous suture line offered a significantly higher bursting strength (P=0.01) compared to an interrupted suture pattern.[4] Although the times for the continuous suture line were shorter, this was not significant. For both suture methods, suture was the main mode of failure and this occurred adjacent to the knot in all the simple continuous patterns and at 71% of the knots for the interrupted suture pattern. The same research group went on to examine the effect of two different suture bite intervals on bursting strength.[48] For this they used the same experimental design and closed celiotomies using USP 2 braided lactomer 9-1 with a suture interval and bite size of 1cm x 1.5cm or 1.5 x 1.5cm apart. No significant differences were found between the two interval patterns for closure time (P=0.32), suture length used (P=0.56) or bursting pressure (P=0.18) with fascial failure being the main method of failure observed.[48] Although no significant differences were observed it was thought that the 1.5cm x 1.5cm interval may have the potential advantages of less suture material, shorter closure times and still offer good strength.

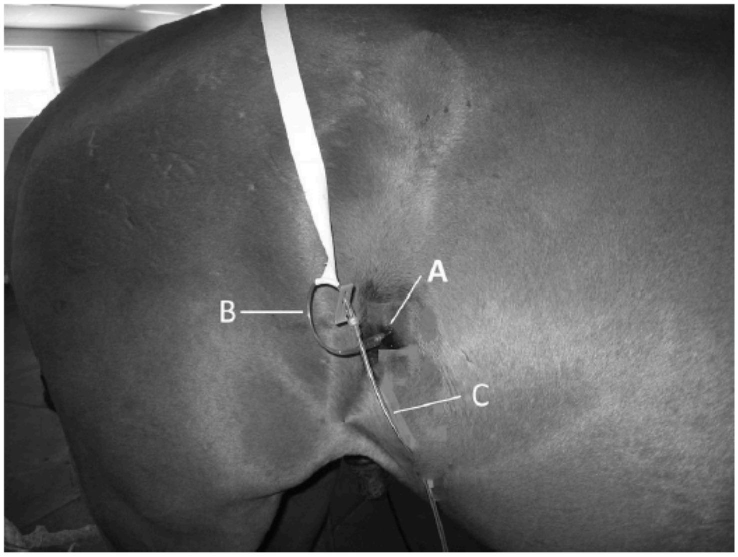
In 2012, Anderson et al. used the same cadaver model as original described by Magee et al. to compare the bursting strength of USP 7 polydioxanone to USP 2 polyglactin 910.[49] It was found that increasing age was negatively correlated with bursting strength in the group closed with polydioxanone. When this was accounted for, closures completed with USP 7 polydioxanone offered a significantly higher bursting strength than those closed with USP 2 polyglactin 910 (P=0.024). In addition, suture failure was the main mode of failure for the group closed with USP 2 polyglactin 910 with these failing at one or both knots, in contrast all those completed with USP 7 polydioxanone failed along the body wall, 4 remotely and the rest adjacent to the suture line. It was concluded that 7 polydioxanone offered a stronger closure. As the suture did not fail, it may be as strong as the tissue (linea alba), therefore fulfilling a basic surgical principle stating that suture should be as strong as the tissue in which it is placed.[35,115] However, one must consider that USP 7 polydioxanone is a larger gauge suture material. Therefore, this may not be fulfilling other basic surgical principles that state: 'the use of an oversized suture may weaken the wound by causing excessive tissue reaction and for a wound under tension it is preferable to increase the number of sutures placed rather than increasing suture size'.[35] The same research group also used this experimental design to examine any difference in bursting strength between ventral midline celitomies and right ventral paramedian celiotomies in horses.[116] Again they found that increasing age had a negative effect on bursting strength along with quarter horse-breed. When accounted for they found that right paramedian celiotomies had a significantly lower bursting strength compared to ventral midline (P=0.039).[116]

d. Intra-abdominal Pressures in the Horse

Excessive increases in intra-abdominal pressures can result in pathological conditions in both humans and animals.[114] This pressure is related to the volume of the abdominal contents and compliance of the abdominal wall. Decreased abdominal wall compliance and an increase in abdominal contents can result in excessive increases in pressure in humans.[117,118] Abdominal wall compliance can be affected by the degree of tightness of abdominal wall closures, support bandages and splinting the abdominal wall muscles from pain. Whereas, an increase in abdominal contents may occur from gastric distension, ileus, intestinal obstruction, enteral feeding and increased abdominal fluid from peritonitis or trauma.[114] It is possible that these conditions may also affect horses undergoing exploratory laparotomies, which may result in increased pressure on the celiotomy incision. It has been previously demonstrated in swine that increases in intraabdominal pressures were significantly negatively correlated to decreases in blood flow to the rectus sheath, which may affect incision healing.[119]

One research group has investigated certain things that may apply to horses post celiotomy, to assess their effect on intra-abdominal pressures.[114,120,121] For these experiments a direct intra-peritoneal pressure was obtained by inserting a peritoneal cannula into the peritoneal cavity in the right flank approximately 10cm caudal to the last rib and at a point midway between the point of the shoulder and the tuber ischii (Figure 5.1). This cannula was attached to a water manometer and zeroed. In 2012, Barrett et al. determined that the normal direct intra-abdominal pressure in the horse was sub-atmospheric at -4.5 ± 3.0 cm H_20. It was determined that intra-abdominal pressures were not significantly affected by transrectal palpation but were significantly increased after the placement of an abdominal support bandage.[114] The following year Barrett et al. reported that the addition of 5, 10, 15 and 20 liters of water to the stomach resulted in gastric distension that significantly increased the intra-abdominal pressure.[120] Following this, the same research group went on to investigate the effect of cribbing on intra-abdominal pressures.[121] It was discovered that intra-abdominal pressures were significantly higher in horses that crib, during active cribbing in comparison to those that do not (P = 0.076). In addition, it was found that these pressures remained significantly elevated after the cribbing behavior had ceased (P=0.0002).[121] Although this research group has demonstrated that gastric distension, placement of an abdominal support band and cribbing result in an increase in intra-abdominal pressure it is likely that the greatest tension on the ventral incision post-operatively is experienced during recovery from general anesthesia.[113] However, it is unknown how this correlates to intraabdominal pressure and a direct measurement of this pressure is unlikely to be obtained.

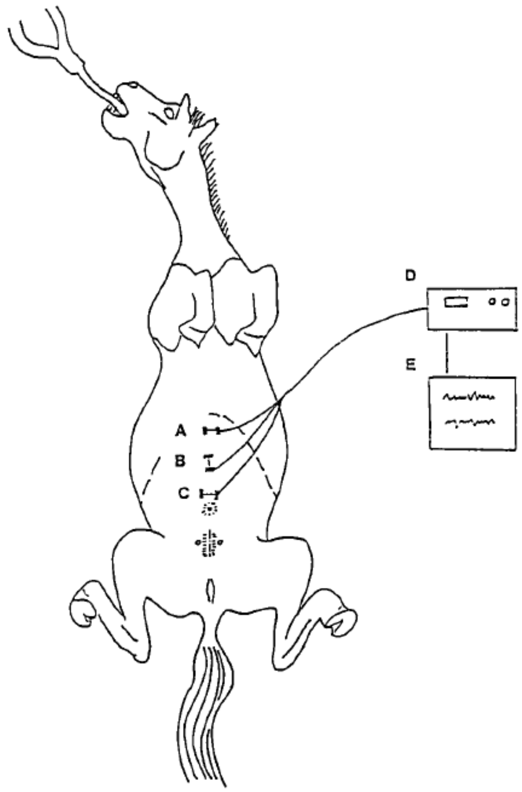
Figure 5.1



Photographic image of the instrumentation for measurement of direct intra-abdominal pressures in the horse, placed in the right flank.[120] (A) 10cm metal cannula, (B) extension set, (C) manometer tubing.

Although not examining direct intra-abdominal pressures, in 1989, Kirker-Head et al. endeavored to determine the different stresses placed on the linea alba in a variety of activities that may occur post celiotomy.[113] For this experiment, three liquid metal strain gauges were implanted surgically at three locations on the linea alba. Horses were placed under general anesthesia and an incision to the superficial fascia of the linea alba was made starting at the umbilicus and extending 75% of the distance between this and the xiphoid. The three locations were cranial, middle, and caudal with the middle one placed longitudinally and the other two placed transversely each separated by 10cm (Figure 5.2).

Figure 5.2



Schematic diagram of the experimental apparatus to measure the stress and strain on the linea alba of horses.[113] Strain gauges are placed in three location within the linea alba fascia, (A) cranial, (B) middle and (C) caudal. (D) Strain gauge amplifier and conditioner, (E) Physiograph.

The strain gauges were calibrated prior to implantation and zeroed at maximal expiration under general anesthesia. The wires were connected to a conditioner and physiography and attached to the horse's side and withers. Linea alba strain, strain rate and tensile stress were recorded under general anesthesia, recovery from general anesthesia, standing, vocalization, rectal palpation and at walk, trot, and canter on a treadmill. The strain was calculated by a change in the length of the specimen divided by its original length, the strain rate was this result expressed as a percentage, and the tensile strength was calculated as the force applied per unit of original cross-sectional area expressed in pascals. It was determined that maximum stress, peak strain and change in strain occurred during recovery from general anesthesia whereas maximum strain rate occurred at the canter. The caudal linea alba experienced higher stress and strain compared to the cranial linea alba. They did not find a consistent relationship between peak strain and weight.[113] In contrast to the results of Barrett et al. they found that applying an abdominal bandage produced no consistent change in mean peak strain, and a rectal exam increased the strain >54% in one horse. However, the study numbers were low and different parameters were investigated (intra-abdominal pressure versus linea alba stress and strain.)[113,114] This begs the question that perhaps intra-abdominal pressures do not directly reflect the pressures experienced on the linea alba. In conclusion, the greatest stress occurred during recovery from general anesthesia and they determined that the likelihood of dehiscence is reduced by minimizing exercise and rectal palpation post-operatively.[113]

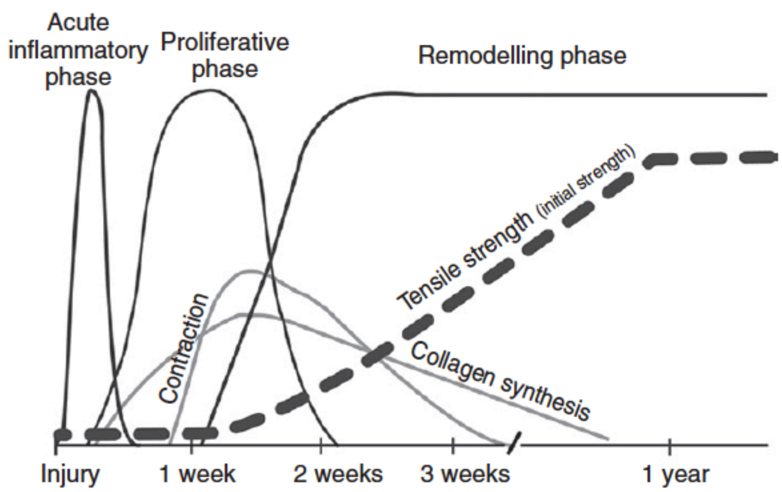
e. Wound Healing

Wound healing is an important topic that every surgeon must be familiar with. This is the biological process by which the body heals itself and it may be following a trauma or a surgical event. It is vital for the surgeon to understand the process of wound healing to improve outcome and reduce patient morbidity. Surgical wounds can be divided into categories based on what structures are involved and entered. Clean wounds are those created under aseptic conditions, whereas clean-contaminated are surgical wounds where the respiratory, alimentary or urogenital tracts are entered. Finally, contaminated wounds are surgical wounds where a major break in sterility has occurred.[122] When a celiotomy is performed it is not uncommon for a clean-contaminated wound to be created. This wound occurs when a resection and anastomosis or enterotomy are performed.

i. Phases of Wound Healing

Wound healing is a complex process involving cellular and biochemical interactions that occur regardless if it is the skin involved or deeper structures such as the linea alba when a celiotomy is performed. The process has been divided into three phases: (1). the inflammatory phase, (2). the proliferative phase and (3). the remodeling phase (Figure 6). It is important that the surgeon is familiar with these phases and understands that these are not isolated as some level of overlap occurs.

Figure 6. Phases of wound healing and gain in tensile strength post wounding.[122]



1. The Inflammatory Phase

The inflammatory phase, otherwise known as the lag phase, occurs immediately, and primarily involves hemostasis and inflammation. This phase typically lasts for several days with the primary function of stopping hemorrhage and preventing infection. In addition, it provides the substrate and cellular signals that facilitate the next steps in the healing process. Hemostasis is initially achieved through compression of the vessels by soft tissue swelling, followed by formation of a fibrin-platelet plug.[122] Both the intrinsic and extrinsic clotting cascade are instrumental to this process with thrombin being a key factor in the formation of the fibrin-platelet plug. Platelet derived growth factor and transforming growth factor beta are key mediators to initiate the influx of leukocytes into the wound. Both of these are released from the storage granules of activated platelets.[122]

2. The Proliferative Phase

The second phase which is the proliferative phase is typically active by day 3 and is characterized by angiogenesis, fibrous and granulation tissue formation, collagen deposition, and contraction.[122] Again it is important to remember that similar to the main phases, these sub-phases also have some degree of overlap. Angiogenesis is essential to provide a blood supply to the healing area as the wound needs a constant supply of oxygen and nutrients to heal in a timely and effective manner. This is stimulated by a lack of oxygen. Fibroplasia and granulation tissue formation is the next step and starts by day 2. Fibroblasts are the main cell type and are crucial as they produce collagen which offers strength to the healing area. Collagen molecules are organized into fibers, followed by bundles that arrange themselves parallel to the wound surface along lines of tension. The production of collagen usually peaks at 1-3 weeks.[123,124] Epithelization starts immediately and is a slow process whose function is key to restoring the skin barrier. Keratinocytes are the main cells involved in this stage. In full thickness wounds such as that of a celiotomy, this process is slow as the basement membrane where keratinocytes reside is disrupted. The final result lacks a dermal layer in full thickness wounds and therefore without the following stage of contraction the result is inferior and less than ideal. Contraction starts in the second week post wounding and can last for several weeks.[124] Fibroblasts are once again an important cell in this stage and it is their differentiation into myofibroblasts that allows contraction to occur. Contraction in loose areas of the skin can occur at up to 0.75mm/day and contraction can reduce the wound surface area as much as 40-80% in humans.[125]

3. Remodeling and Maturation

This is the final stage in wound healing. It begins ~2 weeks following wounding and can last up to 2 years when the scar is formed. During this phase, fibroblasts are once again an important cell type as collagen synthesis continues. This occurs alongside lysis so there is no net gain in collagen. Instead, the collagen fibers that were randomly laid down are now cross-linked in bundles and arranged along lines of tension. This gradually increases the strength over time and this is generally 20% of the original strength at 3 weeks, 50% at 3 months and 80% at completion.[126] In horses, wound healing can continue up to two years.

ii. Wound Healing and Tissue Strength of the Linea Alba

In human medicine there are 6 reported outcomes following wound healing with 5 of these being undesirable: normal healing, dehiscence, herniation, delayed healing, infection and keloid formation.[127] Although keloid formation is rare in horses, the other complications can occur with healing of the linea alba incision. The result of dehiscence may be catastrophic whereas herniation, infection, and delayed healing will significantly increase morbidity and expense. Horses have a considerable weight and pressure placed on their celiotomy incision post-operatively. In addition, many horses are expected to return to an athletic career following surgery. For these reasons amongst others it is important to understand the tissue morphology and tensile strength of the linea alba at different time points post-surgery to best advise clients and improve the patient outcome.

In 2000, Chism et al. examined these properties following ventral midline celiotomy in 18 adult horses.[15] The linea alba of different horses were examined at different time points: 2, 4, 8, 16, and 24 weeks post operatively. Two sections from the cranial and caudal linea were examined histologically with two samples from the cranial, middle or caudal parts of the linea examined for strength by fixing them to clamps and distracting them, using a universal materials testing machine. It was found that the linea alba was significantly thicker at 2, 4, and 8 weeks and was not significantly different from the control linea at 16 and 24 weeks.[15] Interestingly, they found that the thickness of the linea was inversely proportional to its breaking strength and attributed this thickness to edema and granulation tissue which does not provide strength. In relation to tissue strength, they found the 2-week specimens to be significantly weaker (87.7N) than the control group (484.9), and the 24-week specimen to be significantly stronger. No significant differences were seen at 4, 8, and 16 weeks post-operatively. The 2-week sample was very weak and had no evidence of mature collagen formation. This result puts emphasis on the importance of a strong suture and knot combination in the first 2 weeks post-operatively. Although no differences in strength were noticed at 4 weeks post-operatively, the histology demonstrated immature collagen that was not organized into bundles. At 8 weeks, mature collagen was seen. Based on these findings, a horse should not be returned to exercise prior to 8 weeks post operatively as the tensile strength and collagen formation at that time is comparable to that of a normal linea prior to surgery. However, it is important to note, if any complications occur regarding the incision, this will often result in the extension of this time period.

iii. Factors Affecting Wound Healing in the Linea Alba

Various factors can be detrimental to general wound healing such as motion, infection, exposed bone, granulation tissue, and location of the wounds i.e. the distal limb, as they have a reduced blood supply.[122] There are a few studies examining detrimental factors for healing of the linea alba with even less specifically examining the horse. In 1977, Sanders et al. examined the effect of closing rat linea alba with large and small bites and loose and tight suture ties.[98] The breaking strength was weakest in those closed with tight ties and small bites. In addition, on histopathology, evidence of muscle necrosis was noted under the sutures of the group closed with tight ties. Others found similar results, with more extensive inflammation noticed on histology sections of linea closed with tightly tied sutures.[108] To the best of the authors knowledge, this has not specifically been examined in the equine linea alba, it begs the questions if tying knots too tightly would increase inflammation and possibly induce necrosis that may weaken the construct.

Excessive inflammation and tissue reaction may be detrimental to healing. Mechanical irritation may be caused by the presence of a foreign body or in the case of the linea alba, the suture material. This irritation seems to be directly related to suture size, suture stiffness, knot configuration, and volume.[36,37] Looking more specifically at the knot, the addition of extra throws increase knot volume by 150% and increase tissue reactivity by 200%, suggesting it is important to use the lowest throw combination that will produce a secure knot.[37] Various equine papers examine risks for developing incisional infections and incisional complications.[5,8,16,18,24] Unfortunately, these are retrospective papers and do not specifically examine healing of the linea alba based on histological findings in relation to optimum suture and knot combination and the effect on incisional complications. Future research in this area is warranted to determine the effect that suture bite size, knot configuration and knot loop tightness have on the healing of the linea alba.

II. Objectives

Incisional complications post-celiotomy are not uncommon in the equine patient, with reported rates ranging from 4-44%, increasing as high as 88% if a second celiotomy is required.[5,8,25] Multiple research efforts have examined various risk factors, and ways of optimizing ventral midline celiotomy closure.[4,5,9-13,23,25,40,48,49,65] This research has demonstrated that the knot is the weakest point, with the majority of constructs or suture failing at the knot, yet no research has examined different knot options for closure of the equine ventral midline celiotomy incision.[4,9-13] Recently, research in our laboratory has examined the KHC, RKS, weight, and volume of conventional square and surgeon's knots, and self-locking knots, the forwarder and Aberdeen knot using large gauge suture material. The suture tested is commonly used in equine colic surgery: USP 2 and USP 3 polyglactin 910 and USP 3 polydioxanone. A single, cycle to failure testing method was used and all suture was tested using the 'single strand' method. The reason for performing these studies was to determine the optimum number of throws that should be used to produce a secure start and end knot in a continuous suture line using large gauge suture.

Prior to this, one study examined knots completed with large gauge suture, however, they only examined start knots and used the 'loop test'.[79] It is likely that the "single strand' test more accurately mimics the tension placed on the knot and suture that would occur in an *in vivo* scenario as it tests the extrinsic tension on the suture loop. This represents the tension from the length of continuous suture strand. In contrast the 'loop test' tests the intrinsic tension which would arise as a result of excessive inflammation and edema within the knot loop. Israelsson et al. and Schaff et al. reported that self-locking knots outperformed conventional square and surgeon's knots in strength and size, however, these self-locking knots have not been tested in large gauge suture.[66,69] Therefore, our laboratory also compared the findings from self-locking knots to those of the conventional knots to determine if these would offer an advantage when used in equine surgery.

It was determined that the optimum number of throws to complete a secure knot when using USP 2 or USP 3 polyglactin 910 and USP 3 polydioxanone was 6 for a start knot and 7 for a pattern ending knot.[11] In contrast, the strongest forwarder and Aberdeen knots were completed with 4 throws.[12,13] Not only did they require less throws, having a significantly smaller weight and volume, but they both were significantly stronger having a higher KHC and RKS. As previous studies have noted a significant effect of lubrication on the frictional properties of suture, further research at our laboratory investigated the effect of fluid media commonly encountered in equine colic surgery on the strength and size of a square, surgeon's and Aberdeen knot.[10] Interestingly, it was found that exposing suture to fluid media improved the KHC therefore requiring less throws than when completed with dry suture material for the Aberdeen knot and the surgeon's knot between 5 and 6 throws when completed with USP 3 polyglactin.[10] This suggests that an Aberdeen can be completed with 3 throws and 1 turn and a surgeon's start knot 5 throws and a surgeon's end knot, 6 throws when completed with USP 3 polyglactin 910 following exposure to fluid media.

As these studies were performed *in vitro*, it was desirable to test these knot combinations in a scenario that would more closely mimic *in vivo* conditions. In 1999, Magee et al. described an equine cadaver model to test abdominal bursting pressures after the placement of an inflatable bladder within the abdominal cavity. As this model incorporates the entire abdominal cavity and wall and allows for three-dimensional forces to be applied to the celiotomy incision, it more likely mimics an *in vivo* scenario. We elected to use this research model to perform our study. The objective of this study was to compare the bursting strength and failure mode of ventral midline celiotomy closure using a simple continuous suture pattern with one of two knot combinations, a novel self-locking knot combination of a forwarder start with an Aberdeen end knot (F-A) and a traditional knot combination of a surgeon's start with a surgeon's end knot (S-S). We chose to assess USP 3 polyglactin 910, a braided 0.6mm diameter absorbable suture as it is commonly used for closure of ventral midline celiotomies in the horse and is the largest suture available for this in the United States.

a. Hypothesis

This study tested the following hypothesis:

1). Ventral midline celiotomy bursting strength would be significantly higher following closure with the self-locking F-A knot combination compared to closure with the traditional S-S knot combination.

2). Failure of the closure would occur most commonly along the suture line and most commonly at the knot regardless of knot combination used for closure.

3). The F-A closure time would be significantly longer than that of the S-S knot combination.

III. Methods and Materials

a. Horses

For this study, fourteen healthy horses were selected. Selection criteria included age > 2 years, and weight > 300kg and < 650kg. All horses were free of gross abdominal wall abnormalities as assessed by visual examination and digital palpation. Using a random number generator, horses were assigned to one of two knot combination groups; (S-S) or (F-A). Sex, age, weight and breed were recorded. All cadaver horses obtained were euthanized for a separate, unrelated study that was evaluated and approved by the University Institutional Animal Care and Use Committee.

b. Surgical Procedure

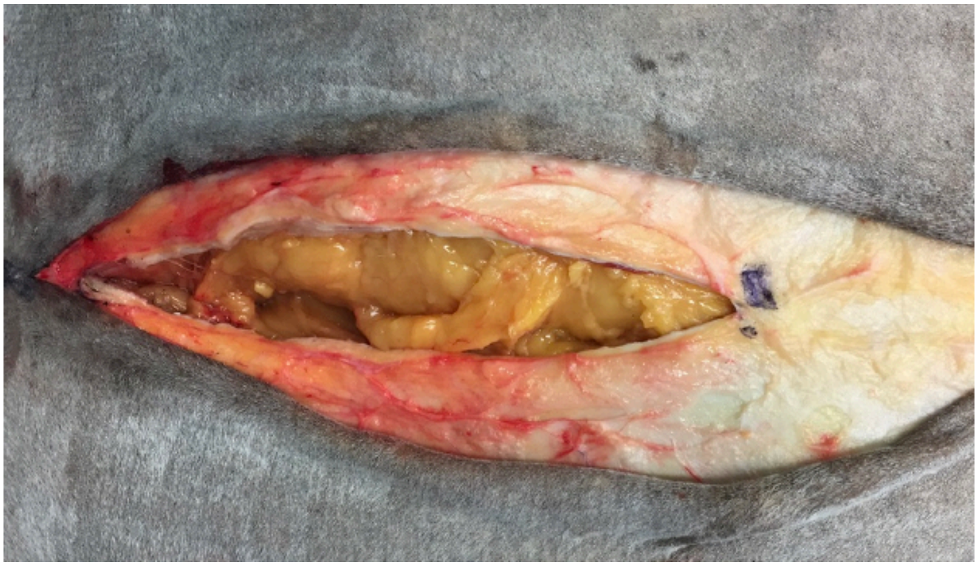
Immediately following euthanasia, cadavers were placed in dorsal recumbency. Using a #10 scalpel blade, a 30-cm skin incision was made extending cranially from the umbilicus, taking care not to score the linea alba over the intended site of a ventral midline celiotomy (Figure 7.1). The subcutaneous tissues and skin were sharply dissected laterally approximately 5 cm from the linea alba to facilitate observation of the suture line and adjacent fascia during failure testing. The abdomen was entered by sharp incision through the umbilicus and extending 20 cm cranially through the linea alba (Figure 7.2). The peritoneum was entered bluntly using the index finger and this defect enlarged. Bilateral stab incisions were made between the 14th and 15th rib, in the ventral third of each intercostal space, to provide access for ingress and egress tubing (Figure 7.3). A custom made, 200 L, polyurethane bladder (New World Mfg., Inc., Cloverdale, CA) was inserted into the abdominal cavity through the celiotomy incision (Figure 7.4i and 7.4ii). The ingress tubing was exteriorized through the right stab incision and attached to an air compressor

(8 gal. 2HP 125 PSI Oil Lube Air Compressor, Central Pneumatic, Harbor Freight, Calabasas, CA) with digital airflow regulator (RMA-24 Dwyer Instruments, Inc., Corporate HQ, Michigan City, IN), while the egress tubing was exteriorized through the left stab incision and connected to a pressure transducer (General Purpose Pressure Transducer PX209, Omega Engineering, Inc., Norwalk, CT), which was connected to a digital display unit (Strain Meter Controller DP25B-S, Omega Engineering, INC, Norwalk, CT). Prior to suture placement, the linea alba was measured and marked with a tissue marker to ensure suture placement 15mm from the linea alba tissue edge with a suture bite interval of 15mm apart (Figure 7.5).

Figure 7.1

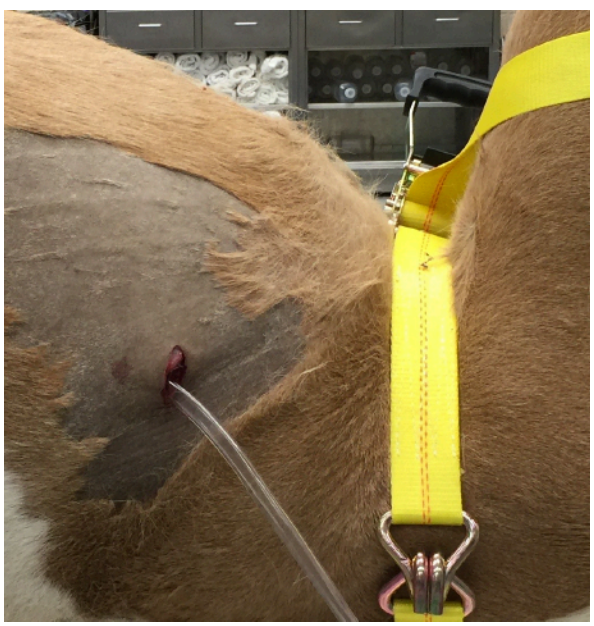
A photographic image of the 30cm skin incision made from umbilicus extending cranially. Left is caudal and right is cranial.

Figure 7.2



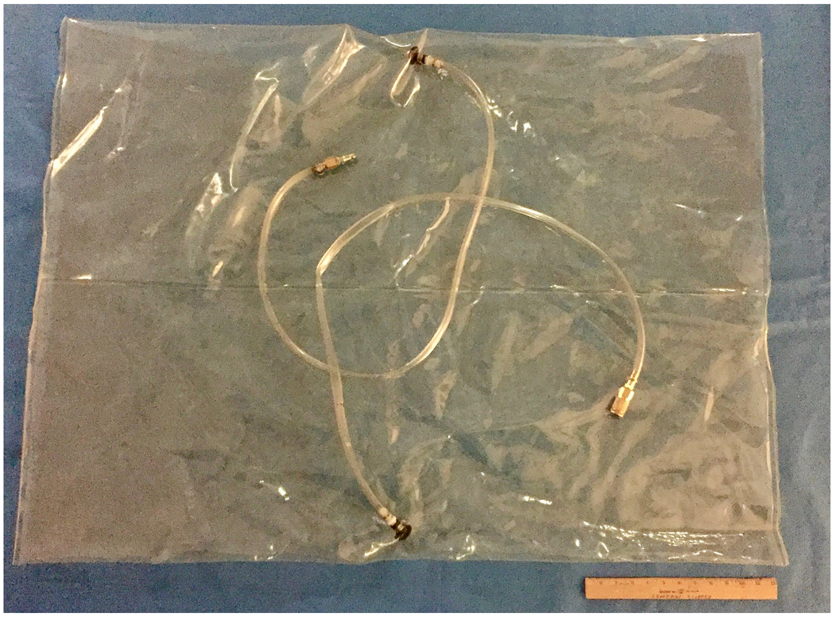
A photographic image of the 20cm incision made through the linea alba. Left is caudal and right is cranial.

Figure 7.3



A photographic image of the ingress tubing placed between the 14^{th} and 15^{th} intercostal space. Left is cranial and right is caudal.

Figure 7.4(i)

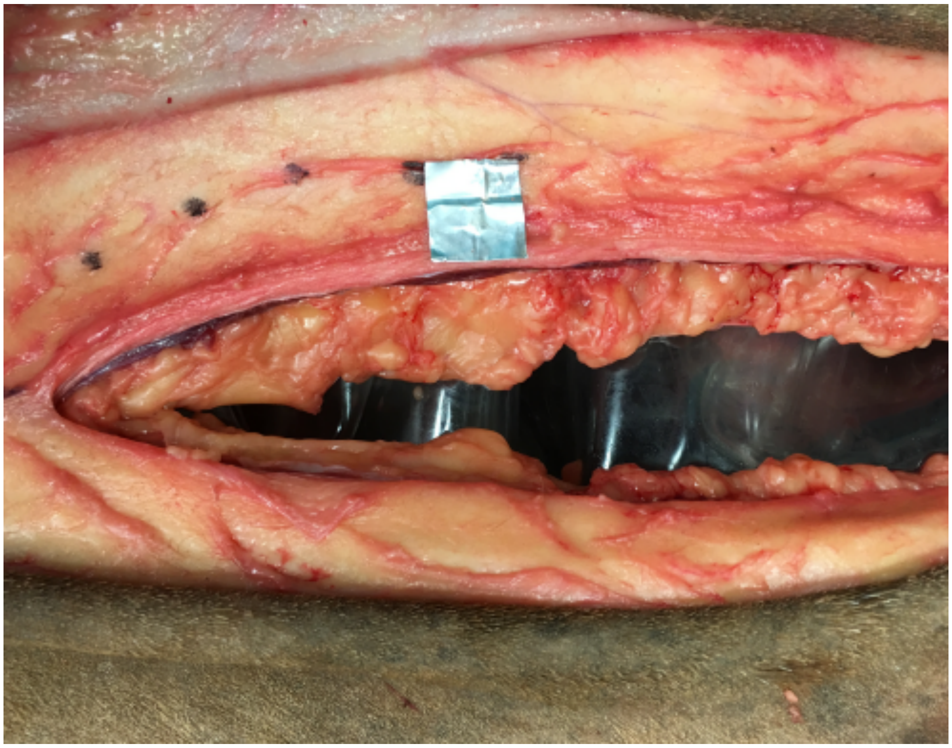


A photographic image of the 200L inflatable bladder

Figure 7.4(ii)

A photographic image of insertion of the 200L inflatable bladder into abdominal cavity. Left is cranial and right is caudal.

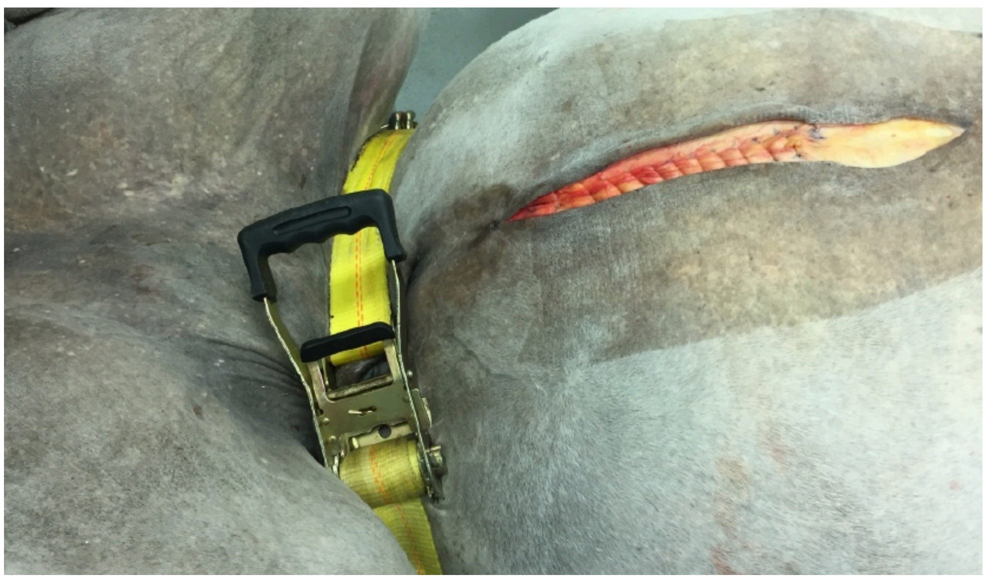
Figure 7.5



Photographic picture of template (15mm x15mm) marking suture bite placement.

All celiotomies were closed with one strand of 3 USP polyglactin 910 (Ethicon US, LLC, Somerville, NJ) in a simple continuous pattern utilizing one of the two different knot combinations. The surgeon's start knot was completed with 5 throws and the surgeon's end knot with 6 throws. The forwarder start knot was completed with 4 throws and the Aberdeen end knot was tied with 3 throws and 1 turn.[10,13] These throw combinations were extrapolated from data previously published by our laboratory that determined these throw combinations displayed the highest knot holding capacity with the smallest knot size.[10,12,13] The surgeon's and forwarder knots were tied using 8" Mayo-Hegar needle drivers, whereas the Aberdeen knots were tied by hand. All celiotomy closures were timed using a stopwatch (Apple iPhone 6, Apple Inc., Cupertina, CA). All celiotomies were created and closed by a single surgeon (LM). After celiotomy closure, a 5-cm wide nylon ratchet strap was placed caudal to the celiotomy, just cranial to the pelvis, and tightened to prevent herniation of the abdominal organs through the inguinal fascia, as previously described (Figure 7.6).[4,49]

Figure 7.6



Photographic picture of nylon ratchet placed over inguinal region. Left is caudal and right is cranial.

c. Biomechanical Testing

All celiotomy constructs were tested within 2 hours of euthanasia (room temperature 65^{o} Fahrenheit, humidity 50%). Bursting strength and failure mode were evaluated by inflating the intra-abdominal bladder with compressed room air at 40 L/min until failure. Bursting strength was defined as the maximum bursting pressure (mmHg) recorded before a sudden decrease in the pressure noted by a video recording (Apple iPhone 6, Apple Inc, Cupertina, CA). The mode of failure and digital pressure reading was recorded by a high-speed camera recording at 240 frames per second (Cannon Power Shot SX40 HS, Tokyo, Japan). Mode of failure was defined as either failure of the suture or failure of the abdominal wall. When failure occurred due to the suture, the location of failure (start knot, end knot, mid-suture line) was recorded. Following failure, all abdominal cavities were thoroughly examined to ensure there was no damage to internal organs.

d. Statistics

The age, sex, breed and weight of the cadavers were described as stratified by closure type. Continuous variables were described as medians, with ranges. The association between the closure time and breaking pressure and closure type were assessed using the Wilcoxon Rank Sum test. Significance was set at $P<0.05$. Descriptive statistics and analyses were conducted using Stata 13.1 (Stata Corp 2013, College Station, TX, USA).

IV. Results

a. Study Population

Fourteen horses were included in the study population, horses had a median weight of 454kg (363- 597kg) and a median age of 14.5 years (range 6-27 years). Five horses were mares and nine were geldings. The horses were of a variety of breeds: five were American Quarter horses, two were American Paint horses, two were Tennessee Walking horses and there was one each of Arabian, Hanoverian, Morgan, Thoroughbred and Welsh Cob breeds. The median weight of S-S horses was 498 kg (range 363-597kg) and the median weight of F-A horses was 431 kg (382-597kg). The median age of S-S horses was 18 years (13- 27) and the median for F-A horses was 13 years (6-25). There were significant differences between the age (P=0.03) and weights (P=0.03) of the groups, with the S-S horses being older and heavier. There were five and four males in the S-S and F-A groups, respectively.

b. Bursting Strength

The median bursting strength of closure with F-A knot combination (388 mmHg; range 276-480mmHg) was significantly greater than the median bursting strength of closure with the S-S knot combination (290 mmHg; range 214- 390 mmHg) (P=0.035) (Table 3 and Figure 8.1). There was no significant correlation between horse's age or weight and the bursting strength of the closure (P=0.10 and P=0.47, respectively).

Table 3

Median bursting strength and closure times		
	Forwarder-Aberdeen (F-A)	Surgeon-Surgeon (S-S)
Bursting strength (mmHg)	388	290
Closure Time (mins)	12.26	10.5
Failure mode for each group		
	Forwarder-Aberdeen (F-A)	Surgeon-Surgeon (S-S)
Knot failure	1	6
Fascial failure	6	1

Bursting strength, closure time and failure mode for each group.

Figure 8.1

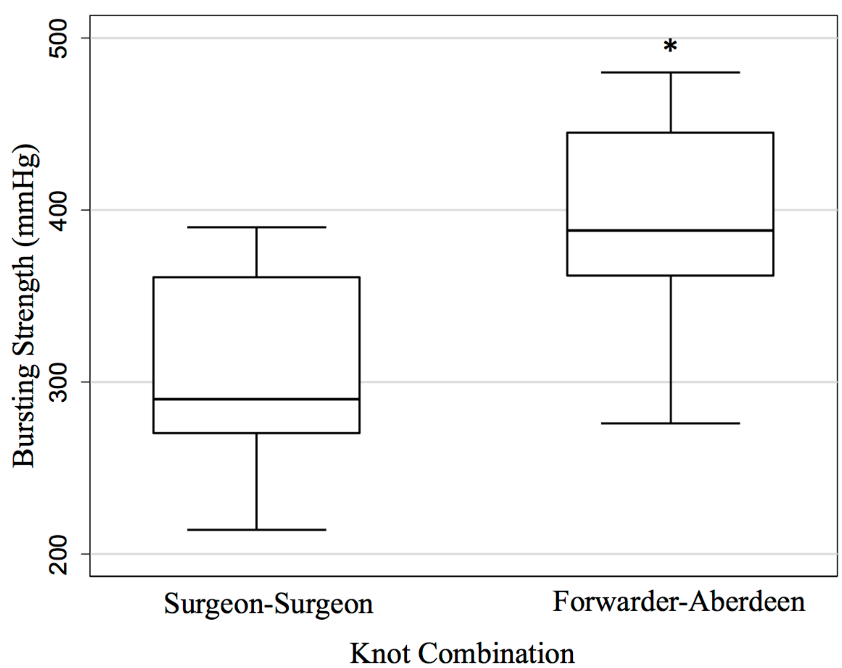


Figure 8.1. Box plots (median, 25% and 75% quartiles, and range) showing the difference in bursting strength of a Surgeon-Surgeon (S-S) and Forwarder-Aberdeen (F-A) knot combinations. The F-A knot combinations demonstrated significantly higher median bursting strength than the S-S combination (P=0.035) as identified by the *

c. Failure Mode

The majority of F-A closures failed at the fascia adjacent to the incision (n=6). In one cadaver completed with the self-locking knots, the failure occurred mid-suture. In contrast, failure occurred most commonly at the knot in S-S closures (n=6) (Figure 8.1). In these S-S closures the end knot failed in two horses and the start knot failed in 4 horses. Failure occurred along the fascia adjacent to the incision in one S-S horse.

d. Closure Time

There was no significant time difference between closure with the F-A knot combination or closure with the S-S knot combination (P=0.48). The median closure time was 12.26 minutes (8.27-14.00 min.) for F-A closures and 10.15 minutes (9.40-13.27 min.) for S-S knot combinations, respectively (Figure 8.1 and 8.3). There was no significant correlation between horse age or weight and the closure time (age: P=0.72; weight: P=0.57).

Figure 8.2

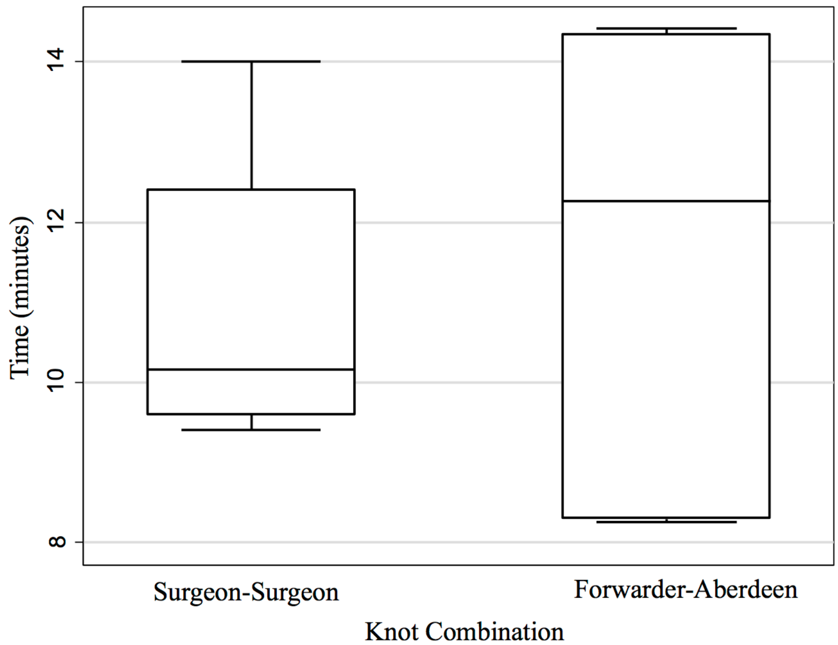


Figure 8.2 Box plots (median, 25% and 75% quartiles, and range) showing the difference in closure times of a Surgeon-Surgeon (S-S) and Forwarder-Aberdeen (F-A) Knot Combinations. No significant differences in closure times were observed between the two knot combinations (P=0.48).

V. Discussion

Our evaluation of a simple continuous suture pattern with a combination of the forwarder start and an Aberdeen end knot (F-A) compared to a traditional surgeon's start and surgeon's end knot (S-S) for ventral midline closure using an *ex vivo* cadaver model found that a F-A knot combination offers a more secure closure than that of a traditional S-S knot combination. When comparing the bursting strength between knot combinations, the use of the self-locking knot combination increased incisional bursting strength by an average of 25%. In addition, the use of the F-A knot combination would not prolong surgical time as we found no significant differences in closure times between F-A closure and S-S closure.

There are 2 large gauge suture materials commonly used in the United States for closure of equine ventral midline celiotomies; USP 2 polydioxanone and USP 3 polyglactin 910. We elected to use USP 3 polyglactin 910 for our testing as it is the largest of these two and has a good size-to-strength ratio demonstrating greater initial breaking strength and stiffness than polydioxanone.[35] To the best of our knowledge, no other authors have specifically examined the effect of different knots on closure of the equine linea alba. Utilizing a surgeon's knot combination, Anderson et al. demonstrated that closure with USP 7 polydioxanone offered a stronger closure than that with USP 3 polyglactin 910. However, this large gauge suture is not available in many parts of the world, and the increased size of this suture would seem to neglect the basic surgical principle that recommends against placing more foreign material in an incision line.[35]

We found no significant correlation in this study between horse age or weight and the bursting strength of closure. However, others have found that increasing age may have a significant negative impact on the celiotomies closed with USP 7 polydioxanone.[49] This report, using a similar experimental model, found a 10.2 mmHg decrease in bursting strength for every year increase in age, suggesting that the strength of the abdominal wall decreases in older horses.[49] These findings are in direct contrast to those reported in our experimental model as well as those by Magee et al., who also reported no significant effect of age on bursting strength using the same research model as our laboratory.[4] Further research is warranted to determine the effect of age and/or co-morbidities associated with aged horses, have on bursting strength of the ventral abdominal wall.

Several different models have been used to test the strength of different variables in closure of the equine linea alba.[4,7,9,48,49,65] The intact cadaver model used in this study was originally described by Magee et al., and has been reported to more accurately simulate *in vivo* conditions.[4,108,128] This is likely as it incorporates the entire abdominal wall and abdominal contents and allows a three-dimensional force to be applied on the incision. It is thought that the highest intra-abdominal pressures experienced post ventral midline celiotomy are during recovery from general anesthesia, however a direct measurement of this pressure has not been performed. The direct intra-abdominal pressures measured in a normal horse are typically sub-atmospheric at approximately -4.5 ± 3.0 cm H_20.[129] Although not directly examining intra-abdominal pressures, Kirker-Head et al. did determine that maximum stress, peak strain and change in strain of the linea alba occurred during recovery from general anesthesia.[113] It must be noted that the experimental design used in our study tested our constructs in a single cycle-failure, which may mimic a sudden increase in pressure experienced during the recovery from general anesthesia. However, it must be acknowledged that some degree or stress and strain is placed on the knot and suture construct in a cyclical manner post-operatively. As previously mentioned, the normal intra-abdominal pressures of the horse are sub-atmospheric and although the supraphysiological pressures experienced in our study would not normally be encountered *in vivo*, this model allowed an even stress to be applied to the closure that would mimic potential sudden post-operative conditions.[4,49,108] It is difficult to correlate an increase in intraabdominal pressure with an increase in strain on the linea alba, although eventually increases in pressure will result in linea alba disruption, and perhaps this is an area that future research could examine.

Rapid completion of surgery is an important aspect of proper surgical technique for small and large intestinal lesions, as increased surgical time of exploratory celiotomy results in reduced survival rates and increased incisional complications.[1,93,130-132] We found no significant difference in the closure time between the F-A and S-S knot combinations, indicating that these novel knots will likely not alter closure times for ventral midline celiotomies. In addition to closure time, previous studies have examined the amount of suture material used, as excessive foreign material placed in a wound can lead to tissue reaction and inflammation which may be detrimental to wound healing.[4,37,48,51] Although we did not specifically examine this, we did utilize 1 suture packet for each closure with little left over so it is unlikely that there was a difference between knot combinations. However, if more than one strand of suture is required, multiple knots may be used which could therefore increase surgical time and suture required for both knot combinations. For this, a closure utilizing the F-A knot combination could be performed by ending the first continuous line with an Aberdeen knot and starting a second continuous line with a forwarder knot as often performed with a surgeon's knot by some surgeons. This may provide increased security in comparison to current methods, and further studies are warranted to investigate additional applications of the F-A knot combination.

Basic surgical principles state that suture should be as strong as the tissue through which it is placed.[35] The celiotomies that were closed with the F-A knot combination most commonly failed at the fascia adjacent to the incision. This suggests that when the F-A knot combination is utilized, the suture/celiotomy construct is as strong as or stronger than the surrounding tissue. One of the F-A knot combinations failed mid suture line. It is difficult to ascertain why this occurred, however it is interesting to note that it failed mid suture rather than at the knot. The knot is typically the weakest point of the construct and to the best of the author's knowledge there has been no prior studies that have reported failure mid suture line in a similar experimental design.[4,48,49] It may be argued that the suture material was defective, however, as the bursting pressure recorded for this particular failure was similar to the other F-A ones recorded, this is less likely.

In contrast to the F-A construct failures, the majority of our S-S constructs (n=6, 86%) failed at the knot. Magee et al. found similar results, with 71% of their constructs completed with a surgeon's knot using a simple continuous suture pattern failing at the knot.[4] It is difficult to determine why 14% (n=1) of our S-S constructs failed adjacent to the fascia rather than at the knot like the other S-S failures. One possible explanation is that the placement of the inflatable bladder allowed for an increase in pressure under the area of the fascia resulting in failure at this location. However, every effort was made to ensure the placement of the bladder allowed for an even pressure to be placed over the abdomen and incision. It has been suggested that it takes approximately 8 weeks for a linea alba incision to be comparable in strength and collagen formation to that of a control linea alba following ventral midline celiotomy.[15] In that study, significant increases in tensile strength were observed between 2 and 4 week specimens, emphasizing the importance of a strong and secure closure in the interim period.[15] During this acute healing phase, when the celiotomy closure is relying heavily on the suture line and knots, the F-A knot combination may provide an advantage over the use of a traditional S-S knot combination, as our results show it to be as strong as the surrounding tissue.

In the study by Magee et al, four knots were used to complete the celiotomy closure, these knots were completed with 6 throws, but the knot which failed was not specified.[4] Fifty-seven percent of our S-S knot combinations failed at the start knot. Our surgeon's start knot was secured with 5 throws and our surgeon's end knot was placed with 6 throws. These throw combinations were based on previous *in vitro* studies from our laboratory that showed these displayed the highest KHC and the smallest knot volume.[10,12,13] The start knot may have failed more frequently due to the variation between *in vitro* and *ex vivo* testing, however the numbers were too low for statistical analysis. In addition to Magee et al., many other studies that used a traditional surgeon's knot combination found that the majority of constructs failed at the suture and more specifically adjacent to the knot.[9,65] One explanation for this finding is that the load applied to the surgeon's knot results in a stress accumulation between the interface of the loop and the first throw, causing failure at the knot. In contrast, when a load is applied to the self-locking knots, the intrinsic properties of these knots allow the suture to slide within the knot dissipating the tension and reducing the coefficient of friction at the knot-suture interface.[12,13,66,69] This property allows the self-locking knot to be more secure and stronger in comparison to traditional surgeon's knot offering a more secure closure.

In contrast to celiotomy and suture constructs failing at the knot or along the suture, constructs may also fail along the body wall adjacent to the suture line or remote to the suture line.[4,48,49] There are a few studies where the majority of failures occurred along the fascia, demonstrating that their construct is as strong as the surrounding tissues, however, they have their limitations. One study investigated the optimum tissue bite interval for celiotomy closure. [48] This model used a double stranded loop suture of USP 2 braided lactomer 9-1 and closed the incisions with two strands of suture starting mid incision, involving a knot at either end. Although the bursting pressures experienced were higher than our study (420.0-479.4 mmHg) and the closures contained the same total number of knots per incision, the disadvantage of this method is placement of a four-stranded knot along with the use of two strands of suture along the continuous suture line. This results in a larger volume of suture material for closure than one-stranded continuous sutured lines.

a. Limitations

Limitations of this study include the fact that we created a relatively small celiotomy incision of 20 cm, to facilitate closure with a single strand of suture material. Often a ventral midline celiotomy incision may be extended past 20 cm, particularly if large colon manipulation is required. In addition, polyglactin 910 suture size USP 3 is only available in a length of 70 cm, and closure with only one suture strand would be difficult, if not impossible, if extended beyond 20 cm. Further studies may need to evaluate the use of size USP 2 Polyglactin 910 with self-locking knots, due to the availability of a longer suture strand (135cm) which would allow for fewer knots to close longer incision lines. Secondly, one could examine the closure with 2 strands of USP 3 polyglactin 910.

An additional complication of the use of both the forwarder and Aberdeen knot is that they are both sliding, self-locking knots. Therefore, it is possible that some level of suture translation may occur within the knot resulting in constriction of the knot around the linea alba tissues, to the detriment of wound healing. Although we did not specifically examine this in our study, the unbroken knot loops were measured post-failure in an attempt to quantify the degree of tissue constriction. Many of the S-S knots were destroyed in the testing, and as a result there were too few knots to statistically compare. It must be recognized that the tension of knot constriction would be opposed by the intrinsic tension of the tissue and also the tension throughout the length of the suture line. To the author's knowledge, there are no published studies that examine either the effect of constriction of the forwarder or Aberdeen self-locking knots, and also surgeon's knots. Studies are warranted to specifically assess the constriction of these knots *in vivo*, and their effect on tissue healing.

VI. Conclusions

The results of this study demonstrate that ventral midline celiotomy closure with USP 3 polyglactin 910 using a self-locking knot combination of a forwarder and Aberdeen knot obtained a greater bursting strength than closure with surgeon's knots. Most of the constructs closed with F-A knots failed along the abdominal wall, suggesting that this suture and knot combination is as strong or stronger than the tissue in which it is placed.[35] In addition, surgical closure times are not significantly increased with the F-A knot combination. As the forwarder and Aberdeen knots have a smaller weight and volume compared to the surgeon's knot, this combination may reduce the volume of foreign material within the closure of the linea alba.[10,12,13] Based on this data, the F-A knot combination appear to be a viable option for improving the bursting strength of ventral midline celiotomy closure in horses. Further investigations, ideally *in vivo* experiments, will be necessary to validate this technique for recommendation in clinical cases.

VII. References

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VIII. Appendix

a. Risk Factors for Incisional Infection

Table 1. List of risk factors and protective factors for the development of an incisional infection post-operatively as determined by various studies.

Author	Year	Infection rate	Criteria	Risk Factors	Protective factors	Closure	Suture
Kobluk et al.	1987	28.6% 1st surgery 87.5% 2nd surgery	-Serosanguineous fluid -Drainage with suspected infection -Confirmed infection	Closure of the linea alba with near-far-far-near suture pattern		Simple interrupted -59.1% NFFN -7.6% NFFN + simple interrupted -30.3% Horizontal mattress -1.5% Continuous -1.5% Unknown -6.1%	Polyglactin 910 -80.3% Polyester -18.2% Gut -1.5% Unknown - 6%
Philips et al.	1993	28%	Incision suppuration	Cecum/large colon lesions		Simple continuous	Polyglactin910
Ingle Fehr	1997	24-44%	Incisional drainage 24% Positive bacterial culture 44%	-Clean contaminated surgery -Preoperative peripheral left shift		Not reported	Not reported

Honnas	1997	25.4%	Purulent discharge +/- bacteria culture	-Increased fibrinogen in peritoneal fluid pre-operatively -Performing an enterotomy -Use of polyglactin 910		Inverted interrupted cruciate - 56% NFFN -26.1%	Polyglactin 910 - 41% Polydioxanone -33% Polyglycolic Acid -2% Unknown -24%
Mair	2005	29% -25% drainage -4% infection	Drainage - serous/ serosanguinous discharge with local edema and no heat or pain Infection - purulent discharge with swelling, heat & pain	-Intraperitoneal contamination -Intraperitoneal antibiotic administration -Repeat laparotomy -Dissection of linea alba prior to closure -Application of stent bandage	-Intraperitoneal heparin -Administration of antibiotics into the wound -Application of incise drape over incision	Simple continuous	Double stranded polyglactin 910
Isgren	2017	25.4%	-Purulent or serous discharge from the laparotomy incision of >24 h duration that developed during hospitalization	-Greater BW -PCV > 48% on admission -Small intestinal resection -Post operative colic -Winter & summer months	3-layer closure	Not recorded	-Braided lactomer single strand – 12% horses -Braided lactomer on loop – 88%

b. Tensile Strength of the Linea Alba

Figure 1. Least Squared Mean (± SEM) Tensile Strength of Healing

Linea Alba in Horses at Selected Times After Ventral Median Celiotomy, taken from Chism et al. 2000

	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*

* Significantly different ($P < .05$) than the control value at that location.

c. Suture Characteristics

Table 2. Structure, absorption times and tensile strength of suture material commonly used in the linea alba during celiotomy closure[35]

Suture Type	Structure	Absorption	Tensile Strength
Polydioxanone	Monofilament	180 days	Reduction 25% at day 14, 30% at day 28, 50% at day 42
Polyglyconate	Monofilament	180 days	Reduction 25% at day 14, 30% at day 28, 50% at day 42
Polyglycolic acid	Braided multifilament	60-90 days	Reduction 35% at day 14, 65% at day 21
Braided lactomer	Braided multifilament	56-70 days	140% of USP requirements at day 0, 80% at day 14, 30% at day 21
Polyglactin 910	Braided multifilament	56-70 days	Reduction by 25% at day 14, 50% at day 21, 100% at day 35

d. Methods of Knot Construction

Figure 2.1 A surgeon's knot

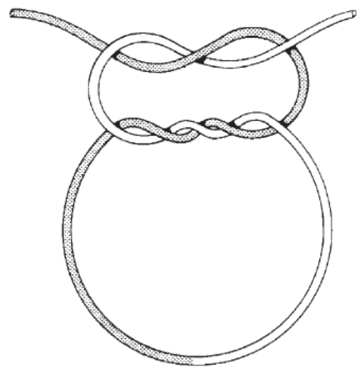


Figure 2.2 A square knot

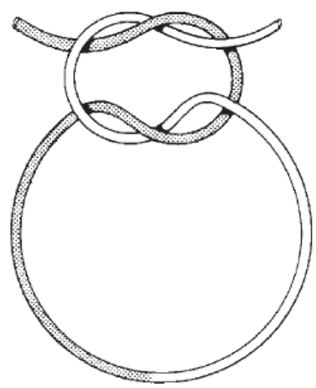


Figure 2.3 A granny knot

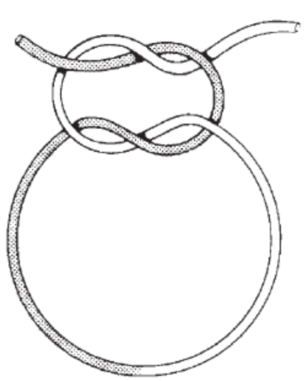
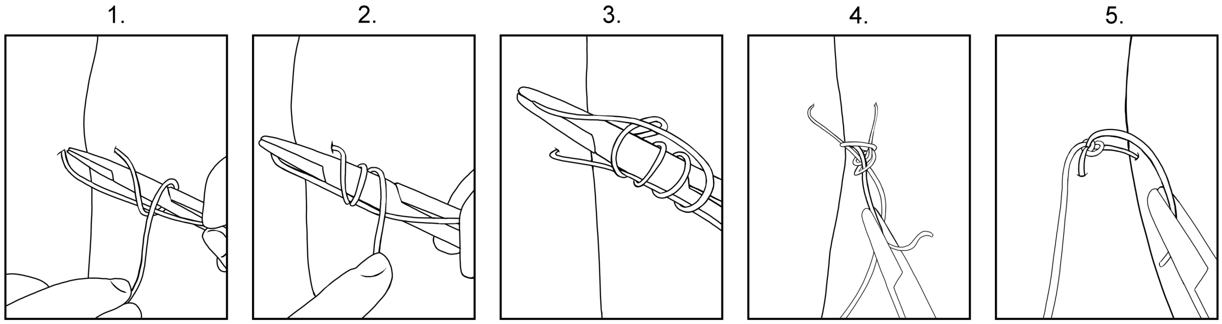
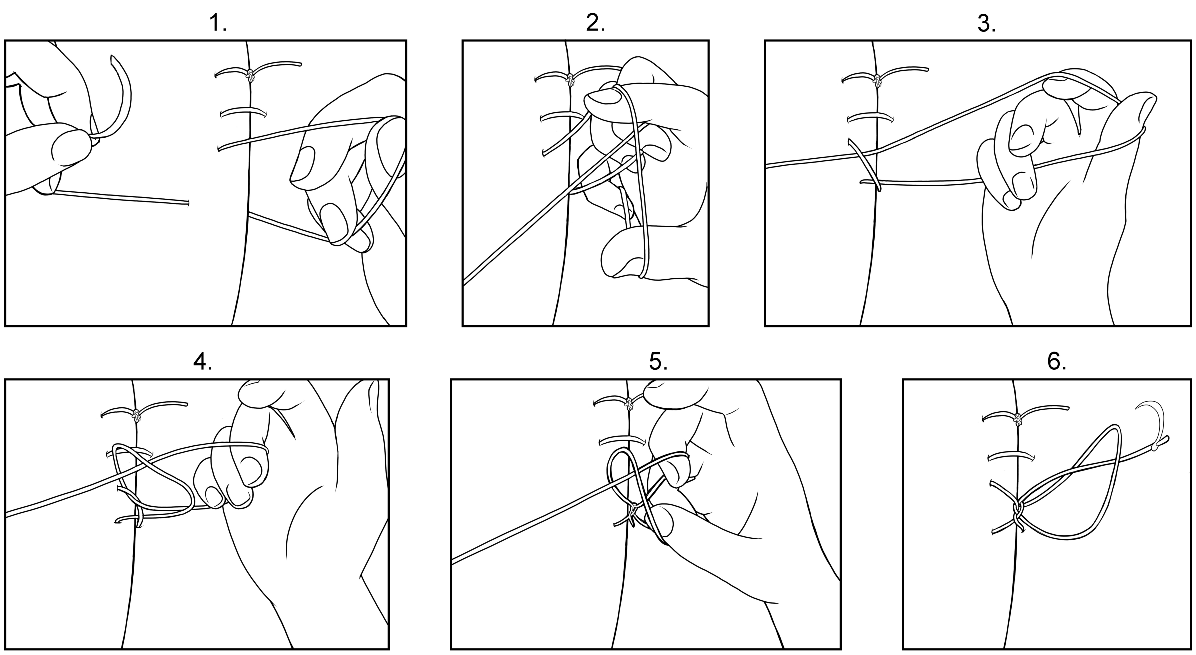


Figure 2.4 Formation of a Forwarder Knot



1) A bite is taken through the tissue from left to right. The free end is in the right hand and the working end is in the left hand. The working end is turned around the needle drivers and the free end to complete one throw. 2) The working end is turned around the needle drivers and free end again, each time representing a throw. 3) The working end is grasped by the needle drivers through the placed throws. 4) The needle drivers are withdrawn pulling the working in through the looped throws. 5) Both the free end and working end are pulled in opposite direction tightening and locking the knot.

Figure 2.5 Completion of an Aberdeen knot



1) The final bite in the continuous suture line is taken from right to left leaving a large loop. The working end with the needle attached is in the left hand. 2) A second loop is created in the working end and passed through the first loop while maintaining tension. 3) The second loop is created and equals one throw. 4 and 5) Step 2 and 3 are repeated to create required number of throws. 6) The working end is pulled through the final loop and tightened to lock the knot.

e. Suture Patterns

Figure 3.1 A simple interrupted suture pattern [35]

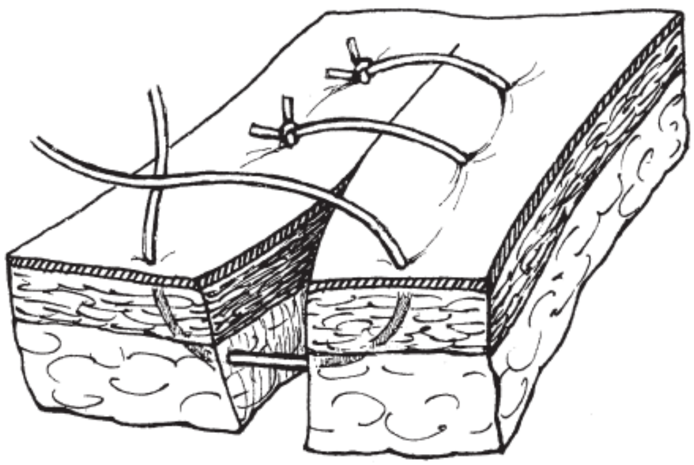
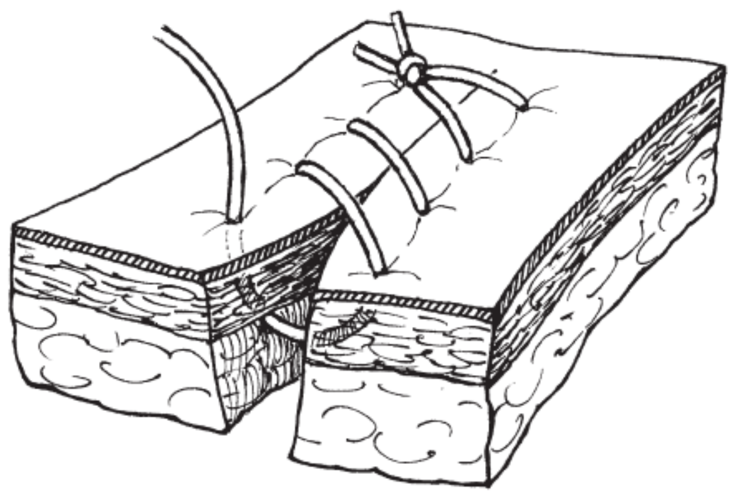


Figure 3.2 A simple continuous suture pattern [35]



f. Methods of Suture/Knot Construction

Figure 4.1

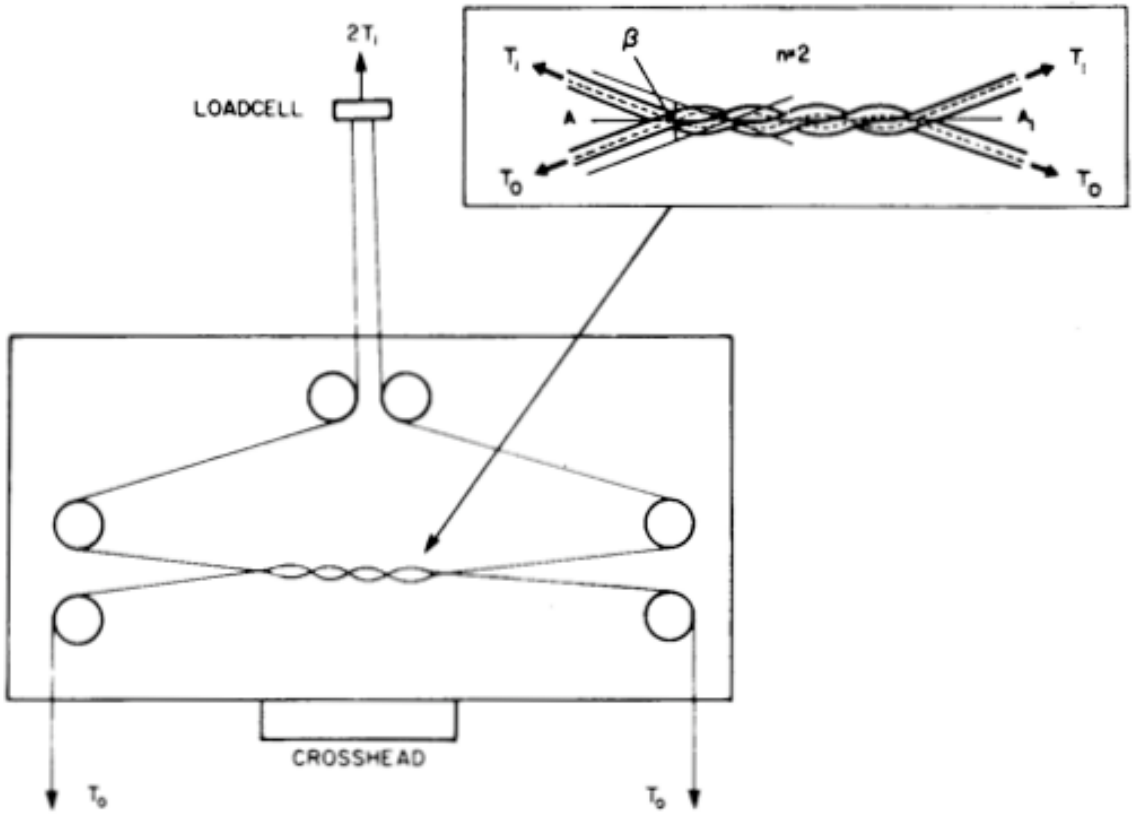
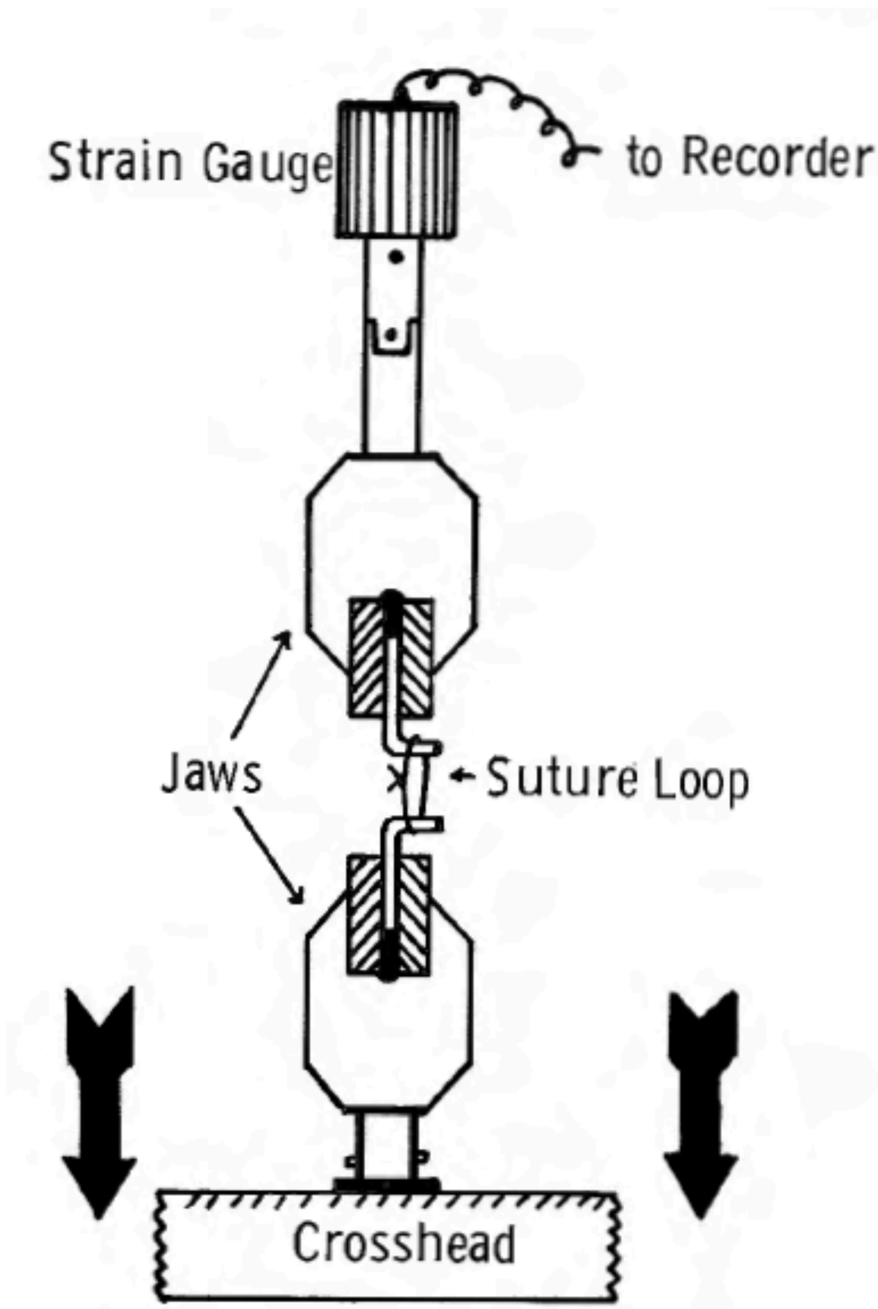


Diagram from Gupta et al. to depict the apparatus used to perform the twist test, load values obtained were used to calculate the coefficient of friction.[44]

Figure 4.2



Schematic depiction of the experimental design for performing the 'loop test' as originally described by Hermann et al.[84]

Figure 4.3

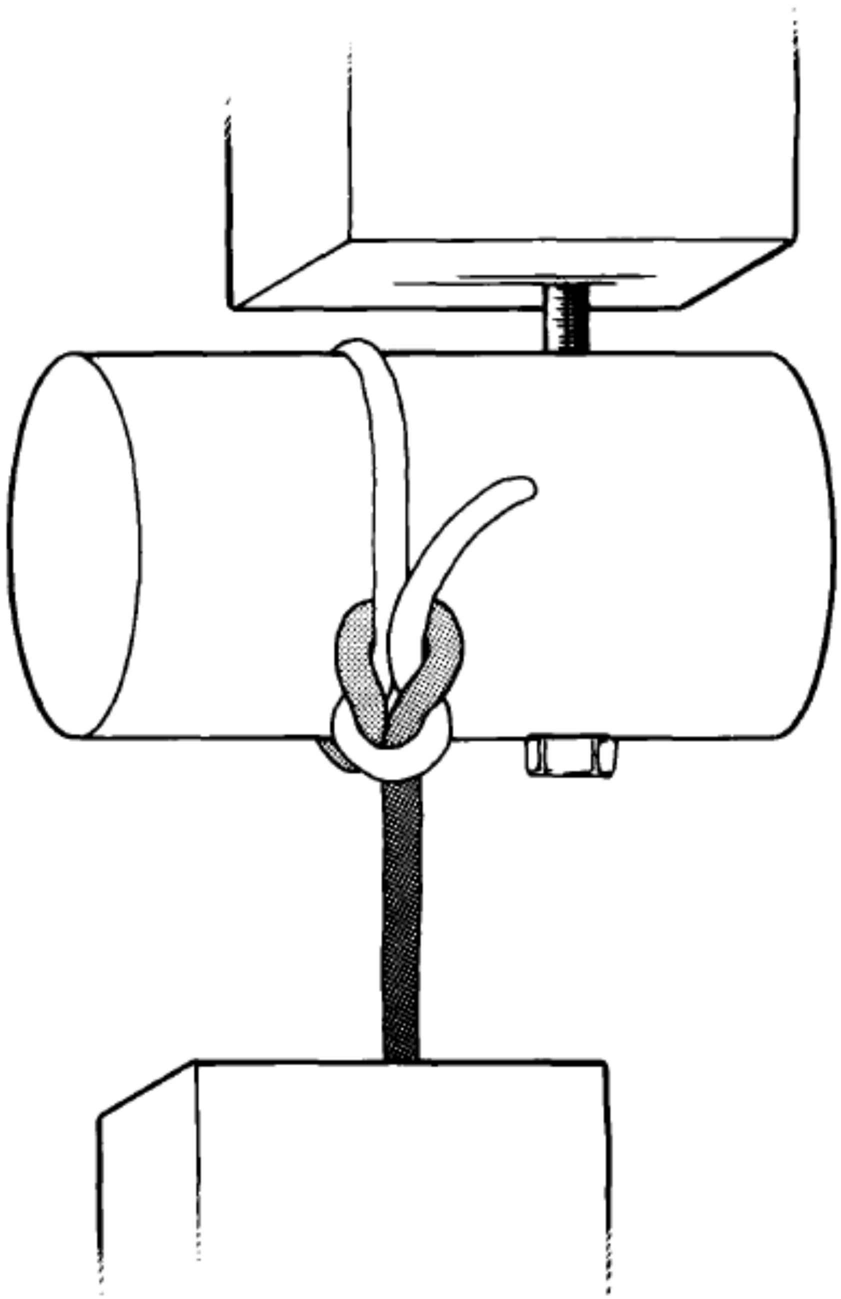
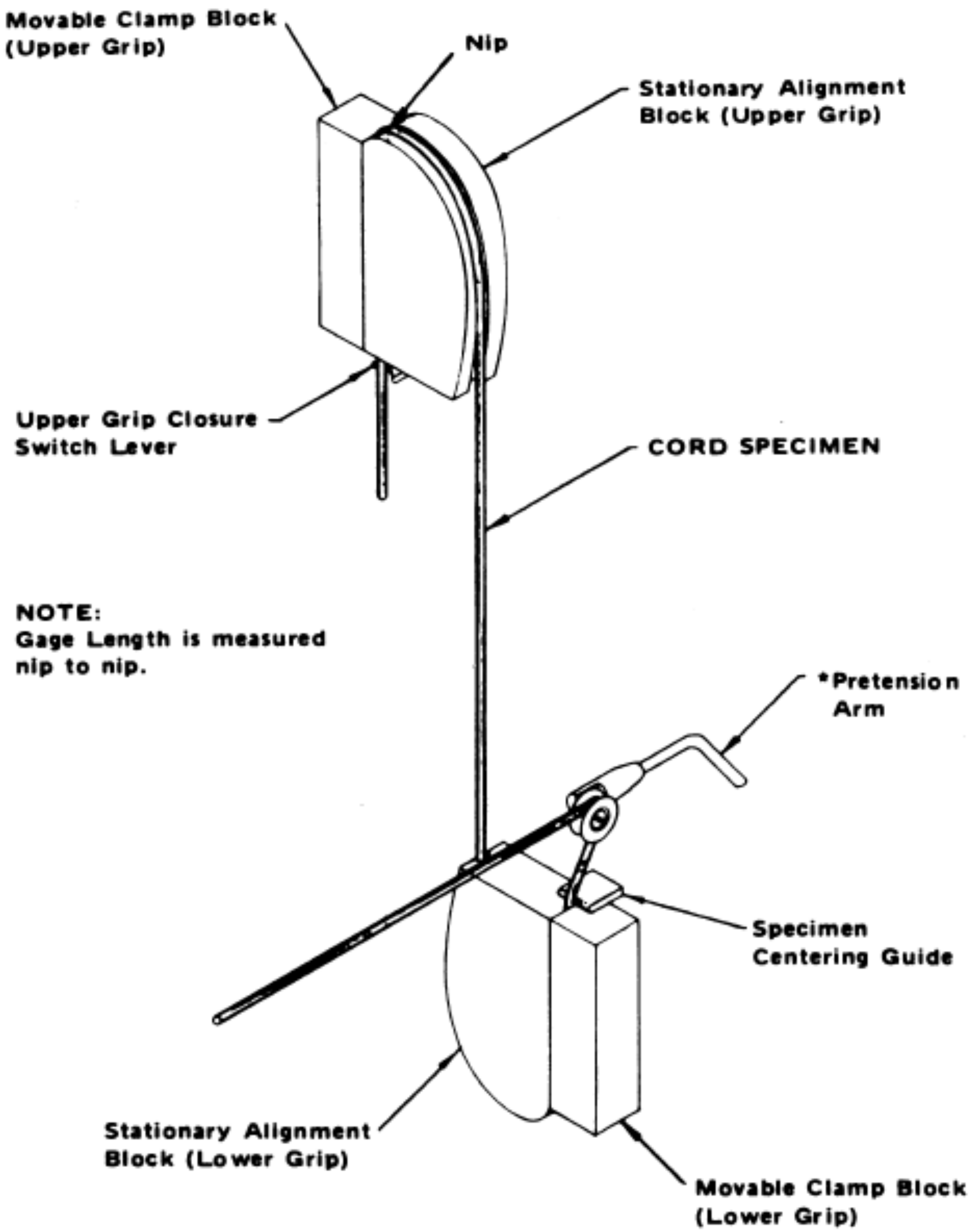


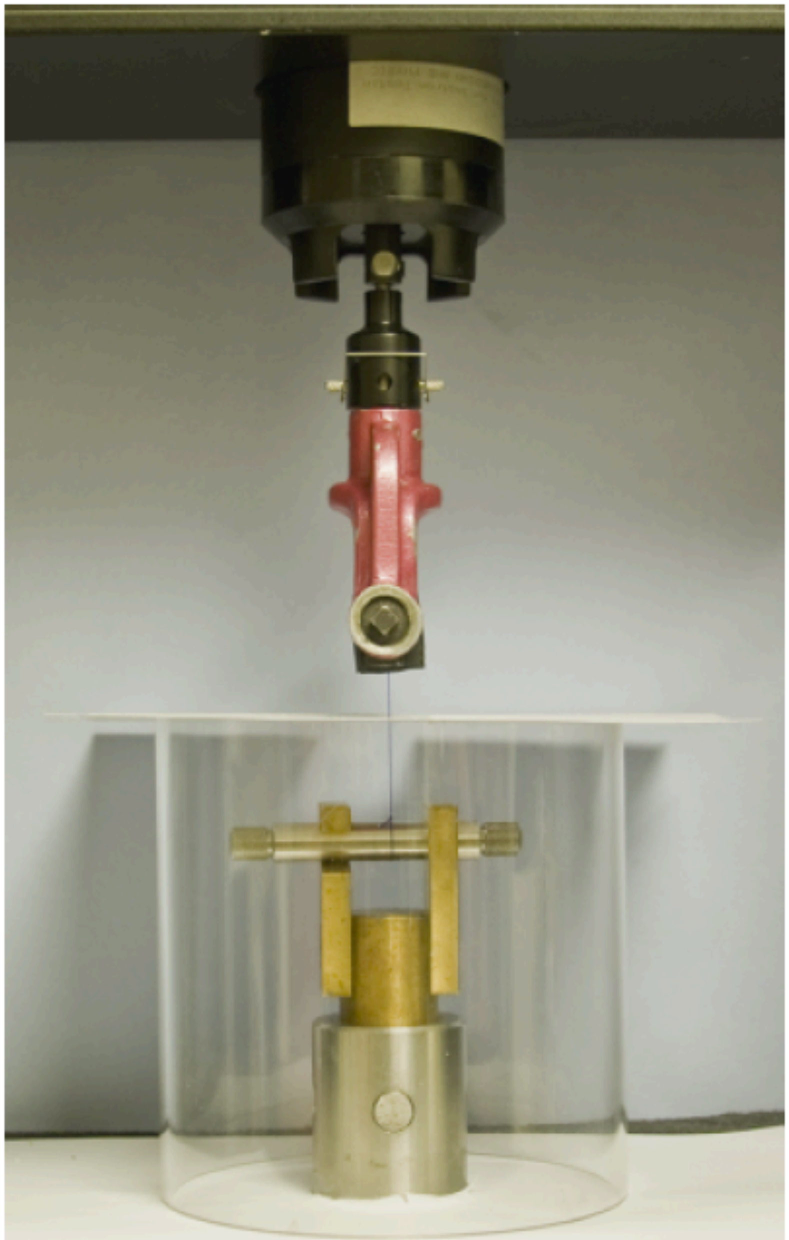
Diagram to depict the testing apparatus used by Roisin et al., to perform a modification of 'the single strand' method for testing knot security and strength.[67] The knot loop is placed around the cylinder that is held in the by the upper grip of the testing machine. The ear is cut to 3mm and the long end is placed in the clamps of the lower grip in the materials testing machine.

Figure 4.4



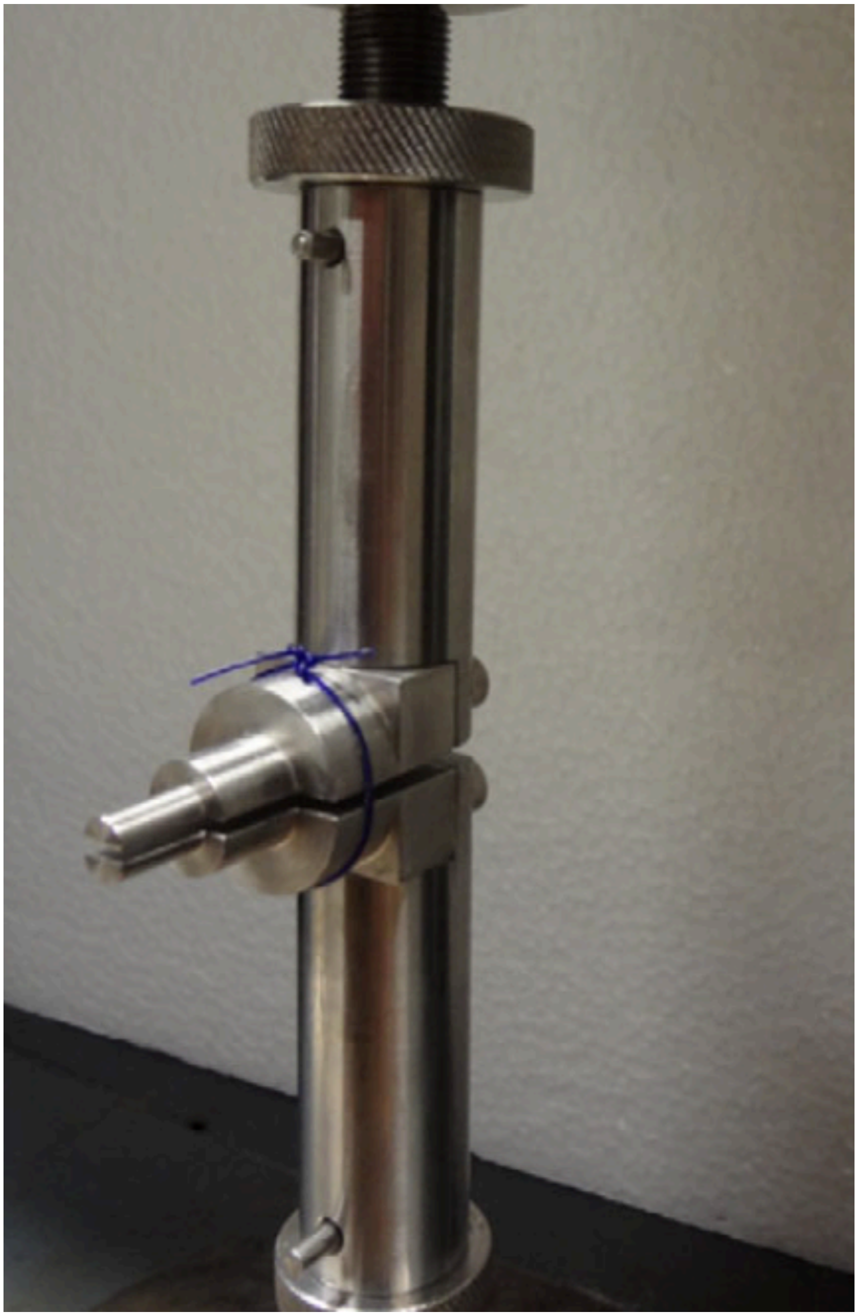
A schematic depiction of the specimen loading apparatus and the yarn grip as originally described by Chu et al.[104] The cord specimen is placed over the specially shaped mandrel of the yarn grip that distributes the force gradually over a length and avoids clamping the specimen creating stress accumulation in a focal point.

Figure 4.5



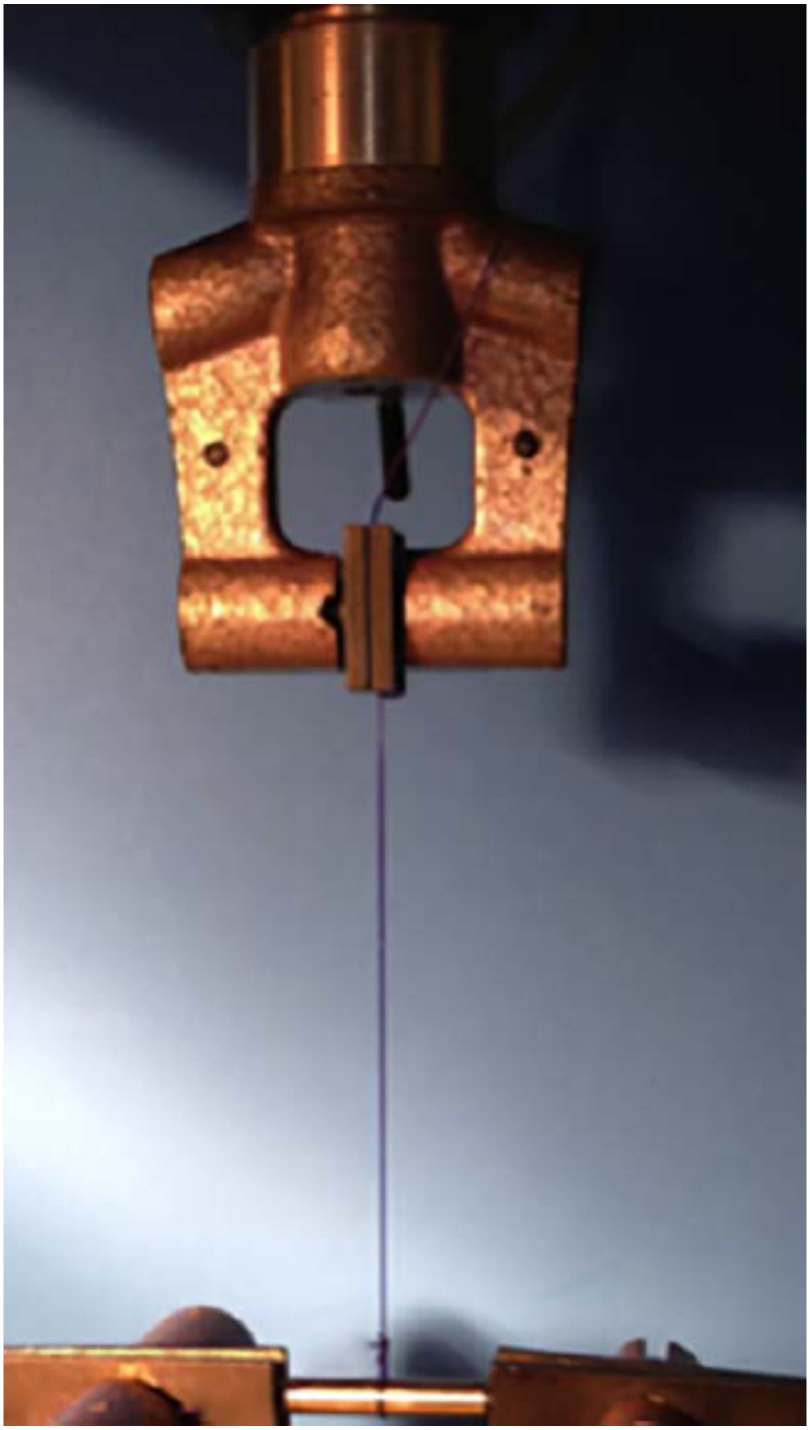
Photographic image of the apparatus used by Schaaf et al.[69] The knot is tied around the metal rod that is fixed to the lower grip of the materials testing machine. The long end of the suture strand is glued between rubber pads that are clamped in the upper grip of the materials testing machine. A catching system is placed around it to capture the knot for further investigation.

Figure 4.6



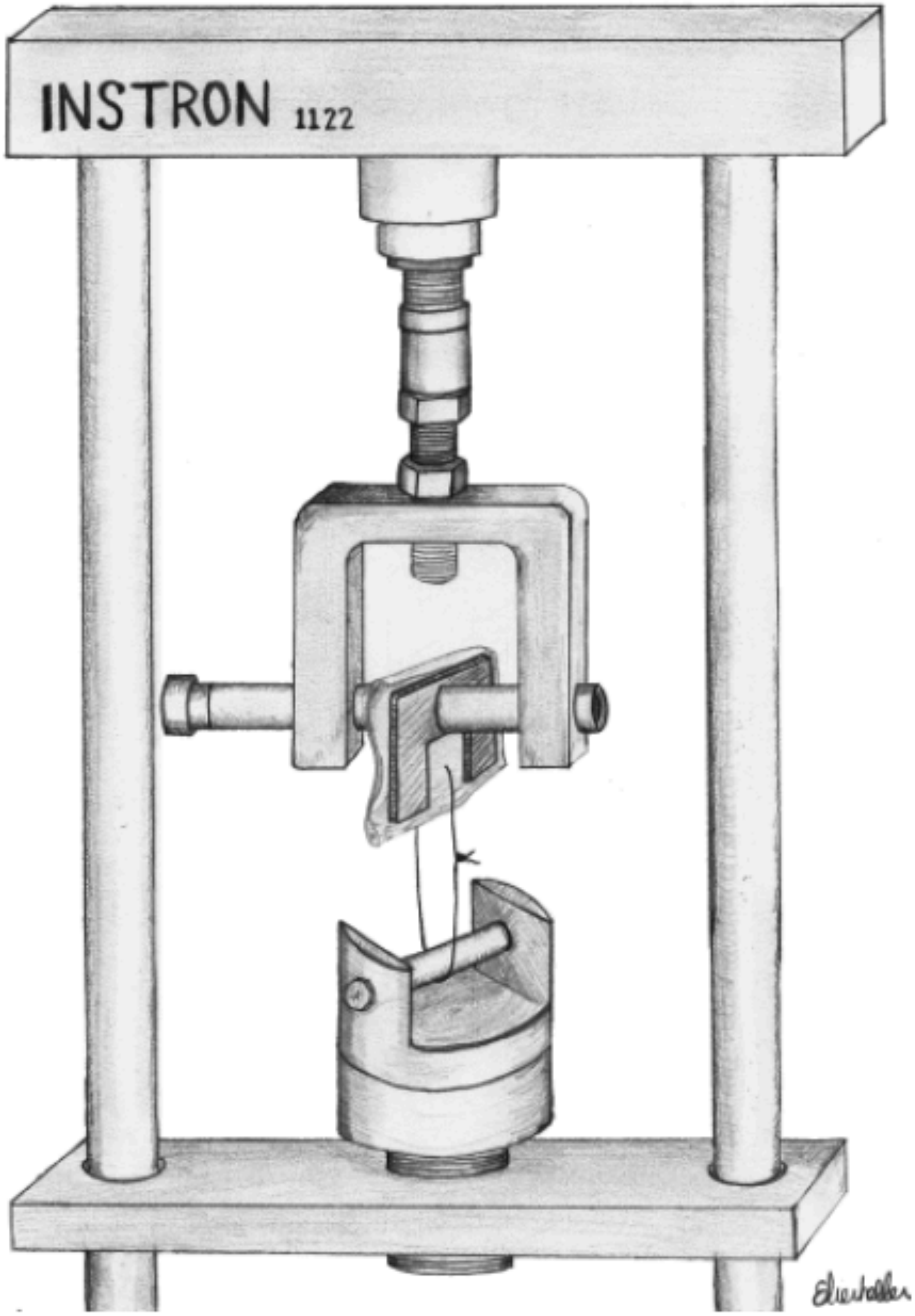
Photographic image of the apparatus used by Saunders et al. to test knot security and strength using the 'loop technique'.[92] Suture loops were mounted on a custom made 20mm diameter split circular jaw in the materials testing machine.

Figure 4.7



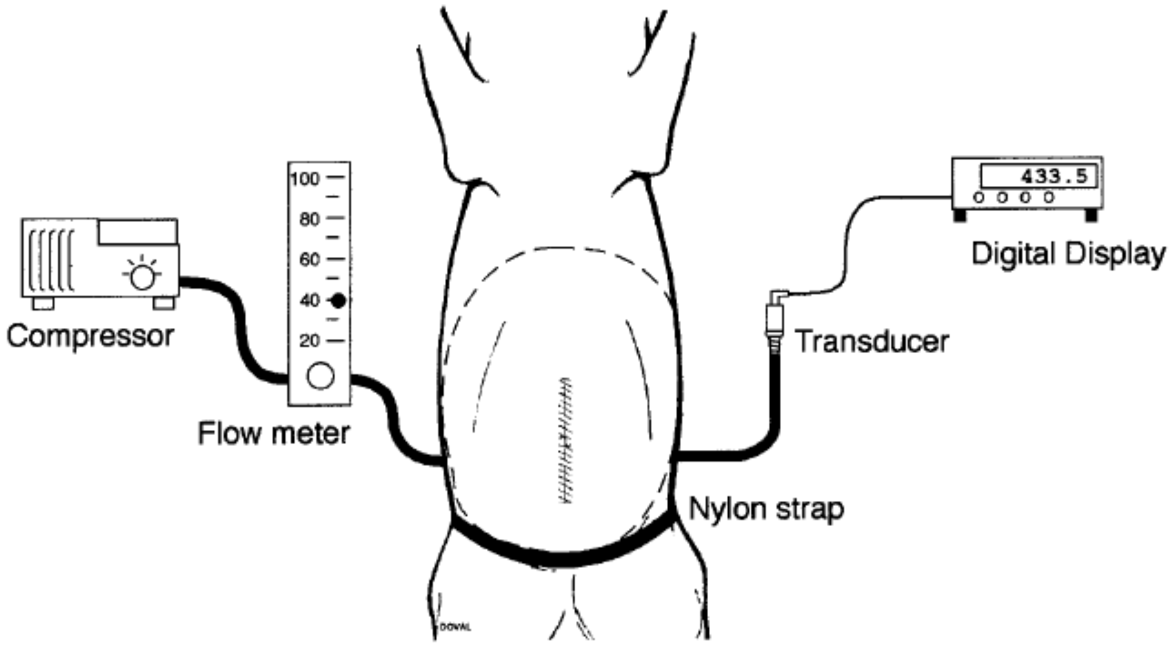
Photographic image of the apparatus used by Gillen et al. to test knot security and strength using the ‘modified single strand’ technique.[11-13] The knot is tied over the rod which is clamped in the lower grip of the testing machine. The free end of the suture is clamped in clamps attached to a load cell.

Figure 4.8



Schematic depiction of the Testing apparatus used by Fierheller et al. to test knot strength after placement in linea alba.[65] The specimen is attached to a U-shaped gripping component of the materials testing machine. A suture loop is placed through the specimen and placed around a rod attached to the lower grips, both grips are distracted.

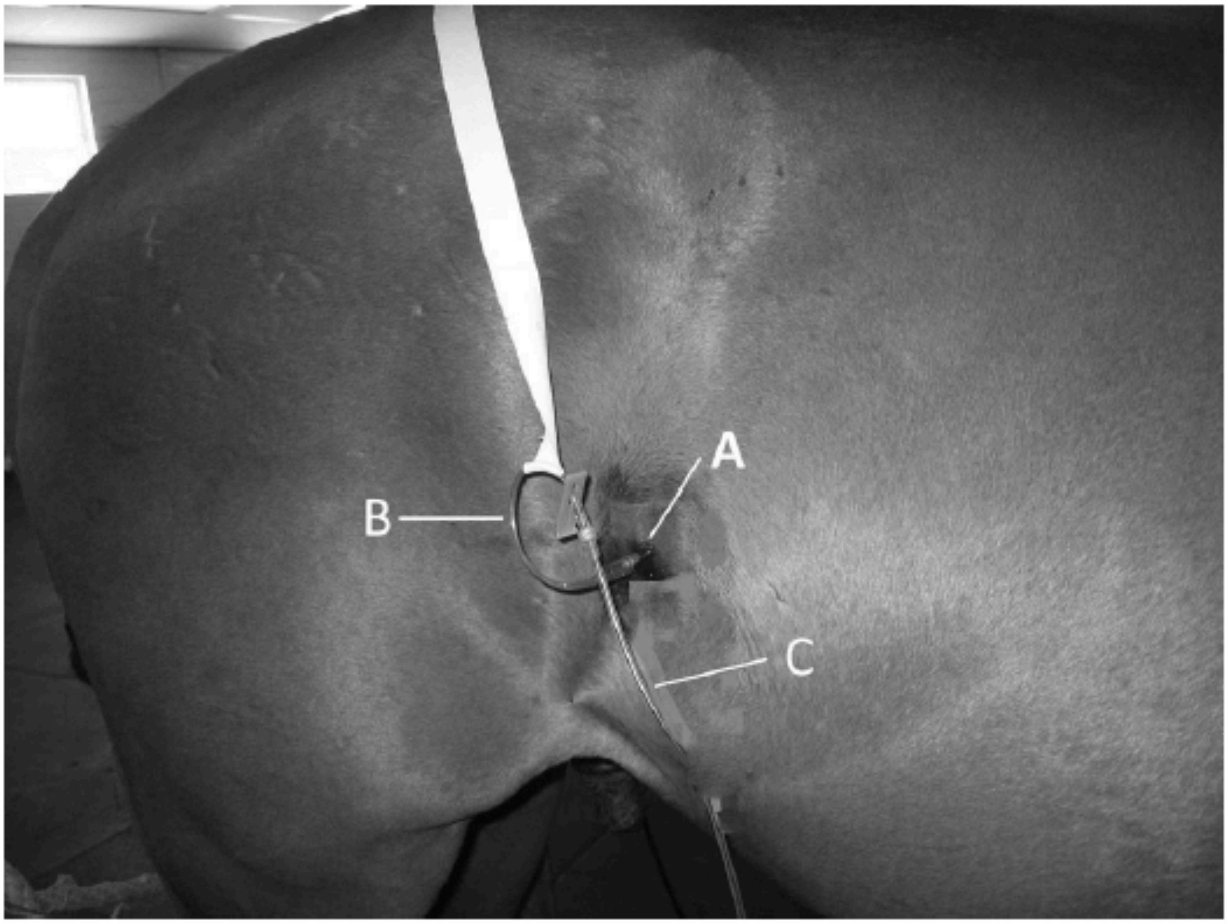
Figure 4.9



Schematic diagram of the experimental apparatus *ex vivo* equine cadaver model to test intra-abdominal bursting pressures, as originally described by Magee et al.[4] The dotted line represents the inflatable bladder within the abdomen.

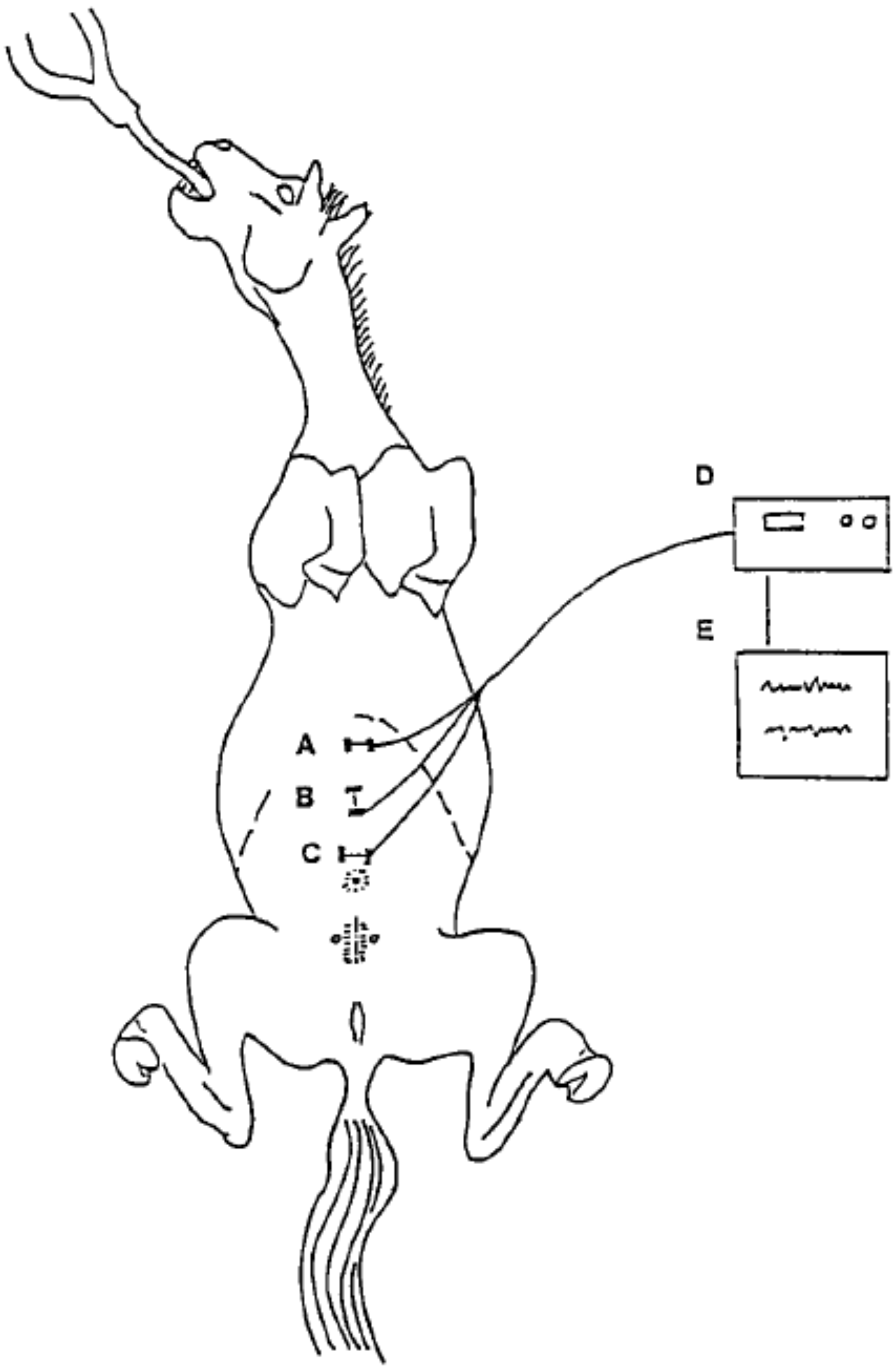
g. Methods of Intra-Abdominal Pressure and Linea Alba Strain Measurements

Figure 5.1



Photographic image of the Instrumentation for measurement of direct intra-abdominal pressures in the horse, placed in the right flank.[120] (A) 10cm metal cannula, (B) extension set, (C) manometer tubing.

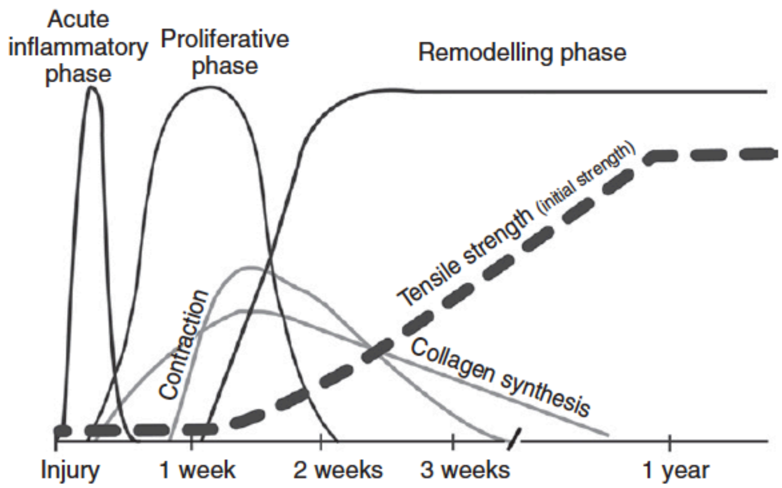
Figure 5.2



Schematic diagram of the experimental apparatus to measure the stress and strain on the linea alba of horses.[113] Strain gauges are placed in three location within the linea alba fascia, (A) cranial, (B) middle and (C) caudal. (D) Strain gauge amplifier and conditioner, (E) Physiograph.

h. Wound Healing

Figure 6. Phases of wound healing and gain in tensile strength post wounding.[122]

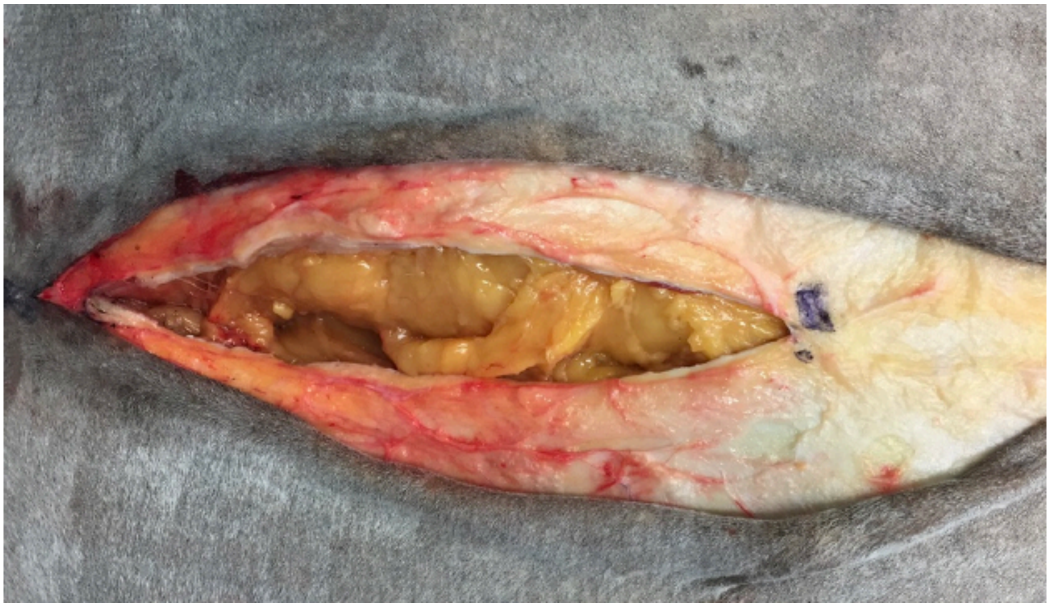


i. Figures from Methods and Materials

Figure 7.1

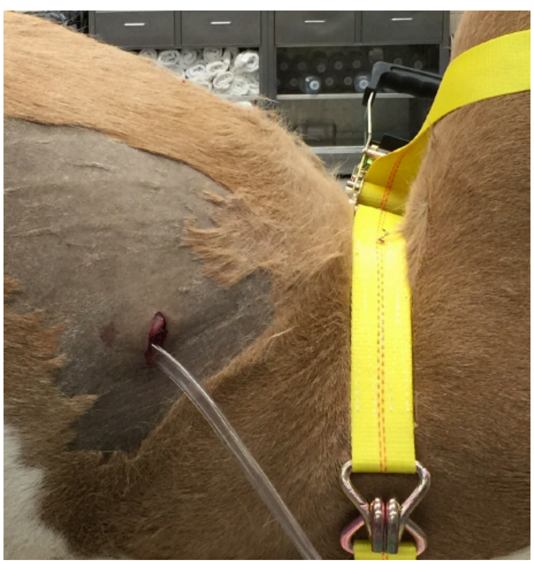
A photographic image of the 30cm skin incision made from umbilicus extending cranially. Left is caudal and right is cranial.

Figure 7.2



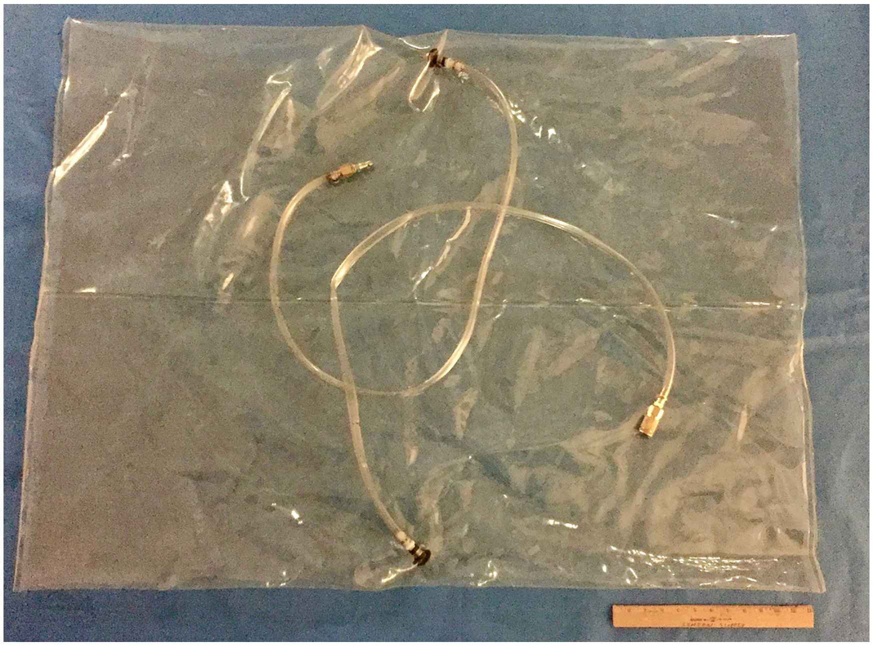
A photographic image of the 20cm incision made through the linea alba. Left is caudal and right is cranial.

Figure 7.3



A photographic image of the ingress tubing placed between the 14^{th} and 15^{th} intercostal space. Left is cranial and right is caudal.

Figure 7.4(i)

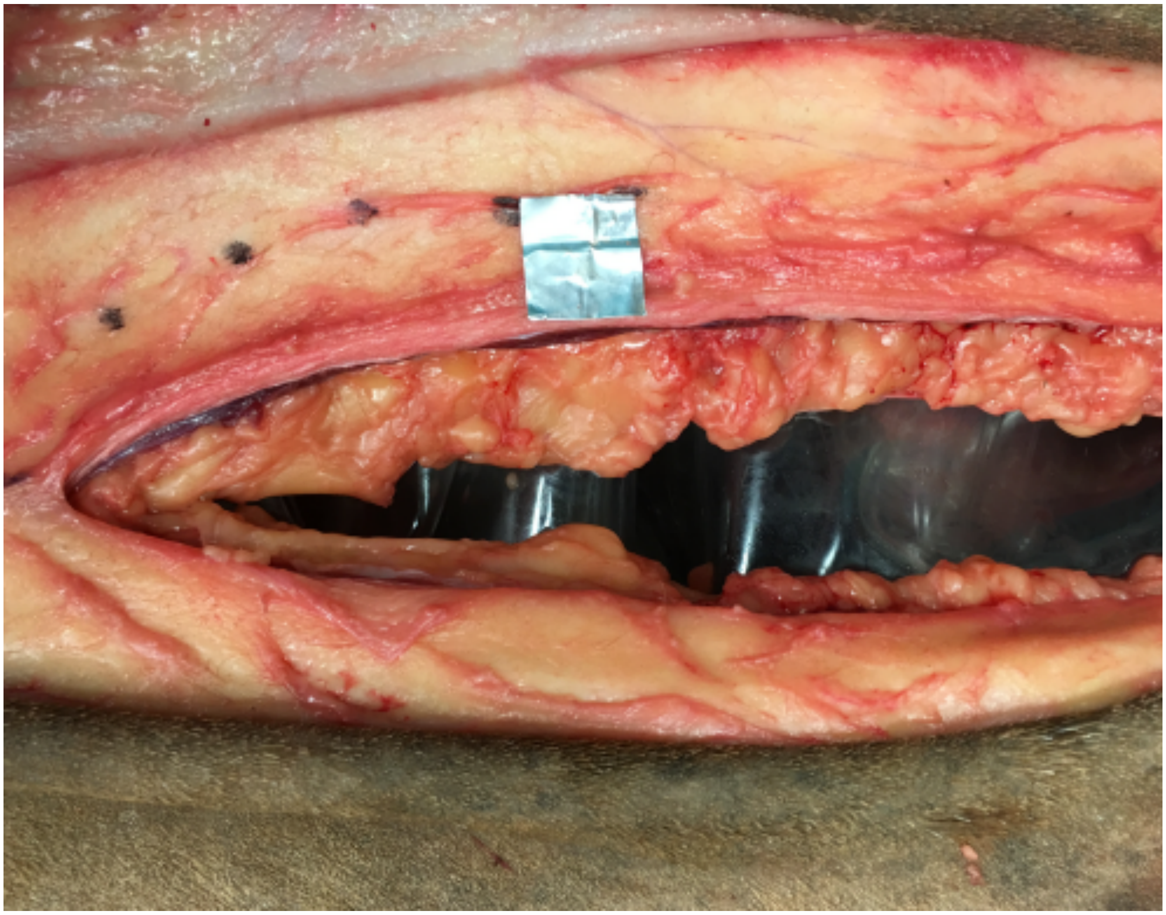


A photographic image of the 200L inflatable bladder

Figure 7.4(ii)

A photographic image of insertion of the 200L inflatable bladder into abdominal cavity. Left is cranial and right is caudal.

Figure 7.5



Photographic picture of template (15mm x15mm) marking suture bite placement.

j. Tables and Figures from Results

Table 3

Median bursting strength and closure times

	Forwarder-Aberdeen (F-A)	Surgeon-Surgeon (S-S)
Bursting strength (mmHg)	388	290
Closure Time (mins)	12.26	10.5

Failure mode for each group

	Forwarder-Aberdeen (F-A)	Surgeon-Surgeon (S-S)
Knot failure	1	6
Fascial failure	6	1

Bursting strength, closure time and failure mode for each group.

Figure 8.1

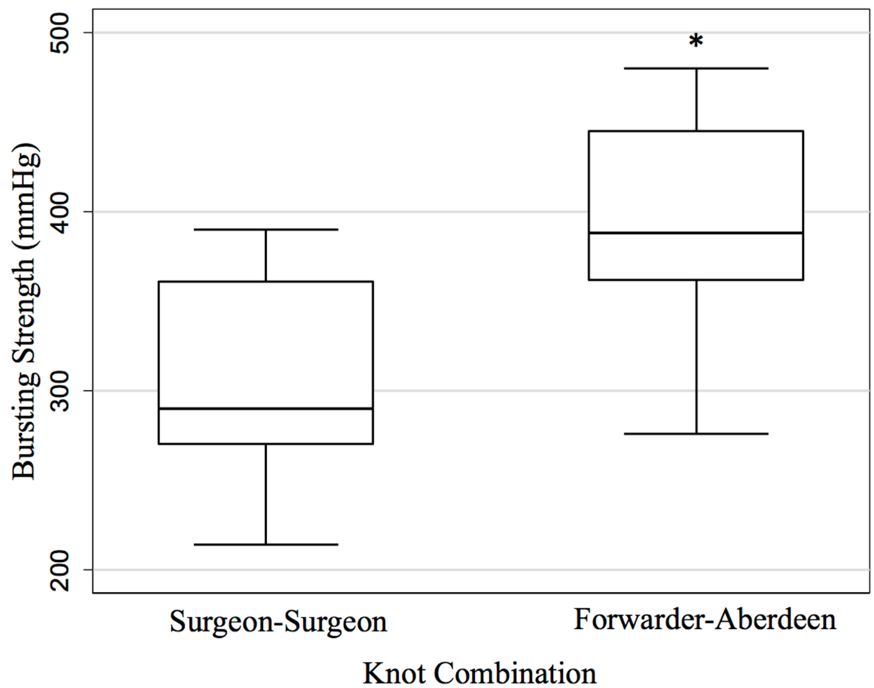


Figure 8.1. Box plots (median, 25% and 75% quartiles, and range) showing the difference in bursting strength of a Surgeon-Surgeon (S-S) and Forwarder-Aberdeen (F-A) knot combinations. The F-A knot combinations demonstrated significantly higher median bursting strength than the S-S combination (P=0.035) as identified by the *

Figure 8.2

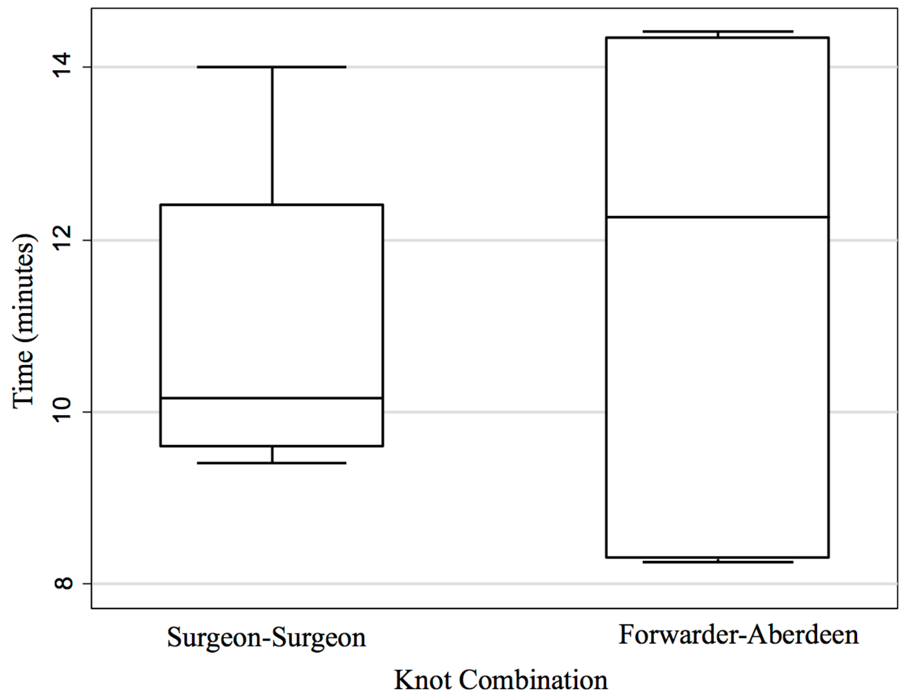


Figure 8.2 Box plots (median, 25% and 75% quartiles, and range) showing the difference in closure times of a Surgeon-Surgeon (S-S) and Forwarder-Aberdeen (F-A) Knot Combinations. No significant differences in closure times were observed between the two knot combinations (P=0.48).