Evaluation of the Aberdeen and Forwarder Knots in Large Gauge Suture

by

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Abstract

Methods: Forwarder, Aberdeen, surgeon's and square knots were tested using large gauge suture under linear tension on a universal testing machine, recording knot holding capacity (KHC). Relative knot security (RKS) was calculated as a percentage of KHC. Knot volume and weight were assessed by a digital micrometer and balance, respectively. An ANOVA and post hoc testing compared strength between number of throws, suture, suture size, and knot type. $P \leq 0.05$ was considered significant.

Results: Forwarder and Aberdeen knots had a higher KHC and RKS than surgeon's or square knots for all suture types and number of throws ($P < 0.001$). For all suture materials, no Forwarder or Aberdeen knots unraveled. Forwarder and Aberdeen knots had a smaller volume and weight than surgeon's and square knots with equal number of throws ($p < 0.001$).

Conclusions: The Forwarder and Aberdeen knots were shown *in vitro* to be stronger, more secure, and smaller than surgeon's and square knots.

Acknowledgments

Table of Contents

List of Tables

List of Illustrations

List of Abbreviations

KHC   Knot Holding Capacity

RKS   Relative Knot Security

TS   Tensile Strength

AVNOA   Analysis of variance

a. Ventral Midline Celiotomy Incisions

i. Comparisons of Methods of Access for Abdominal Surgery

Surgical access to the abdomen is required for a variety of reasons in both humans and domestic species. In humans, the most common reasons for abdominal surgeries include appendectomy, cesarean section, hysterectomy and exploratory surgery (Rath *et al.* 1996). In horses, the most common reason for performing abdominal surgery is abdominal pain (colic), with 1.4-10% of colic cases requiring surgical management (Traub-Dargatz *et al.* 2001; Tinker *et al.* 1997; Mair *et al.* 2007). Other common procedures requiring access to the abdominal cavity are cesarean section, ovariectomy and cryptorchidectomy (Slone 1988; Collier 1980).

There are multiple routes by which access to the abdomen can be obtained. In human surgery, the most common method of obtaining access to the abdomen is via a ventral midline celiotomy (Rath *et al.* 1996). However, this is largely being replaced by laparoscopy for many procedures, such as appendectomy (Hall Long *et al.* 2001), hysterectomy (Reich *et al.* 1989) and colorectal resection (Maggiori *et al.* 2012).

Laparoscopy is used in many equine abdominal procedures, especially ovariectomy and cryptorchidectomy (Hendrickson 2006). However, given the weight (150lbs) and volume of the large colon (75-90 liters of ingesta), and the length of the small intestine (on average, 30 meters),

1

laparoscopic intervention for many colic surgeries is not a possibility (Carr 2012). Therefore, equine surgeons rely on other approaches to the equine abdomen. Methods of accessing the abdomen include the ventral midline, ventral paramedian, inguinal, flank, parainguinal, caudal ventral midline, modified low flank and transcostal approaches (Kummer 2012).

A ventral paramedian incision is most commonly used for exploratory celiotomies, either when a ventral midline incision has previously been performed, or when surgical access to the right or left side of the abdomen is preferred, depending on the expected condition (Kummer 2012). The majority of surgeon's will choose to perform a ventral midline celiotomy due to the ability to access to the majority of the gastrointestinal tract via one incision (Kummer 2012). The caudal ventral midline approach can be utilized when a previous ventral midline incision has been made further cranially, or during bladder surgery, as this aids in exteriorization of the organ (Schott and Woodie 2012).

Inguinal incisions can be performed for a variety of procedures, including cyrptorchidectomy, or surgery on the aboral portion of the small colon. These incisions are generally smaller and do not provide visualization or access to the majority of the gastrointestinal tract (Kummer 2012). Parainguinal incisions can also be used to perform cryptorchidectomies or ovariectomies and can again be used to exteriorize the caudal portion of the small colon. Use of this approach to the abdomen is comparatively less common.

The flank and modified low flank approaches are most commonly used in laparoscopic surgery, such as ovariectomy, cryptorchidectomy, uteropexy or nephrosplenic space ablation (Fischer *et*

al. 2012). However, a flank approach for manual correction of nephrosplenic entrapment of the large colon has also been described (Krueger *et al.* 2015). Despite these other approaches, the ventral midline approach gives superior access to the abdomen, allowing 75% of the gastrointestinal tract to be exteriorized, while still maintaining a sterile surgical field, and is the most frequently utilized approach for exploratory celiotomies (Kummer 2012).

ii. Complications Following Ventral Midline Celiotomy in the Horse

Common post-operative complications following abdominal surgery include persistent post-operative colic (32.1%), ileus (18.2%) and endotoxemic shock (13.9%) (Mair and Smith 2005a; Mair and Smith 2005b). Other documented complications include laminitis, colitis, jugular vein thrombophlebitis, anastomotic leakage and septic peritonitis (Proudman *et al.* 2002a, 2002b; Ducharme *et al.* 1983; Philips and Walmsley 1993; Freeman *et al.* 2000; Hunt *et al.* 1968; French *et al.* 2002). Post-operative incisional complications remain a commonly documented complication (Mair and Smith 2005b).

Post-operative incisional complications include incisional swelling, incisional infection, suture sinus formation, hematoma formation, incisional drainage, hernia formation, and most seriously, incisional dehiscence (Klohnen 2009). Incisional complications have a high incidence following ventral midline celiotomy, with as many as 40% of cases undergoing intestinal surgery developing such complications in one report (Wilson *et al.* 1995). Other studies give a variable, but still relatively high incidence of incisional complications. Philips and Walmsley (1993) reported an incidence of incisional complications of 37%, whereas two more recent studies found

similar incidences of 26% and 26.9% respectively (Philips and Walmsley 1993; Mair and Smith 2005; Bischofberger *et al.* 2010). Other studies found lower but significant rates of incisional complications, with Proudman *et al.* (2000) and Freeman *et al.* (2000), reporting 16% and 7.4% respectively. The highest rates of complication are reported in older retrospective studies, possibly showing a trend towards improvement in the rates of post-operative complications. Alternatively, the different hospitals, surgeons and geographic locations may contribute to the different rates of incisional complications.

iii. Consequences of Incisional Complications

The most devastating incisional complication, occurring in approximately 1.1% of equine ventral midline celiotomies, is dehiscence, either partial or total, of the surgical incision (Wilson *et al.* 1995). When dehiscence occurs, eventration can follow, leading to significant damage to the viscera, resulting in death of the horse (Wilson *et al.* 1995). The method for attempted salvage is copious lavage of the viscera, resection of intestine where necessary and closure of the abdomen using stainless steel suture in an interrupted horizontal mattress pattern, under general anesthesia (Mair *et al.* 2007).

Incisional edema is a common post-operative complication and while swelling in itself has minimal consequences for the overall health of the horse, swelling is associated with other post-operative complications including incisional infection and drainage (Klohnen 2009). Incisional infection can delay post-operative healing and increase the incidence of incisional hernia formation (Klohnen 2009). Incisional hernia formation can occur is as many as 16% of colic

surgeries (Gibson *et al.* 1989). While many of these hernias are only of cosmetic consequence, they can potentially lead to intestinal strangulation and can be of significance in breeding mares (Gibson *et al.* 1989).

iv. Risk Factors for Incisional Complications

There are several identified risk factors for incisional complications. Many of these risk factors, such as an elevated packed cell volume, leukopenia, hypoproteinemia, concurrent infection, and an elevated fibrinogen content of the peritoneal fluid are related to the presence of endotoxemia, which is known to be involved in many post-operative complications (Mair *et al.* 2007). There are also several factors specifically related to the surgical procedure which can increase the risk of incisional complications. Increased manipulation of the gastrointestinal tract may traumatize the edges of the incision, particularly if the incision is not of sufficient size to accommodate distended large intestine (Kummer 2012; Honnas and Cohen 1997). An increased risk of incisional complications can occur if the tissues are exposed to sources of contamination, which can occur during an enterotomy or resection and anastomosis (Philips and Walmsley 1993; Honnas and Cohen 1997). Other risk factors for post-operative incisional complications are related to the specific horse involved. These factors include horse weight, with greater than 300kg being a risk factor (Wilson *et al.* 1995), elevated intra-abdominal pressure, previous abdominal muscle injury and a poor nutrition status (Honnas and Cohen 1997; Protopapas *et al.* 2000; Stone *et al.* 1990).

Factors related to the tissues of the incision are also important in the incidence of incisional complications (Mair *et al.* 2007). A ventral midline celiotomy is performed through the linea alba. This tissue can withstand high tensions placed on suture material due to the fact that the linea alba fibers are orientated at 90 degrees to the suture (Trostle *et al.* 1994). This property is crucial in abdominal wound closure as the suture material is placed under high tensile strength (Magee and Galuppo 1999). Paramedian incisions do not involve the linea alba, however, it has been established that the external rectus sheath is the strongest muscle layer and must be incorporated into any closure (Kummer 2012). Although the many surgeons prefer to perform all exploratory celiotomies via a ventral midline approach, a study performed by Boone *et al.* (2014), found there to be no difference between the strength of right paramedian celiotomies and repeat ventral midline celiotomies when tested using an intra-abdominal inflatable bladder. However, both of these approaches showed inferior biomechanical and histological properties when compared to primary ventral midline incisions. Further evidence for this is shown in an *ex vivo* study, which revealed right paramedian incisions to have a lower bursting strength than ventral midline celiotomies when a polyurethane bladder was placed in the abdomen (Anderson *et al.* 2013).

It has been established that the recovery stall is a major source of contamination and therefore a risk factor in the development of incisional infection (Mair *et al.* 2007). Several methods have been utilized to reduce contamination of the incision in the recovery stall. It has been shown that a film-forming iodophore complex reduced the number of bacterial colony forming units in a retrospective study (Galuppo *et al.* 1999) and an elasticon belly band was found to reduce the incidence of infection by 45%, compared to horses without a belly band during recovery from

anesthesia (Smith and Mair 2005). While the horse is on stall rest following exploratory celiotomy, the incision should still be protected. Often, this involves keeping the horse in a clean, dry stall (Smith and Mair 2005). Although many surgeon's prefer to maintain a belly bandage post-operatively, this has not been shown to improve the incidence of post-operative incisional complications. Alternatively, a stent bandage, placed over the incision for up to five days led to a reduction in incisional complications from 21.8% to 2.7% (Tnibar *et al.* 2013).

Horses that undergo a repeat celiotomy before discharge from the hospital are at significantly higher risk of developing incisional complications, due to the transfer of bacteria from the raw edges of the wound and decreased resistance of the edematous tissues to bacteria colonization (Freeman *et al.* 2002; Philips and Walmsley 2003; Mair and Smith 2005a; Mair and Smith 2005b). An additional reason for the increase in complication rates is that the incision is in the remodeling phase at the time of the second celiotomy, during the inflammatory or proliferative phases of wound healing. The normal course of wound healing causes disruption of the provisional wound matrix and granulation tissue, leading to increased complications (Provost 2012).

The increased risk of incisional complications after repeat celiotomy is supported by clinical reports. Mair and Smith (2005a) found the incidence of incisional herniation to increase from 7.2% to 25% when a repeat celiotomy was performed. Mair and Smith (2005b) also report an increase in incisional drainage from 23.8% to 44.0% when a repeat celiotomy was performed during the same hospitalization period. Philips and Walmsley (2003) found a 57% rate of incisional infection in horses who had undergone repeat celiotomy, compared to 29% in horses

who underwent only one celiotomy. An alternative to performing a repeat ventral midline celiotomy is to perform a second incision, either via a left or a right paramedian approach. However, when a second celiotomy incision was performed through the muscle wall, an 88% incisional complication rate was reported, compared to a 40% incidence when the second incision was made through the linea alba (Wilson *et al.* 1995). The numbers in this study were small, therefore further research is needed in this area.

It has been well established at a bacterial colonization of 10^5 bacteria per gram of tissue is defined as contaminated tissue (Provost 2012). However, Ingl-Ferr *et al.* (1997) found no association between bacteria culture of the incision at the end of surgery and the incidence of incisional infection. Ingl-Ferr *et al.* (1997) found the most common cultures to be *Staphylococcus spp., Streptococcus spp.* and *Escherishia coli*, and it is, therefore, likely that incisional infections are caused by endogenous flora on the skin. It is recommended that topical or systemic antimicrobials be employed in cases of incisional infections, based on appropriate culture and sensitivity (Freeman *et al.* 2002; Ahern and Richardson 2012).

b. Wound Healing

i. Phases of Wound Healing

Wound repair or surgical incision repair involves multiple interactions between cellular and biochemical events. Wound healing can be divided into three phases: the inflammatory

phase, the proliferative phase and the remodeling phase (Provost 2012). It is important to note that all phases of wound healing are overlapping.

The inflammatory phase lasts up to 5 days and involves hemostasis and inflammation. The primary functions are to stop blood loss, protect against infection and provide the substrate and cellular signals for the remaining phases of healing (Franz *et al.* 2007). Immediately following the surgical incision or the time the wound is sustained, vasoconstriction, platelet aggregation and fibrin deposition occur. The early fibrin clot forms the provisional wound matrix and is instrumental in the further stages of wound healing.

The proliferative phase of tissue repair begins within 3 days of injury or surgical incision (Provost 2012). During this phase, angiogenesis, granulation tissue formation, collagen deposition, epithelialization and wound contraction occur (Provost 2012).

The remodeling and maturation phase begins within 2 weeks of the injury and ends in the formation of a scar 1 to 2 years later. The scar which forms remains 15-20% weaker than the original tissue (Theoret 2008). Specifically regarding the equine linea alba, Chism *et al.* (2000) established that by 4 weeks post-operatively, the strength of the incised equine linea alba was not significantly different from control samples which had undergone no surgical incision. It is therefore particularly important that the tissue is supported by appropriate closure methods during this time.

c. __Principles of Suture Materials, Suture Patterns and Knots__

i. Suture Materials

There are a multiple methods of categorizing suture material. Suture can be described as naturally occurring or synthetic, as well as being divided into both absorbable and non-absorbable categories. In addition to this, suture material can be comprised of monofilament or multifilament fibers (Kummerle 2012).

The majority of suture materials are now synthetic fibers as these fibers cause minimal inflammatory reaction in comparison to natural fibers (Kummerle 2012). It has been shown that an increased tissue reaction can predispose to incisional swelling and incisional drainage by extending the inflammatory phase of wound healing, making naturally occurring fibers an unusual choice for closure following ventral midline celiotomy (Richey and Rose 2005).

When choosing between absorbable or non-absorbable suture, the main determining factor is whether, and how quickly, the suture material will degrade. Degradation of absorbable suture material can occur in two ways. Synthetic absorbable sutures are degraded by hydrolysis, whereas natural absorbable sutures are degraded by proteolytic enzymatic digestion or phagocytosis by leucocytes (Rousch 2003). Non-absorbable sutures undergo no appreciable degradation and are therefore utilized where support for an incision or wound is required for an extended period of time and when sterile, foreign material in the wound is unlikely to be detrimental (Kummerle 2012).

Monofilament sutures are formed from a single suture strand, whereas multifilament suture materials are formed from multiple filaments braided together (Kummerle 2012). Multifilament and monofilament sutures have different surface profiles, which determines the degree of drag as the suture is pulled through the tissue. Monofilament suture provides low tissue drag and this is particularly important when suturing structures such as a thin walled viscus but less important when suturing the linea alba (Kummerle 2012). Conversely, multifilament suture has an irregular surface prolife and can therefore achieve better tissue apposition under a lower degree of tension, due to increased tissue drag (Kummerle 2012). Due to the additional fibers in multifilament suture, there is an increased risk of capillarity. The capillarity of a suture is the process by which bacteria and fluid are wicked into the fibers of a multifilament suture. Bacteria located in these fibers can be largely protected from the host's immune system and can therefore set up a persistent infection. To reduce this risk, coating is frequently applied to multifilament suture. Commonly used coatings include polyglactin 310 and calcium stearate (Todtmann 2008). Multifilament sutures without coating should be avoided in a surgical site where contamination is of concern (Kummerle 2012). A further advantage of multifilament suture is that the irregular surface profile increases the coefficient of friction when a knot is tied, thereby achieving increased knot security when compared to monofilament suture (Fossum 2007). Although coating multifilament suture reduces the irregular surface profile, these materials still have a more irregular surface profile than monofilament sutures, providing increased knot security (Fossum 2007; Gupta et al. 1985; Alzacko and Majid 2007).

There are several additional properties to be considered in suture material selection. The flexibility of a suture is particularly important for good handling characteristics and this is essential in ligating vessels or performing a continuous suture patterns (Kummerle 2012). The flexibility of a suture increases as the diameter of the suture decreases (Fossum 2007). Another important property is the elasticity of a suture. This is defined as its capacity to undergo elastic deformation under tension but then return to its original length once the stretching force has ceased. This is particularly beneficial in cases of wound or incisional edema when tissue swelling can cause stretching of the suture material (Kummerle 2012). Finally, suture memory describes the suture's ability return to its original shape after deformation. This characteristic has a large effect on its handling properties, with increased memory making the suture material more difficult to handle. Sutures with a high memory have poor knot tying properties as they are more difficult to cinch down into a tight knot (Kummerle 2012).

Regarding suture strength characteristics, the suture selected should be as strong as the normal pre-operative strength of the tissue through which it is placed. The reduction in tensile strength of the suture over time should relate to the healing characteristics and gain in strength of the tissue involved. The suture should retain its tensile strength while wound healing occurs and once the wound has healed, the suture should be degraded. The strength of a wound is in fact more dependent on the involved tissue's ability to hold the suture material, than the strength of the suture material itself. Elastic suture materials are required for areas of high tissue edema, which can occur in the subcutaneous and skin layers when closing the equine abdomen. Alternatively, suture materials with a high stiffness are required for abdominal closure, herniorrhaphy or joint imbrications (Kummerle 2012).

When choosing the ideal suture material for a given closure, the fiber's structure, absorption properties and tensile strength should be considered. The following describes the major properties of the suture materials currently in use:

Surgical gut is formed from collagen obtained from bovine intestinal serosa or ovine intestinal submucosa and treated with a chromic salt solution. It is a multifilament structure which creates a moderate inflammatory reaction in tissue as it is broken down by proteolytic enzymes and phagocytosed by macrophages. It has a lower degree of tensile strength than the majority of synthetic absorbable sutures, with the exception of polyglytone 6211. Surgical gut is inexpensive and has good handling characteristics unless it has been treated with a chromic salt solution (Kummerle 2012).

Polyglactin 910 (Vicryl) is frequently used in the closure of the equine linea alba (Kummerle 2012). It is a copolymer of glycolide and L-lactide and is frequently coated with polyglactin 310 and calcium stearate. Polyglactin 910 is reabsorbed within 56-70 days, which, due to the healing rate of the linea alba, is appropriate in closure this layer. Polyglactin 910 loses 25% of its tensile strength after 14 days. A further 50% of the tensile strength is lost at 21 days and by 35 days, the suture retains none of its tensile strength. Polyglactin 910 has a good tensile strength with a greater initial stiffness than polydioxanone. Polyglactin 910 causes minimal tissue reaction and has excellent handling properties. As a multifilament suture, increased tissue drag can cause polyglactin 910 to cut through friable tissue. While this is usually not a concern in an

uncomplicated ventral midline celiotomy closure, it can make this suture material a poor choice for wounds where the tissue is friable (Kummerle 2012).

Polyglycolic acid (Dexon) is a polymer of glycolic acid, which can be coated with polycaprolate. This multifilament suture is reabsorbed in 60-90 days and undergoes a tensile strength reduction of 35% by day 14 and 65% by day 21. Polyglycolic acid has good handling characteristics but tends to drag through tissues. It has a lower knot holding capacity than polyglactin 910, which can be of particular consequence in closure of the linea alba (Kummerle 2012).

Braided lactomer (Polysorb) is a copolymer of glycolide and lactide, coated with a mixture of caprolactone, glycolide copolymer and calcium stearoyl lactylate. This multifilament suture undergoes reabsorption in 56-70 days. This material shows excellent tensile strength characteristics, and in this respect is superior to polyglactin 910. Although tensile strength is initially excellent, this decreases to 80% at day 14 and 30% at day 21. This suture material has good knot security and excellent handling properties (Kummerle 2012).

Glycomer 631 (Biosyn) is a combined polymer of glycolide, dioxanone and trimethylene carbonate. This monofilament suture is reabsorbed within 90-110 days. The tensile strength provided is 75% of the minimum knot strength requirements at day 14 and 40% at day 21 (Kummerle 2012). Unusual for a monofilament suture, Glycomer 631 has minimal memory and excellent handling properties; it also invokes minimal tissue reaction. However, its poor knot security makes this an inappropriate choice in closure of the linea alba (Kummerle 2012).

Polyglytone 6211 (Caprosyn) is a copolymer of glycolide caprolactone, trimethylene carbonate and lactide. This is a monofilament suture that is reabsorbed completely within 56 days, with 50% of its tensile strength being lost within 2 weeks. All tensile strength is lost within 21 days. This suture material is only beneficial where short-term tensile strength followed by reabsorption is required and is an inappropriate choice in closure of the equine linea alba (Kummerle 2012).

Polydioxaone (PDS II) is frequently used in closure of the equine linea alba (Kummer 2012). It is formed from a polymer of poly-*p*-dioxanone and a monofilament suture. It is reabsorbed in 180 days, undergoing a tensile strength reduction of 25% at day 14, 30% at day 28 and 50% at day 42. Polydioxanone maintains tensile strength over a prolonged period and has less memory than polyglyconate. However, this suture remains moderately difficult to handle, and provides only moderate knot security due to its high memory. Although rare, polydioxanone has been noted to have discrepancies in its strength (Lieurance *et al.* 2003), which may lead to suture breakage. However, despite these discrepancies, its maintenance of tensile strength over time makes polydioxanone a suitable material for closure of the equine linea alba (Kummerle 2012).

Polyglyconate (Maxon) is a copolymer of glycolide and trimethylene carbonate. It is a monofilament suture that undergoes reabsorption in 180 days. Its tensile strength is reduced by 25% at day 14, 50% at day 28, and 75% at day 42. The suture has a slow resorption and loss of tensile strength, resulting in polyglyconate being three times stronger than polyglactin 910 (which retains on 50% of its tensile strength) at day 21 of wound healing. Polyglyconate has good knot security, but significant memory and limited pliability make the suture material more difficult to handle (Kummerle 2012).

Polyglyconate (Monosyn) is a copolymer of glycolide, trimethylene carbonate and carprolactone. Its resorption time is 60-90 days, with a tensile strength reduction of 30% at day 7, 50% at day 14, and 80% at day 21. Although it is a monofilament suture, it has very good handling properties and provides good knot security (Kummerle 2012).

Poliglecaprone (Monocryl) is a copolymer of glycolide and caprolactone. It is a monofilament suture which has a resorption time of 90-120 days. It loses 50% of its tensile strength at day 7, and 80% by day 14. Complete loss of strength occurs at 21 days. The suture has very little tissue drag and only minimal memory, giving it excellent handling qualities. It invokes minimal tissue reaction and is rapidly reabsorbed. Although this suture material has many excellent properties, it's rapid loss of tensile strength make it an inappropriate choice of suture material for closure of the equine linea alba (Kummerle 2012).

There are also several non-absorbable materials, which although rarely used in the equine linea alba still merit discussion. Silk is a natural, braided material which can be coated or uncoated. It has good handling characteristics, especially when performing ligatures and is therefore used primarily in cardiac, ophthalmic or neurological procedures. Silk is able to maintain tensile strength for 6 months. Unfortunately, silk may potentiate infection and invokes a significant tissue reaction. It should therefore be avoided in contaminated sites (Kummerle 2012).

Surgical steel (Steelex) is an alloy of iron which can be used as a monofilament or multifilament twisted wire. It has the greatest tensile strength and knot security of any suture material used. It

also invokes no inflammatory reaction of the surrounding tissue, except by mechanical trauma where the sharp ends may touch tissues. It has poor handling characteristics and requires minimal manipulation to prevent breakage. It should also be noted that multifilament wire can fragment and migrate, leading to sinus tract formation (Kummerle 2012). However, despite the difficulties with handling of this suture, it has excellent strength and is therefore the material of choice in closure of the equine linea alba following dehiscence of the incision (Kummer 2012).

Nylon (Dafilon) is a polymer of polyamide and can be used as a monofilament or multifilament suture. It has intermediate tensile strength, with monofilament nylon losing 30% of it tensile strength after 2 years, and multifilament nylon losing complete tensile strength by 6 months. It is suitable for use in contaminated wounds as degradation products act as antibacterial agents. It has poor handling characteristics and poor knot security. It is not recommended for use within serous or synovial cavities due to the fact that buried sharp ends may cause frictional irritation (Kummerle 2012).

Polycaprolactam (Supramid) is a polymerized caprolactam which forms a multifilament structure with a polyamide coating. It has improved tensile strength compared to nylon and excellent handling properties, providing high knot security. There is intermediate tissue reactivity and the suture has a tendency to form sinuses on implantation in tissues and is therefore best suited for use in the skin (Kummerle 2012).

Polyester (Mersilene, Synthofil, Ethibond) is formed from polyethylene terephthalate and can be used as a monofilament suture or a multifilament suture. The multifilament suture is coated with

polybutilate, silicone or polyethylene/vinyl acetate. It retains its high tensile strength for over 2 years. Although non-coated polyester fibers have a high coefficient of friction, coating dramatically decreases the knot security, which has significant consequences in wounds or incisions under tension. This suture also invokes a marked tissue reaction and should therefore not be used in contaminated wounds (Kummerle 2012).

Ultra-high molecular weight polyethylene (FiberWire) is a multifilament suture with a polyethylene/polyester coating. It has superior strength compared to many other suture materials as it undergoes less elongation under tension than polyester sutures. It has high abrasion resistance and good knot security, with less tissue drag than polyester sutures (Kummerle 2012). Although its rate of loss of tensile strength has not been reported, Wright (2009), reported FiberWire to have a higher strength than stainless steel sutures of the same gauge.

Polypropylene (Premilene, Prolene) is formed from polyolefin plastic. It is a monofilament suture with moderate tensile strength. It has the greatest knot security of all synthetic monofilament suture and is the least thrombogenic suture, making it useful for vascular surgery. It causes minimal tissue reactivity and it least likely to potentiate infection. However, its memory makes suture handling and knot tying difficult (Kummerle 2012).

Polybutester (Novafil) is a copolymer of butylene, terephthalate and polytetramethylene ether glycol. It is a monofilament suture with good handling characteristics and knot security. It is flexible and elongates under wound tension but has memory to return to its original form. It

invokes minimal tissue reaction and is therefore beneficial in edematous tissue (Kummerle 2012).

Many suture materials have been evaluated for closure of the equine linea alba. Nylon has been used for this procedure, however, due to its low breaking strength, it is not ideal for use (Trostle *et al.* 1994). Large gauge polyester (5 USP) has been used following ventral midline celiotomy. Although the large gauge suture maintains good tensile strength, it has poor knot security and is therefore not recommended for this procedure. Although 0 USP stainless steel suture and polypropylene suture provide excellent tensile strength and have been used in dogs (Kirpensteijn *et al.* 1993), the large gauges (2 and 3 USP) required in horses makes handling of the suture and knot tying more difficult. Stainless steel suture is therefore primarily used for complicated incisional closures, for instance, following incisional dehiscence (Kummerle 2012). Polyglactin 910 and polydioxanone remain the most common suture materials used in closure of the equine linea alba (Mair *et al.* 2007). The most useful properties of polyglactin 910 are that it is an absorbable suture with high tensile strength, good handling properties and excellent knot security. Polydioxanone is also an absorbable suture material used in closure of the equine linea alba. Polydioxanone maintains its tensile strength longer than polyglactin 910, however, it has reduced knot holding capacity and only moderate handling abilities (Kummerle 2012).

ii. Suture Size

The suture gauge is the cross-sectional diameter of the suture. The units of measurement are the United States Pharmacopoeia (USP). This method uses 0 (3.5mm) as the baseline suture size. For

suture materials larger than 0, the numbers 1, 2, 3, etc. are applied. For materials smaller than 0, the sizes are termed 2-0, 3-0, 4-0, etc. as the size decreases (Kummerle 2012). The European Pharmacopoeia uses the metric system and suture size is expressed as 1, 2, 3, 4, etc., corresponding to 1/10 of the suture diameter in mm (Kummerle 2012). Sizes of suture range from 11-0 USP and 0.1 metric (0.010-0.019 mm diameter) to 8 USP and 10.0 metric (1.000-1.099 mm diameter).

The size of suture material selected is based on the tensile strength of the suture material required, as well as the fragility and strength of the tissue being sutured. Suture material which is overly large may weaken the repaired wound by causing excessive tissue reaction. For incisions or wounds under tension, increasing the number of sutures placed has a less detrimental effect on the tissue whereas increasing the gauge of the suture has a greater detrimental effect (Celeste and Stashak 2008; Kummerle 2012). Another important implication of the suture diameter is that knot security decreases as the suture gauge increases. It is thought that this is likely to be due to the fact that large gauge suture is more difficult to cinch down into a tight knot (Mulon *et al.* 2010; Schubert *et al.* 2002). In closure of the equine linea alba, the choice of suture gauge will depend on the suture's tensile strength, the tissue reaction surrounding the suture and the degree of knot security.

iii. Suture Pattern

Suture patterns are either interrupted (multiple, single sutures, each tied with a knot) or continuous (one suture used to close the entirety of the incision with a knot at each end).

Interrupted patterns theoretically have increased security because failure of one knot does not jeopardize the whole suture line. However, this has been disputed in a variety of tests, including tests in which the equine intra-abdominal pressure is increased using a polyurethane bladder (Rosin 1985; Kummerle 2012; Magee and Galuppo 1999). Another advantage of an interrupted pattern is that it is less disruptive to the blood supply of the tissue. Further advantages which are less applicable to the linea alba are that irregular wound margins can be more precisely reconstructed using interrupted sutures, providing accurate control of tension at each point of the wound margin is possible (Moy 1992). Again, although not applicable to the linea alba, should drainage of a wound be necessary, one or two interrupted sutures can be removed to allow escape of fluid without disrupting the whole line (Kummerle 2012). Double stranded sutures are rarely used in equine surgery, however, Hassan *et al.* (2006) found no difference in incisional bursting strength when loop sutures were used to close the equine linea alba, when compared to inverted cruciate and simple continuous patterns (Magee and Galuppo (1999). It should be recognized that although these studies were conducted using the same experimental method, and largely by the same authors, they were separate investigations and cannot be directly compared. Conversely, when assessing 3-0 and 4-0 polyamide sutures, Netscher *et al.* (2015) found double stranded tendon repair to have a significantly lower force to failure than single strand repair. Based on this evidence, double stranded sutures should be used with caution in the equine linea alba.

There are several advantages to performing a continuous suture pattern. A continuous suture pattern places a smaller volume of suture material and fewer knots in the tissues, thereby reducing the tissue reaction and likelihood of incisional drainage (Richey and Rose 2005;

Israelsson 1994; Brown 1992). Due to the fact that only two or three knots are placed when performing a continuous suture pattern, the surgery time is significantly reduced, often by as much as 50% (Fossum 2007). A continuous suture pattern also distributes the tension along the whole incision, providing an increased incisional bursting strength (Magee and Galuppo 1999) and a tighter seal (Kummerle 2012).

Suture patterns can also be classified as appositional, inverting or everting. For the purposes of the linea alba, the suture pattern placed is generally everting or appositional (Kummer 2012). Appositional sutures are beneficial as they provide an anatomically precise closure (Kummerle 2012).

iv. Knot Type

An *ex vivo* evaluation of simple continuous suture patterns used in closure of ventral midline celiotomies showed that 71% of suture lines failed at the knot, as opposed to breakdown of the tissues and suture pull through (Magee and Galuppo 1999). A further *ex vivo* study showed that when a sutured incision fails, 98.7% of the time it is the suture rather than the tissue that tears, with 90.4% of these disruptions occurring at the knot-suture junction (Fierheller and Wilson 2005). This suggests the knot may be a limiting factor for a secure celiotomy closure. The optimal suture pattern and knot technique that minimizes the risks of dehiscence, infection and herniation of the linea alba in the horse has not been established (Fierheller and Wilson 2005). However, the ideal closure would consist of a knot which is both secure, and supports a high load to failure (Schaaf *et al.* 2009). It has been shown that a knot which is incorrectly tied, is

more prone to slippage (Ching *et al.* 2013). It is therefore particularly important that the knot is tied correctly as to prevent weakening of the knot and potential incisional dehiscence (Kummerle 2012; Schaaf *et al.* 2009; Ching *et al.* 2013).

To form a correct square knot, at least two throws are placed on top of each other and the direction of the suture ends is reversed between each throw. If the direction is not reversed, this forms an asymmetrical ("granny") knot, which is incorrect and weaker (Kummerle 2012; Mulon *et al.* 2010). Another error that can be made in knot placement is reversal of throw direction combined with pulling mainly on one strand of suture, which results in a half hitch, which is also weaker and more prone to slippage than a square knot (Kummerle 2012; Mulon *et al.* 2010).

A superimposition of square knots is considered to be a reliable knot configuration (Fossum 2007). However, when the wound edges are under tension, for example when closuring the equine linea alba, a surgeon's knot can be tied (Kummer 2012). A surgeon's knot can be more successful when holding the wound edges in apposition, however, it places more suture material in the wound than a square knot and can invoke a greater tissue reaction. In the majority of studies, there was no significant difference between the knot holding capacity of surgeon's and square knots (Rosin and Robinson 1989). However, Huber *et al.* (1999) found surgeon's knots to have altered structural properties in non-absorbable monofilament sutures.

Instrument ties are more commonly used than hand ties for the majority of knots as it results in less suture wastage (Kummerle 2012). Knots formed at the start of a continuous suture line are tied in the same way as an interrupted suture. In forming a knot at the end of a continuous suture

line, a loop of suture is used to tie the knot. It is essential to use the instruments to grasp the loop in its center to avoid asymmetric loads placed on either side of the loop and the formation of a weaker, asymmetric knot (Kummerle 2012, Schaaf *et al.* 2009).

In human surgery, to form a knot which is both secure and has a low volume, self-locking knots have been applied (Stott 2001). A possible substitute for a square or surgeon's knot at the start of a continuous suture line is the forwarder knot. The forwarder knot is a self-locking knot consisting of unique throws that is used to secure the start of a continuous suture line (Figure 1) (Perez *et al.* 2014). To form a forwarder knot, a bite is taken through the tissue first, before holding the needle holders and the working end of the suture adjacent to each other. The working end of the suture is then turned around the needle holders and standing end of the suture. The number of times this is performed depends on surgeon preference. The working end of the suture is then grasped by the needle holders through the placed loops. The needle holders are then withdrawn, pulling the working end of the suture through the loops and locking the knot.

The forwarder knot has been applied successfully in human surgery, and is anecdotally preferred in certain laparoscopic surgeries to conventional square knots by some surgeons (Perez *et al.* 2014). Recently, the forwarder knot has been described in suture reinforcement of the laparoscopic gastric sleeve staple line in human bariatric surgery (Daes 2013). The surgeons who employ this knot anecdotally report that the use of self-locking knots reduces the likelihood of fatal gastric leakage following this surgery, with no reported incidents since this knot was used (Perez *et al.* 2014). The forwarder knot can be performed using either a sliding or a non-sliding

technique (Perez *et al.* 2014; Daes 2013). The sliding technique provides clear advantages for laparoscopic surgery, allowing for extracorporeal knot tying when this knot is used. At the time of this report, the forwarder knot has not been reported in use in veterinary surgery, and has not been tested either *in vivo* or *in vitro* under controlled conditions using suture commonly used in closure of equine ventral midline celiotomies. A search of the literature also was not able to show any report of comparison between the strength of forwarder knots and surgeon's or square knots.

Although the forwarder knot is a possible knot for starting a continuous suture line, another self-locking knot can be used to end a continuous suture line. The Aberdeen knot, has been used in human surgery for completing gastric closures and small animal surgery for ending continuous suture patterns in the subcutaneous tissue and skin (Perez *et al.* 2014; Schaaf *et al.* 2009). However, this knot also has not yet been used in equine surgery and has not been evaluated in large gauge suture materials.

The Aberdeen knot is a self-locking knot consisting of a combination of unique throws and turns that is used to secure the end of a continuous suture line (Figure 2) (Stott 2009). To form an Aberdeen knot, a loop is formed in the suture and passed through the tissue on each side of the incision line. A second loop is then passed through the first loop. This is termed one throw. This step can be repeated any number of times to achieve the desired number of throws. To lock the

knot, an additional throw called the turn is performed by passing the end of the suture through the final loop. The turn can be repeated depending on surgeon preference.

The Aberdeen knot has been applied successfully in human and small animal surgery, and was shown to be stronger and smaller than square knots using 2-0 polydioxanone (Schaaf *et al.* 2009). At the time of this report, the Aberdeen knot has not been reported in use in equine surgery, and has not been tested in knots using suture materials larger than 1 USP polyglyconate (Fong 2008). Comparisons have been made between Aberdeen and square knots in 2-0 polydioxanone (Schaaf 2010), with the Aberdeen knot having a relative knot security of 81.08% and square knots have a relative knot security of 56.69%. No comparison has been made to evaluate the differences in strength and security of Aberdeen knots versus surgeon's knots.

v. Size of Knot

There are several factors related to the strength of a knot, with one of the most important factors being knot security. Knot security is defined as the effectiveness of the knot at resisting slippage when load is applied (Kummerle 2012; Alzacko and Majid 2007). Knot security is related to the structural configuration of the knot and the type of suture material (Schubert *et al.* 2002). Suture memory and the coefficient of friction both affect knot security but it has been well documented that body fluids can coat the surface of the suture material and affect the coefficient of friction and therefore knot security (Gupta *et al* 1985; Schaaf *et al.* 2009).

The number of throws and suture end length also affect knot security. It has been shown that a suture end length of at least 3 mm is recommended to optimize knot integrity (Mazarrese *et al.* 1997; Muffly *et al.* 2009). It has also been shown that greater than 3 mm of slippage of a knot is associated with increased incisional complications due to increased motion between the apposed tissue edges (Fugazzi *et al.* 2013). The minimal number of throws needed to form a secure square knot using 2-0 USP suture is three for polyglycolic acid, polyglactin 910 and polypropylene and four for nylon and polydioxnanone (Rosin and Robison 1989; Schaaf *et al.* 2010). Muffly *et al.* (2011) demonstrated that a square knot with five throws provided a secure knot in 0 USP polyglactin 910 and 2 USP polydioxanone, however, other numbers of throws were not evaluated. The work by Muffly *et al.* involved knots at the start of the suture line. It has also been shown that knots at the end of a continuous suture line require either one, two or three additional throws compared to the start of a suture line to achieve knot security (Schaaf *et al.* 2009; Rosin and Robinson 1989). Therefore, the position of the knot has an effect on the number of throws required.

It is essential that the knots tied are correctly formed. Incorrectly tied knots require additional throws to be placed to achieve a higher security. When knots such as a half-hitch or asymmetric "granny" knot are formed, they require two additional throws to achieve knot security in polyglactin 910 (Rodeheaver *et al.* 1988). However, this characteristic is dependent on the suture material used, as the same configuration of asymmetric knots in braided lactomer did not require additional throws to achieve knot security (Rodeheaver *et al.* 1988).

Another factor to consider is the environment the suture is placed in. A wound environment containing abundant amounts of lipid can increase the number of throws needed to achieve a secure knot. Schaaf *et al.* (2010) found that one additional throw was required in fat coated suture to form a secure knot at both the start and end of a continuous suture line, in 2-0 USP polydioxanone, when compared to plasma coated suture.

d. Techniques in Ventral Midline Celiotomy

<u>i. Techniques for Closing a Ventral Midline Celiotomy Incision in Humans</u>

<u>1. Suture Material</u>

The choice of suture material used should be related to the rate of abdominal healing (Ceydeli *et al.* 2005). In human surgery, if the abdominal closure is performed through the linea alba, it has only 51-59% of its original strength at 42 days post-operatively. After 120 days, the abdominal wall is at 70-80% of its preoperative strength. By 140 days, this has increased to 73-93%. This is in contrast to the equine linea alba, where there is no significant difference between incised and non-incised linea alba at 28 days (Chism 2000). In humans, the abdominal wall does not regain more than 93% its original strength, regardless of the post-operative duration. (Rath and Chevrel

1988; Douglas 1952). The chosen suture material is, therefore, required to maintain strength for several months.

In human surgery, non-absorbable suture materials are frequently used as they maintain their tensile strength while the abdominal wall regains its strength. The most common materials used are polyethylene, polypropylene, polyamide and nylon (Ceydeli *et al.* 2005). Silk and stainless steel wire have been used historically, but due to handling difficulties, high infection rates and inflammatory reactions, are rarely used today (Ceydeli *et la.* 2005; Bucknall *et al.* 1983; Bucknall 1983; Sharp *et al.* 1982). Unlike silk suture, multifilament, non-absorbable sutures retain their tensile strength but are more susceptible to bacterial colonization compared to monofilament or absorbable sutures (Bucknall *et al.* 1983; Bucknall 1983; Sharp *et al.* 1982) and are therefore rarely the suture of choice for closure of the abdominal wall (Ceydeli *et al.* 2005). Monofilament non-absorbable sutures have less tissue reactivity and are less susceptible to infection than absorbable suture (Ceydeli *et al.* 2005). These suture materials are a logical choice for closure of the abdominal wall in human surgery. Unfortunately, these materials are also associated with increased risk of sinus formation, post-operative pain and button-hole hernias (Wissing *et al.* 1987; Bucknall *et al.* 1983; Bucknall 1983; Sharp *et al.* 1982; Krukowski *et al.* 1987; Larson *et al.* 1989; Corman *et al.* 1981; Knight *et al.* 1983). Due to these complications, monofilament, absorbable sutures are the most appropriate choice in closure of the human linea alba (Ceydeli *et al.* 2005).

Multiple studies in human surgery have investigated the effect of using non-absorbable versus absorbable suture material. While several studies found a lower rate of incisional complications

(Bucknall *et al.* 1982; Wissing *et al.* 1987; Bucknall and Ellis 1981; Askew 1983; Lewis and Wiegand 1989), a greater number found no significant difference in the incidence of incisional infection or hernia formation when absorbable versus non-absorbable sutures were used (Gys and Hubens 1989; Pollock *et al.* 1979; Rengard *et al.* 1988; Cameron *et al.* 1980; Corman *et al.* 1981; Krukowski *et al.* 1987; Donaldson *et al.* 1983; Ellis *et al.* 1982; Hoffman *et al.* 1991). It has, however, been suggested that although non-absorbable suture material may not give rise to an increased incidence of herniation in the immediate post-operative period, it may result in the formation of late 'button-hole' hernias, where the hernia forms in a narrow slip, which can form more than one year following the procedure (Carlson *et al.* 1995). Button-hole hernias can form due to the non-absorbable suture tearing through fascia during the remodeling phase of wound healing (Read and Yoder 1989; Krukowski and Matheson 1987). The use of nylon, due to its absorbable nature, appears to result in an increased incidence (up to 9%) of suture sinus formation due to the suture material sawing through the fascia (Bucknall and Ellis 1981; Wissing *et al.* 1987). Conversely, the use of polyglactin and polyglycolic acid does not appear to predispose to suture sinus formation, with the incidence being as low as 0.2% in a retrospective study (Carlson *et al.* 1995; Bucknall and Ellis 1981; Wissing *et al.* 1987; Corman *et al.* 1981).

The benefit of absorbable suture materials is that they approximate the healing muscle tissues early on in inflammatory and proliferative phases of wound healing but are then reabsorbed before complications such a suture sinus formation can occur (Ceydeli *et al.* 2005). Absorbable suture material has been shown to have a significantly decreased incidence of sinus formation and chronic incisional pain when compared to non-absorbable suture (Rucinski *et al.* 2001; Bucknall *et al.* 1983; Larsen *et al.* 1989; Corman *et al.* 1981). Absorbable suture materials are

therefore more commonly used in closure of the human abdomen than non-absorbable sutures (Ceydeli *et al.* 2005).

Polyglycolic acid and polyglactin 910 are the most commonly used, rapidly absorbable suture materials in closure of the human abdomen (Ceydeli *et al.* 2005). Resorption time for these materials is 60-90 and 56-70 days respectively, however, the tensile strength of both suture materials is markedly reduced by 14-21 days, with the tensile strength of the suture materials be reduced by 35% and 25%, respectively, at 14 days (Kummerle 2012). Both polyglycolic acid and polyglactin 910 are less reactive than chromic gut or silk because they are absorbed by hydrolysis (Kummerle 2012). Absorbable suture material, including polyglactin 910, in some studies, has been associated with an increased incidence of hernia formation (Rucinski *et al.* 2001; Luijendiik 2000; Wissing *et al.* 1987). This may be due to the fact that, braided suture materials have a greater likelihood of becoming a nidus for infection and monofilament materials. Infection is known to be associated with other incisional complications (Weiland *et al.* 1998; van't Riet *et al.* 2002; Rucinski *et al.* 2001; Hodgson *et al.* 2000; Bucknall 1983).

Polydioxanone and polyglyconate are the most commonly used slowly absorbable sutures used in closure of the human abdomen (Ceydeli *et al* 2005). Absorption of these suture materials takes 180 days and they retain 50% of their tensile strength for 28 days (Kummerle 2012). These suture materials are absorbed slowly by hydrolysis and are less susceptible to bacterial colonization due to their monofilament nature (Ceydeli *et la.* 2005). Currently, there is no evidence suggesting any differences in the incidence of hernia formation, incisional infection or incisional dehiscence between polydioxanone or polyglyconate and non-absorbable suture

materials in human surgery (Weiland *et al.* 1989; van't Riet *et la.* 2009; Rucinski *et al.* 2001; Hodgson *et al.* 2000). However, multiple prospective studies, as well as meta-analyses, show slowly absorbable suture material to be associated with lower rates of chronic incisional pain and suture sinus formation when compared to non-absorbable suture (Ceydeli *et la.* 2005; Bucknall *et al.* 1982; Wissing *et al.* 1987). Therefore slowly absorbable suture, which is absorbed over approximately 180 days, is recommended for closure of the human linea alba.

2. Suture Gauge

In human and veterinary surgery, as the suture gauge increases, the tensile strength and knot holding capacity of that suture increases (Muffly *et al.* 2013). Most studies in the literature evaluate 0 or 1 USP suture. The gauge of the suture material is important for several reasons. First, the smaller the suture gauge, the more likely the suture is to break. Also, smaller gauge sutures are more prone to tearing through the fascia (Ceydeli *et al.* 2005). Conversely, larger gauge sutures place more foreign material within the suture line, increasing the incidence of tissue reaction (Wallace *et al.* 1980; Gallup *et al.* 1980). Interestingly, Larsen *et al.* (1989) found no significant difference between the rate of incisional dehiscence when 2-0 suture, compared to 0 USP suture was used to close the fascia. It is therefore preferable to use the smallest gauge of suture that will provide high knot security when challenged with the physiologic pressures of the human abdomen.

3. Suture Pattern

In human patients, a continuous suture line remains the preferred method of closure for the linea alba. One reason for this is that abdominal distention in humans can cause a 30% increase in wound length (Jenkins 1976). A simple continuous suture pattern is more able to accommodate lengthening of the linea alba (Poole 1984; Poole *et al.* 1985; Jenkins 1976). Although this is yet to be proven in horses, this factor should be considered in equine patients. Wound lengthening during periods of increased intra-abdominal pressure can occur in humans and it is therefore recommended that the suture used is four times the length of the incision, to accommodate for any wound stretching that occurs (Jenkins 1967).

Although there are benefits for performing a continuous suture pattern, in human surgery, there has no evidence suggesting that this creates and improved outcome for the patient (Irvin *et al.* 1977; Wissing *et al.* 1987; Fagniez *et al.* 1985; Orr *et al.* 1990; Richards *et al.* 1983; Sahlin *et al.* 1993; Cleveland *et al.* 1989; McNeill and Sugarman 1986). However, the simple continuous closure technique is more rapid, requiring half the time and less suture material (Carlson *et al.* 1995; Weiland *et al.* 1988; Cleveland *et al.* 1989; McNeill and Sugarman 1986; Fagniez *et al.* 1985; Richards *et al.* 1983; Gislason *et al.* 1995; Trimbos and Rooji 1995; Colombo *et al.* 1997; Brolin 1996; Trimbos *et al.* 1992; Sahlin *et al.* 1993). A further advantage to performing a continuous closure technique is that this technique has greater incisional bursting strength in *ex vivo* studies compared to a simple interrupted closure (Rodeheaver *et al.* 1987; Poole *et al.* 1984). Similar findings have been shown *ex vivo* in horses (Magee and Galuppo 1999). A simple continuous suture pattern will also result in less foreign material in the incision, reducing the incidence of tissue reaction, infection and sinus tract formation (Ceydeli *et al.* 2005). These benefits are supported by the result of four meta-analyses, showing a simple continuous closure

33

results in a lower rate of incisional hernia formation compared to simple interrupted closures (Weiland *et al.* 1988; van't Reit *et al.* 2002; Rucinski *et al* 2001; Hodgson *et al.* 2000).

When using only one strand of suture in a continuous suture pattern, it has to be a consideration that disruption of one of the knots or the suture can have catastrophic consequences for the suture line. However, in contrast to equine patients (Magee and Galuppo 1999), the majority of incisional failures in human patients are due to tearing of the muscle rather than disruption of the suture line (Gislason *et al.* 1995; Alexander and Prudden 1996).

In order to avoid tying a knot at the start of a continuous suture line, a double loop closure method (involving doubling the suture strand so a loop is formed at one end of the suture in place of the knot) can be employed. It has been shown that the double loop closure method provides increased incisional strength, however, one study reports an increased incidence of post-operative death from pulmonary complications (Niggebrugge *et al.* 1999). This was hypothesized to be due to decreased compliance of the abdominal wall, therefore, a double loop may not be the ideal method for closure of the linea alba. Double stranded sutures are rarely used in equine surgery, however, Hassan *et al.* (2006) found no difference in incisional bursting strength when loop sutures were used to close the equine linea alba, when compared to inverted cruciate and simple continuous patterns (Magee and Galuppo (1999). It should be recognized that although these studies were conducted using the same method, largely by the same authors, they were separate investigations.

Although many human surgeons prefer to perform a layered closure (Carlson *et al.* 1995), a mass closure incorporating all layers of the abdominal wall with the exception of the skin, is becoming more widely accepted, with a layered closure now being considered dated in human surgery (Ceydeli *et al.* 2005). The potential benefits of a layered closure include decreased abdominal adhesions, decreased leakage of abdominal fluid, increased hemostasis and increased wound strength (Brennan *et al.* 1987; Gilbert *et al.* 1987; Donaldson *et al.* 1982a; Donaldson *et al.* 1982b). A mass closure is not only faster than a layered closure, it also provides a decreased incidence of incisional dehiscence (Goligher *et al.* 1975; Blomstedt and Welin-Berger 1972; Ellis and Heddle 1977; Askew 1983). Bucknall *et al.* (1982) performed a prospective study where either a layered closure or a mass closure was performed. Patients receiving a layered closure had a significantly higher rate of incisional dehiscence (3.8%), compared to patients undergoing a mass closure (0.76%). However, there was no effect on the rate of hernia formation. Based on the results of two meta-analyses, mass closure is considered a favorable option in human surgery (Weiland *et al.* 1998; Rukinski *et al.* 2001). The mass closure technique has not been employed in equine patients. It is hypothesized that movement of the different layers during ambulation of the patient would place additional strain of the suture material. Currently the linea alba is closed separately to the skin. The subcutaneous tissues may also be closed if desired (Coomer *et al.* 2005).

Debate has previously existed as to whether or not the peritoneum should be closed (Ceydeli *et al.* 2005). However, following multiple investigations, it has been determined that closure of the peritoneum results in an increased incidence of abdominal adhesions (Lewis and Wiegand 1989) and an increase in the duration of surgery time (Hugh *et al.* 1990; Kendall *et al.* 1991; Stark

1993; Ellis and Heddle 1977; Chana *et al.* 1993; Spencer and Akuma 1988; Kieley and Spitz 1985; Hoerr *et al* 1951; Humphries *et al.* 1964; Leaper *et al.* 1977; Irvin *et al.* 1977). Current evidence regarding closure of the peritoneum is conflicting in horses with some reports recommending closure using 0 chromic gut (Becker 1985; Boles 1975) and other reports suggesting the closure is not necessary (Swanwick *et al.* 1973). Currently, the majority of surgeons choose not to close the peritoneum as this results in a more lengthy closure of the abdomen (Kummer 2012).

Retention sutures are a method used to suture the entire abdominal wall to the subcutaneous tissues and skin. The proposed benefit is that these sutures provide additional security (Ceydeli *et al.* 2005). However, it has been shown that these sutures not only fail to reduce the incidence of incisional dehiscence but are also associated with increased post-operative pain (Wasijew and Winchester 1982). For these reasons, retention sutures have largely fallen out of favor in the last decade (Ceydeli *et al.* 2005). Although retention sutures can be used in the management of infected incisions or hernias in equids, their use is uncommon (Kummer 2012).

4. <u>Incision</u>

There are several risk factors for incisional complications in human patients. As with all species wound infection increases the risk of incisional dehiscence and hernia formation (Blomstedt and Welin-Berger 1972; Bucknall *et al* 1982; Greenall *et al.* 1980; Irvin *et al.* 1977). Male patients have a predisposition to hernia formation and incisional dehiscence (Bucknall *et al* 1982; Greenall *et al.* 1980). As expected, increased intra-abdominal pressure is also related to an

increased incidence of incisional hernia formation (Bucknall *et al.* 1982; Bucknall and Ellis 1981; Pollock *et al.* 1979). Additional variables related to abdominal distention, including obesity (Bucknall *et al.* 1981; Renegard *et al.* 1988) and pleuritis (Bucknall *et al* 1982; Greenall *et al.* 1980; Pollock *et al.* 1979; Bucknall and Ellis 1981) are also related to an increased incidence of incisional complications. As in veterinary surgery, abdominal surgery performed on an emergency basis has been found to be a risk factor in humans for the occurrence of a range of complications, including incisional infection and herniation (Renegard *et al.* 1988). This is particularly pertinent where equine colics are considered, as the vast majority of procedures are performed on an emergency basis. Similar to equine patients (Mair *et al.* 2007), Renegard *et al.* (1988) found that performing a second laparotomy prior to discharge of the patient resulted in an increased risk of incisional complications.

It has been shown that in human patients, a ventral midline incision yields a higher rate of ventral hernia formation than paramedian incisions (Carlson *et al.* 1995; Blomstedt and Welin-Berger 1972; Bucknall *et al* 1982; Renegard *et al.* 1988; Ausobasky *et al.* 1985; Cox *et al.* 1986; Wissing *et al.* 1987; Cameron *et al.* 1980; Corman *et al.* 1981; Krukowski *et al.* 1987; Hugh *et al.* 1990; Kendall *et al.* 1991). A comparison of the incidence of incisional hernias due to incision location in equine patients has not been performed. However, in contrast to human studies, it has been shown that ventral midline celiotomies have a decreased rate of complications overall in comparison to right paramedian incisions (Mair *et al.* 2007).

In human patients, the use of a transverse abdominal incision may result in a lower rate of incisional complications than a longitudinal incision (Blomstedt and Welin-Berger 1972),

however, more recent studies show minimal difference in the outcome between longitudinal and transverse incisions (Bucknall *et al* 1982; Greenall *et al.* 1980; Pollock *et al.* 1979; Ausobasky *et al.* 1985). Transverse incisions have not been recorded in equine patients. It is hypothesized that the orientation would provide inadequate access to the abdomen and tension on the muscle layers during closure would result in wound dehiscence or failure to achieve closure.

ii. Techniques for Closing a Ventral Midline Celiotomy Incision in Small Animals

1. Suture Material

Similar to other species, the canine linea alba remains at only 20% of its pre-operative strength for the first two weeks following surgery (Miller 1970). The suture materials of choice when closing the canine linea alba are slowly absorbable monofilament suture materials, namely polydioxanone, polyglyconate and polypropylene (Slatter 2003). Although cost effective, chronic gut is now rarely used in closure of the linea alba as it's decreased tensile strength make it inferior to other suture materials for this purpose (Slatter 2003; Bellenger and Meek 1990). When polydioxanone and polypropylene were used to close ventral midline celiotomies of rats, there was no difference between the strength of the closure using both suture materials at 1, 2 and 6 months post-operatively, making both materials equally suitable choices for closure of the linea alba in this species (Kon *et al.* 1984). Poliglecaprone is unsuitable for closure of the linea alba as it loses 50% of its tensile strength within 7 days, making dehiscence of the incision more likely (Kummerle 2012). Stainless steel has been used as an alternative to suture material,

however, its poor handling characteristics and the time consuming nature of suture placement mean that it is rarely used for uncomplicated closures (Kirpenstein *et al.* 1993).

Another important consideration when selecting suture material is the tissue reaction elicited by each material. In a study evaluating the use of non-absorbable suture in the canine linea alba, stainless steel and nylon resulted in the least degree of tissue inflammation. In the same study, granulomas were observed around fragments of polypropylene suture, and in clinical cases this has been shown to lead to drainage and incisional discomfort (Wood *et al.* 1984). Polyamide elicited slightly greater reactions and the most severe reactions occurred with silk (Wood *et al.* 1984). A comparison of polyglactin 910 and polydioxanone showed comparable tissue reaction in the linea alba of cats at days 1, 7, and 14 post-operatively for both suture materials (Freeman *et al.* 1987). Similar to equine patients, both polyglactin 910 and polydioxanone can be used in closure of the canine linea alba, however, polydioxanone is more slowly absorbable and maintains it tensile strength for an additional three months, which can be beneficial in some cases (Kummerle 2012).

2. Suture Gauge

The suture gauge chosen can vary depending on the size of the patient. As the majority of incisions in small animals fail at the tissue, or due to suture pull out, breakage of the suture is a rare source of incisional failure (Slatter 2003). It has also been shown that a smaller suture gauge is more likely to undergo suture tear out through the muscle (Slatter 2003). Conversely, large gauge suture causes increased tissue trauma and results in an increased quantity of foreign

material within the incision (Kummerle 2012). Therefore, the suture gauge should be selected to minimize tissue trauma, but should not be of such a small gauge that it can cut through the tissue of the linea alba. It is also necessary that the suture has sufficient tensile strength to withstand physiologic pressure. Therefore, it is widely accepted that 2-0 to 1 USP suture should be used in canine patients and 3-0 to 2-0 USP suture should be used in feline patients (Slatter 2003).

3. Suture Pattern

As with all domestic species, complications of a ventral midline celiotomy include infections, herniation and incisional dehiscence (Slatter 2003). The ventral midline is usually closed in three layers. Closure of the subcutaneous layer has been shown to reduce the incidence of incisional complications in dogs (Bellenger and Meek 1990) but there is conflicting evidence in cats (Freeman *et al.* 1987; Muir *et al.* 1993). The peritoneum is now rarely sutured closed (Slatter 2003), following the results of two *ex vivo* studies showing this had no effect on the bursting strength of incisions (Kapur *et la.* 1979; Karipineni *et al.* 1976).

Some surgeons prefer to close the canine linea alba in a simple interrupted suture pattern (Slatter 2003). However, there are now multiple reports of successful use of a continuous suture pattern using polypropylene and polydioxanone (Crowe 1978; Johnson 1989; Rosin 1985). A simple continuous pattern is now the most common method used by small animal surgeons (Johnson 1989). In an experimental model, there was no significant difference in incisional bursting strength between canine models closed in simple continuous, simple interrupted or simple cruciate patterns using polyglycolic acid (Poole *et al.* 1984). Similar to human patients, but in

contrast to equine patients, most closures of the linea alba dehisce at the tissue-suture interface rather than by suture breakage, suggesting that multiple knots are not required to create a secure closure (Poole *et al.* 1984).

Another aspect of suture placement is choosing which layers of linea alba are incorporated in the closure. In a case series of feline patients whose linea alba were closed incorporating only the external layer of the rectus sheath, ventral midline herniation occurred in only 1 out of 91 patients (Rosin 1985). In an *ex vivo* study involving 12 canine patients, where the linea albae were closed either with or without incorporation of the internal rectus sheath, no difference in the breaking strength of the wounds was found between the two groups (Rosin and Richardson 1978). Although the practice of including on the external layer of the rectus sheath is evidence based (Kummerle 2012), a further study has recommended closure of both the external and internal rectus sheaths cranial to the umbilicus where the linea alba is thinner (Johnson 1989).

It is currently recommended to place sutures 5-10 mm from the incision edge in canine patients (Slatter 2003). The reason for this is extrapolated from human studies, where placement of sutures between the linea alba and rectus sheath resulted in a 50% increase in the suture strength of the wound (Tera and Nberg 1976). The distance of 5-10mm from the incision line means that the rectus sheath is not penetrated.

Regarding suture placement, it is recommended to place the sutures 3-12 mm apart depending on the size of the patient (Slatter 2003). This recommendation is based on the fact that sutures

placed close together increase the tensile strength of the wound (Tera and Nberg 1976) but will also be detrimental to the blood supply of the tissues (Poole 1987).

4. Knot Type and Knot Size

The majority of surgeons prefer to place square or surgeon's knots when closing a ventral midline celiotomy in small animal patients (Fossum 2007). The number of throws selected is important, as the surgeon should choose the minimum number of throws to produce a secure knot. This ensures that maximum knot holding capacity and also minimum knot size are achieved, so as not to increase foreign material within the incision (Israelsson 1994). Previous work using a mechanical testing model illustrated that polydioxanone 3-0 suture requires four throws to form a secure simple interrupted suture (Schaaf *et al.* 2009). When using a similar model for a simple continuous suture pattern, 3-0 polydioxanone required 5 throws at the start of a continuous suture line and at least 6 throws at the end of a continuous suture line (Rosin and Robinson 1989).

In order to place a knot which is both secure and small, the Aberdeen knot has been used to end continuous suture lines (Regier *et al.* 2015). The Aberdeen knot has been shown to be both stronger and smaller than square knots formed in 2-0 USP polydioxanone (Schaaf *et al.* 2009). The Aberdeen knot has recently been shown to provide similar strength to square knots when used at the end of a continuous suture line in an *ex vivo* study using 4-0 polyglyconate (Regier *et al.* 2015). The same study revealed the knots to be smaller than their corresponding square knots.

The Aberdeen knot is, therefore, becoming an increasingly popular choice for a variety of small animal surgical procedures (Regier *et al.* 2015).

iii. Considerations for Closing a Ventral Midline Celiotomy Incision in the Horse

1. History of closure of the equine linea alba

Over recent decades, marked changes have occurred in the methods used to close the layers of the equine linea alba. Historically, Messervy (1969) discussed the use of 2 USP chronic gut to close the peritoneum in a continuous suture pattern. Closure of the peritoneum has since fallen out of favor due to the increased incidence of adhesions (White *et al.* 1999). Again, in 1969, Messervy reported the use of simple interrupted sutures using terylene polyester yarn to close the aponeurosis of the linea alba. The majority of surgeons currently choose to employ a simple continuous suture pattern due to the improved bursting strength and speed of application (Magee and Galuppo 1999). Messervy (1969) reported the use of 2 USP chronic gut to place simple interrupted subcuticular sutures, followed by closure of the skin with 3 USP monofilament nylon suture in a continuous horizontal mattress pattern. Chromic gut is now rarely, if ever, used for closure of the equine linea alba due to its low tensile strength (Kummerle 2012). Two and 3 USP suture is also rarely used to closure subcuticular and skin layers due to the large volume of suture material creating a possible nidus for infection (Kummerle 2012).

2. Incision

A longitudinal incision through the linea alba is the most common route by which surgical access to the abdomen is obtained (Kummer 2012). The linea alba is a fibrous structure which extends from the xiphoid to the pubic symphysis in humans and from the xiphoid to the pre-pubic tendon in horses (Kummer 2012). It contains the median fibrous raphe of the external and internal abdominal oblique and the transversus abdominus muscle aponeuroses and also separates the left and right rectus abdominis muscles (Dyce *et al.* 2010). The equine linea alba consists of dense connective tissue composed of sheets of cross-linked collagen bundles and fibroblasts and is largely devoid of blood vessels and nerves (Dyce *et al.* 2010). In the horse, the linea alba ranges in thickness from 10 mm at the umbilicus caudally, to 3 mm at the xiphoid cranially. The width of the linea alba is also greatest closer to the umbilicus but decreases in width closer to the xiphoid (Trostle *et al.* 1994).

The advantages of performing a ventral midline incision are improved accessibility, strength, and reduced hemorrhage. Only 25% of the abdominal gastrointestinal tract (stomach, duodenum, distal ileum, dorsal body and base of the cecum, distal right dorsal colon, transverse colon and terminal descending colon) is inaccessible from this incision during surgery (Kummer 2012). The remaining 75% of the gastrointestinal tract can be exteriorized and assessed. Due to the lack of vessels in the linea alba, there is minimal hemorrhage during the incisional process (Dyce 2010) and the fibrous nature of the linea alba creates a strong closure (Kummer 2012).

To perform a ventral midline celiotomy, the patient should be placed in dorsal recumbency. In male patients, the urethra is catheterized and the prepuce suture closed to reduce the incidence of contamination of the surgical site (Kummer 2012). The ventral abdomen can then be clipped and

aseptically prepared. A number 10 or 22 scalpel blade, depending on surgeon preference, is used to incise the skin and subcutaneous tissues longitudinally. A small incision is then made in the linea alba just cranial to the umbilicus, where the linea alba is thickest and most palpable, to avoid accidental paramedian incision (Kummer 2012). The incision is extended cranially, taking care to raise the linea alba away from the abdominal contents to avoid penetration of the viscera. It is also important to ensure the incision is continued along the linea alba and does not penetrate the rectus abdominis muscle. The incision should be large enough to allow exteriorization of the viscera without applying excessive pressure on the intestine or creating unnecessary trauma to the edges of the incision. On average, the incision in approximately 20cm in length, however, this varies depending on the size of the horse, the distension of the intestine and the reason for performing a celiotomy (Kummer 2012).

3. Suture Material

Studies investigating wound strength and tissue morphology have shown that, following ventral midline celiotomy, the equine linea alba remains significantly weaker that the original tissue for up to 8 weeks post-operatively (Chism *et al.* 2000). The same authors found the linea alba to be at its weakest (80% of its original tensile strength) up to 2 weeks post-operatively. It is therefore essential that the suture material selected is one that retains its tensile strength for a minimum of two weeks, and ideally, for eight weeks post-operatively.

Polyglactin 910 is an absorbable, multifilament suture material and is currently recommended as the suture material of choice for closing equine celiotomy incisions (Mair *et al.* 2007). The braided nature of polyglactin 910, together with the coating of polyglactin 310 and calcium stearate, makes this suture material less susceptible to instrument damage, compared to monofilament suture (Trostle *et al.* 1994). Three USP polyglactin 910 has been shown to have similar tensile strength to 5 USP polyester, and a greater tensile strength than 2 USP polyglycolic acid and 2 USP nylon, thereby making this a suitable choice in closure of the linea alba (Trostle *et al.* 1994). One drawback of polyglactin 910 is that it is a braided suture and this allows the possibility for increased bacterial colonization, however, this is reduced by the presence of a coating. This suture is therefore not recommended where significant contamination has occurred during surgery (Ducharme *et al.* 1983). In such instances, polydioxanone, a monofilament suture, is recommended as it is less likely to create a nidus for infection (Ducharme *et al.* 1983; Trostle *et al.* 1994). In agreement with this principle, Honnas and Cohen (1997) found polyglactin 910 to be associated with an increased number of incisional infections, whereas polydioxanone and polyglycolic acid were associated with fewer infections.

Polydioxanone is a monofilament, absorbable suture material that is frequently used in closure of the linea alba. The monofilament nature of the suture means that it is less likely to potentiate infection than other multifilament sutures used for closing the equine linea alba, including polyglactin 910 (Honnas and Cohen 1997). One drawback is that suture formed from polydioxanone remains in the incision for 180 days, which is longer than the four to eight weeks required for the linea alba to regain 80% or more of its strength (Kummerle

2012; Chism 2010). Suture material remaining in the incision for longer than required can result in an increased duration of infection should bacterial colonization of the suture material occur (Kummerle 2012). It has also been shown that monofilament suture can be susceptible to instrument damage during knot tying and manipulation, which may then weaken the suture compared to multifilament strands (Trostle *et al.* 1994).

Polyglycolic acid, which is an absorbable multifilament suture material, has a slightly increased, but comparable, tensile strength to polyglactin 910 in one study (Trostle *et al.* 1994) but is also reported to have a decreased knot holding capacity when compared to the same suture material (Kummerle 2012). Polyglycolic acid retains 65% of its tensile strength for 14 days post-implantation and is reabsorbed after 60-90 days. Polyglactin 910 fairs slightly worse, retaining 50% of its tensile strength after 14 days, with the majority of the material reabsorbed after 56-70 days. However, either of the suture materials can be considered as providing adequate suture strength for the equine linea alba. Further studies are required to evaluate polyglycolic acid suture material in the equine linea alba.

Polypropylene, a non-absorbable suture material, is associated with the formation of incisional infections and suture sinus formation in equine patients, due to the fact that the suture remains in the incision site indefinitely (Trostle and Hendrickson 1995). Due to the fact that the suture remains in the patient, greater tissue reactivity can also occur, predisposing the patient to incisional complications (Poole 1984; Poole 1985; Richey and Rose 2005; Israelsson 1994; Brown 1992; Trostle 1998). Polypropylene, while forming secure knots, has poor suture handling characteristics due to its increased memory,

frequently requiring a greater number of throws to achieve knot security (Kummerle 2012). Increasing the number of throws increases the quantity of foreign material at the incision site, leading to increased tissue reaction and an increased incidence of sinus tract formation (Israelsson 1994).

For patients who have undergone previous incisional dehiscence, monofilament stainless steel can be placed in an interrupted horizontal mattress pattern or near-far-far-near pattern to reduce tension on the incision (Mair *et al.* 2007). Monofilament stainless steel has excellent knot holding capacity (Schumacher *et al.* 1981) and creates minimal nidus for infection (Tulleners and Donawick 1983). However, due to the increased closure time and the continued presence of the stainless steel within the incision, this method is not used in routine closures of the equine linea alba.

4. Suture Gauge

Suture material should be of a large enough gauge to have sufficient tensile strength to withstand the force placed on the suture during both formation of the knots and from the abdominal viscera (Mair *et al.* 2007). However, the suture material should be of as small a gauge as possible to achieve this, to reduce the quantity of foreign material in the wound and therefore the degree of tissue reaction (Richey and Rose 2005; Israelsson 1994; Brown 1992; Trostle 1998). The largest suture gauges for use in equine surgery are 7 USP polydioxanone and 6 USP polyglactin 910. These suture materials are not available in the United States, however, they have been shown to have higher breaking strengths than the more commonly

used 2 USP polydioxanone and 3 USP polyglactin 910 (Fierheller and Wilson 2005). A more recent study has shown that 7 USP polydioxanone does not yield a higher rate of incisional complications than 2 USP polydioxanone, despite being of larger gauge (Anderson *et al.* 2015). Although 2 USP polydioxanone and 3 USP polyglactin 910 are the most commonly used suture materials in use in the equine linea alba (Mair *et al.* 2005), further work is required to evaluate the use of 7 USP polydioxanone and 6 USP polyglactin 910 for closure of the equine linea alba. Size 2-0 USP polyglactin 910 can be used in a continuous suture pattern in closure of the peritoneum (White *et al.* 2009). Although peritoneal closure has been associated with increased adhesion formation, it does maintain a barrier between the linea alba and the bowel during closure, as well as preventing peritoneal fluid form contacting the incision (White *et al.* 2009).

5. Suture Pattern

A simple continuous suture pattern has been shown to have a 17% greater incisional bursting strength, using an intra-abdominal inflation device, than an interrupted inverted cruciate pattern when 3 USP polyglactin 910 is used (Magee and Galuppo 1999). In this *ex vivo* study, all knots tied were surgeon's knots with 6 throws, which is the most commonly used number of throws (Mair *et al.* 2007). The greater incisional bursting strength, combined with the increased speed of closure, reduced quantity of suture in the incision and therefore reduced cost, make a simple continuous suture pattern the most common method for closure of the equine linea alba (Mair *et al.* 2007; Turner *et al.* 1988; Looysen *et al.* 1988). A simple continuous suture pattern also

ensures even tension distribution across the entire length of the wound, which decreases forces on the knots, the part of the suture line most likely to fail (Poole *et al.* 1984; Magee and Galuppo 1999). Conversely, Looysen *et al.* (1988) found a simple interrupted suture pattern in 3 USP polydioxanone to have similar breakage strength compared to a simple continuous pattern in the equine linea alba. However, given the decreased suture material and increase speed of suture placement, a simple continuous pattern is preferred by most surgeons (Mair *et al.* 2007).

When performing a simple continuous suture pattern, placement of the suture is crucial. If the suture is pulled too tight through the linea alba, focal necrosis can occur at the tissue margins, increasing the likelihood of suture disrupting the tissue and incisional failure (Sanders and Clementi 1976). Tissue bites of 15 mm from the tissue edges provides the maximum strength when 3 USP polyglactin 910 is used in the horse (Trostle *et al.* 1994; Freeman *et al.* 2002). Fifteen millimeters is sufficiently close to the tissue edges to prevent incorporation of the rectus abdominis muscles, which can undergo focal necrosis when the suture material is pulled tight (Kummer 2012). In human patients, it has been shown that suture interval should equal tissue bite size (Jenkins 1976). Although this has yet to be shown in equine patients, this principle is still generally applied in closure of the equine linea alba, with the sutures being placed approximately 10-15 mm apart (Mair *et al.* 2007; Trostle *et al.* 1994). Trostle *et al.* (1994) evaluated a variety of tissue bite sizes and found that bite sizes greater or smaller than 15mm accounted for up to 44% of a loss in strength of the closure of the linea alba.

Although previously a popular method of closure, performing a near-far-far-near suture pattern in closure of the equine linea alba has been associated with an increased incidence of incisional

complications when compared to a continuous suture pattern (Kobluck *et al.* 1989). This is thought to be due to undermining of the tissue creating dead space and therefore a greater propensity for incisional drainage. Use of the near-far-far-near pattern also leads to an increased quantity of suture material in the incision. Conversely, a more recent study found there to be no increased risk of incisional complications when a near-far-far-near suture pattern was used, however no undermining of the linea alba occurred in this study (Honnas and Cohen 1997). Further support that undermining of the linea alba is detrimental to the outcome of an incision is provided by Mair and Smith (2005b), who found undermining of the linea alba made horses four times more likely to experience incisional complications, including incisional swelling and infection.

Two USP multifilament, lactomer 9:1 loop material has previously been used to create a double-stranded continuous suture pattern (Mair *et al.* 2007; Hassan *et al.* 2006). Using this method, 7% of closures failed at the suture loop, compared to 71% of continuous suture patterns in 3 USP polyglactin 910,which failed at the knot (Hassan *et al.* 2007; Magee and Galuppo 1999). Critically, there was also no significant difference in the incisional bursting strength between the two methods of closure.

Following closure of the linea alba, the subcutaneous tissues and skin can also be closed. Coomer *et al.* (2007) found that closing the linea alba and skin decreased rates of incisional complications compared to three layer closures, including the subcutaneous tissues. Equine patients that were closed in two layers had an 18.7% incidence of incisional complications, whereas patients closed in three layers, including the subcutaneous layer, had a 23.9% incidence

of complications, however, this difference was not statistically significant. Currently, the placement of a subcutaneous layer depends largely on surgeon preference (Kummer 2012; Mair *et al.* 2007).

6. Knot Type

Ventral midline celiotomy incisions in the horse have been reported to fail because of infection, suture failure, knot slippage, and/or suture tear-out through failure of the tissues holding the implanted suture (Wadstrom and Gerdin 1995). An *ex vivo* evaluation of simple continuous suture patterns used in closure of ventral midline celiotomies showed that 71% of suture lines failed at the knot, as opposed to breakdown of the tissues and suture pull through (Magee and Galuppo 1999). The same study demonstrated that suture failure occurred at the knot in 100% of inverted cruciate pattern closures of the equine linea alba. An *ex vivo* study further study showed that when a sutured incision fails, 98.7% of the time it is the suture rather than the tissue that tears, with 90.4% of these disruptions occurring at the knot-suture junction due to shear stress (Fierheller and Wilson 2005; Trostle *et al* 1994; Carlson 1997). This suggests the knot may be a limiting factor for a secure celiotomy closure. The optimal suture pattern and knot technique that minimizes the risks of dehiscence, infection and herniation of the linea alba in the horse has not been established (Fierheller and Wilson 2005). However, the ideal closure would consist of a knot which is both secure and supports a high load to failure (Schaaf *et al.* 2009).

It has been shown that formation of an asymmetrical or poorly configured knot can lead to knot slippage or unraveling of the knot (Schaaf *et al.* 2009; Rosin and Robinson 1997). Slippage in itself is still significant as it has been shown that greater than 3mm of slippage is detrimental to an incision, due to increased motion between the two incision edges (Fugazzi 2014). For some operators, it can be difficult to form knots correctly in the linea alba as tension on the wound edges can contribute to slippage as the knots are being tied (Mair *et al.* 2007).

Square and surgeon's knots are by far the most commonly used knots in closure of the equine linea alba. Previous work has shown that there is no significant difference in knot holding capacity between square and surgeon's knots formed in 3-0 polyglactin 910 (Rosin and Robinson 1997). Square knots, having only one overhand twist on the first throw, create a smaller knot than surgeon's knots, which have a double overhand twist on the first throw (Hermann 1971). However, a square knot does not maintain tissue apposition under tension, and, given the fact that the linea alba is frequently under tension, the surgeon's knot may be preferable for closure of the equine linea alba (Thacker *et al.* 1975). Use of a surgeon's knot can assist in prevention of knot slippage by maintaining tissue apposition under tension (Mair *et al.* 2007) however, it has also been shown that a surgeon's knot may alter the structural properties of the suture material, creating unknown consequences for the tensile strength and knot holding capacity of that suture (Trostle *et al.* 1994).

It has been demonstrated in both human and small animal surgery that self-locking knots have both a higher knot holding capacity and smaller size than conventionally used square knots

(Israelsson 1996; Stott *et al.* 2007; Schaaf *et al.* 2009). Israelsson (1996) discusses the use of self-locking knots to start and end a continuous suture line. The report discusses the use of the forwarder knot for starting a continuous suture line and the Aberdeen knot for ending a continuous suture line. At the time of this report, the Aberdeen knot has not been reported in use in equine surgery, and has not been tested in knots using suture materials larger than 1 USP polyglyconate (Fong *et al.* 2008). The forwarder knot has rarely been documented and has not yet been used in veterinary surgery. Comparisons have been made between Aberdeen and square knots in 2-0 polydioxanone (Schaaf *et al.* 2009), but no comparison has been made to evaluate the differences in strength and security of Aberdeen knots versus surgeon's knots.

7. Knot Size

It has previously been shown that using 2-0 polydioxanone and 3-0 USP polyglactin 910, at least one additional throw is required for square knots at the end of a continuous suture line compared to the start of a continuous suture line to achieve maximum KHC (Schaaf *et al.* 2009; Rosin and Robinson 1997). This principle has not yet been evaluated in large gauge suture.

v. Current techniques for Closure of a Ventral Midline Celiotomy in the Horse

Currently, the majority of surgeons choose to perform closure of the equine linea alba using 3 USP polyglactin 910 or 2 USP polydioxanone in a simple continuous suture pattern (Mair *et al.* 2007). The majority of surgeons place surgeon's rather than square knots at both the start and end of the suture line to counter the tension at the wound edges of the linea alba (Freeman 2005;

54

Mair *et al.* 2007). Current anecdotal recommendations are to place six throws at the start and end of a continuous suture line (Mair *et al.* 2007).

d. Methods of Evaluating Knots and Suture Material

i. Nomenclature

There are several measurements that can describe the strength of a suture and the strength of knots tied within that suture. The tensile strength (TS) of a suture is the maximum longitudinal force that can be placed on a single strand of suture just before it breaks (Kummerle 2012; Schaaf *et al.* 2009). The knot holding capacity (KHC) is the maximum longitudinal load that can be applied to the knotted suture material before the suture breaks or the knot slips (Kummerle 2012). Relative knot security (RKS) is a standardized method of describing the knot-holding capacity as a percentage of the suture's tensile strength, using the formula: RKS (%) = (KHC/TS)x100 (Kummerle 2012). This allows knots in different suture materials to be compared.

ii. Methods of *in vitro* testing

Testing can be undertaken by a variety of methods. The majority of testing of suture is undertaken *in vitro*. Most commonly, this is done on a loading apparatus such as a universal testing machine equipped with a load cell or tensiometer. Schaaf *et al.* (2009; 2010) tied suture

around a smooth metal bar perpendicular to the direction of tension placed on the suture. This method aims to represent the forces acting on a continuous suture line. Mulon *et al.* (2010) use two low-friction pulleys to distract the suture. Muffly (2010; 2011) used two hooks to achieve the same purpose. These testing methods place the tensile forces on different parts of the knots. The studies performed by Mulon *et al.* (2010) and Muffly *et al.* (2010) effectively result in the KHC being increased as the suture is doubled, by forming a loop of suture, rather than a continuous strand. This makes it possible to achieve RKS value of greater than 100%.

It has previously been shown that greater than 3mm of slippage on a knot is detrimental to the outcome of an incision (Fugazzi *et al.* 2013). Therefore, in such *in vitro* studies, the tag ends of the suture are cut to 3mm to improve identification of this amount of slip. Tension is applied to the knot using the universal materials testing machine or tensiometer until the knot slips or the suture breaks. The software coupled to the load cell measures the KHC. RKS can then be calculated. In studies where a tensiometer is used (Muffly *et al.* 2010), software is unnecessary for recording the KHC.

Tension can be placed on the suture at a variety of rates. In the current literature the rate varies from 5mm/min (Muffly *et al.* 2010) to 100mm/min (Mulon *et al.* 2010). An increase in the rate of distraction will result in a higher knot holding capacity recorded. The majority of studies use a distraction rate of 10-50mm/min (Schaaf *et al.* 2009; Stott *et al.* 2007). While the majority of studies apply a single cycle to failure test (Schaaf *et al.* 2009, 2010; Mulon *et al.* 2010; Marturello *et al.* 2014). However, some studies, such as Kim *et al.* (2014) perform cyclic loading

to more closely mimic *in vivo* forces. The advantages of performing a single cycle to failure test, however, is than an absolute value for KHC can be obtained.

The majority of *in vitro* studies are performed using dry suture with no other media (Mulon *et al.* 2010; Schaaf *et al.* 2009). These studies can also be performed in a variety of media, such as plasma and fat, to mimic the local wound environment (Schaaf *et al.* 2010). Using these solutions in an *in vitro* testing environment, these authors found Aberdeen and square knot tied in 2-0 USP polydioxnanone to require an additional throw for security in fat, but not in plasma. Other authors have evaluated surgeon's and square knots is plasma and saline (Muffly *et al.* 2010; Marturello *et al.* 2014). Although comparisons of KHC values cannot be made between studies, it does appear as though tying knots in a media, especially fat, can reduce knot holding capacity (Schaaf *et al.* 2010: Ching *et al.* 2013).

Another aspect of knots that can be assessed *in vitro*, is their size. As the number of throws on a knot increases, so too does the KHC, up to an optimum number of throws (Kummerle 2012). Previous studies (Schaaf *et al.* 2009; Schaaf *et al.* 2010; Regier *et al.* 2015) have measured knot size using a digital micrometer and have approximated knot volume to that of a cylinder using the formula $V = \pi r^2 h$. The ideal knot will have the maximum KHC the knot can provide while having the fewest possible number of throws and therefore the smallest size, to create a secure knot (Schaaf *et al.* 2009).

   iii. <u>Methods of testing *ex vivo*</u>

Ex vivo studies have also been undertaken, these studies have assessed incisional bursting strength using different suture patterns (Magee and Galuppo 1999; Anderson *et al.* 2014). These methods can provide a more accurate data for the use of specific knots within an abdominal closure as this will also show how the tissue behaves when the closure is performed. These methods used a polyeurethane bladder which was inflated within abdomen until incisional bursting occurred. The pressure used in inflate the polyurethane bladder was recorded to provide a relative value of incisional bursting strength.

iv. Methods of testing *in vivo*

Retrospective or prospective case controlled studies can be performed using different techniques for closing the equine linea alba. Although large meta-analyses have been performed in the human literature (van't Riet *et al.* 2002; Weiland *et al.* 1988), such numbers have not been evaluated in the equine literature. However, a retrospective study involving 300 surgical colic cases (Mair and Smith 2005a,b) provided evidence-based information regarding risk factors in incisional complications. Anderson *et al.* (2011) established that right paramedian incisions resulted in an increased risk of incisional complications. While the majority of such studies are retrospective, Coomer *et al.* (2007) performed a prospective case-controlled study and found the placement of a subcutaneous layer of sutures to increase the incidence of incisional complications from 18.3% to 28.9%. Further prospective case-controlled studies evaluating the type of knot used and the number of throws placed on the knot are required before guidelines regarding closure of the equine linea alba can be formed.

e. Objectives

The objective of this study was to compare the forwarder and Aberdeen self-locking knots to surgeon's and square knots, using a combination of throws and types of suture materials (2 USP and 3 USP polyglactin 910, and 2 USP polydioxanone) commonly used in equine surgery for closure of a celiotomy incision. The purpose of our investigation was to determine the ideal combination of throws, suture size and type, and knot construction that would provide both the strongest and smallest knot possible in large gauge suture materials. We hypothesized that 1) the forwarder and Aberdeen knots would have increased strength (knot holding capacity (KHC) and relative knot security (RKS)) compared to surgeon's and square knots; 2) the strength of each knot would increase with an increase in the number of throws for all types of knots and suture materials; 3) the forwarder and Aberdeen knots would have a smaller volume and weight compared to surgeon's and square knots with the same relative strength; and 4) knots formed from polyglactin 910, would have a higher KHC than knots formed from polydioxanone, when the same suture gauge, same knot type, and number of throws were placed.

II. Materials and Methods

Testing of the knots was performed in a controlled environment of 20°C ±2°C and 65% ±5% relative humidity at the Auburn University Polymer and Fiber Engineering Laboratory. To ensure all sutures were tied using a force consistent with *in vivo* tension, a preliminary study was performed. Three board certified large animal ACVS surgeons tied 10 square knots each, using Mayo-Hegar 8" needle holders, for a total of 30 knots. The tension applied to the suture was measured during the first throw using a tension measurement technique, similar to previous studies (Kitagawa *et al.* 1998). Briefly, the INSTRON® 5565 frame (Norwood, MA) equipped with a one-degree-of–freedom load cell that clamped one end of the suture. A second suture clamped in a fixed grip was used to orient the applied force in a direction parallel to the axis of the load cell. The average of all forces placed on the suture was 21N for all three surgeons, and was established as the standard force used for all knots in the study (Schaaf *et al.* 2010).

To ensure all sutures were of similar strength, one suture from each box was subjected to a single, cycle-to-failure test to identify the intrinsic tensile strength (TS). Each suture was tested to failure with a distraction rate of 20mm/minute with a 21N +/- 0.01% static preload, consistent with the calculated standard force. Those sutures with a tensile strength outside of the acceptable range (10% of the mean tensile strength) were considered defective, and the entire box of suture associated with a defective strand was removed from the study.

Over a four week period, 40 knots were tied and tested each day by a single operator (AG), to prevent fatigue. A new packet of suture was used for each knot. The knots were tied using Mayo-

Hegar 8" needle holders on the loading device, consisting of a smooth metal bar distally and a clamp with metal inserts proximally. The standard tension of 21N was used to tie each knot. Tension was applied by the operator, and an INSTRON® (Norwood, MA) 1kN load cell connected to the standing end of the suture ensured equal tension on each throw. The standing end is defined as the suture which would be placed to close the incision in a simple continuous pattern preceding the knot that terminates the suture pattern.

Surgeon's knots consisted of a double overhand throw on the first throw, followed by single throws; square knots comprised of only single throws. Half (300) of the square and surgeon's knots were performed using a single strand of suture as would be performed when beginning a continuous suture line. Half (300) of the square and surgeon's knots were tied by grasping a loop of suture with the needle holders, as would be performed when ending a continuous suture line. Surgeon's and square knots were performed with a range of four to eight throws for each suture type, creating a total of 150 square and 150 surgeon's knots representing the start of a continuous suture line, and 150 square and 150 surgeon's knots representing the end of a continuous suture line.

Forwarder knots were performed with a range of three to six throws in each suture material, creating a total of 120 Forwarder knots. Aberdeen knots were performed with a range of three to six throws and either one or two turns for each suture type, creating a total of 240 Aberdeen knots. Including the square and surgeon's knots, each type of knot was tied ten times, resulting in a total of 960 knots. (Table 1)

Once tied, the knots were removed from the device. One of the tag ends, not including the line representing the preceding continuous pattern, was cut to 3mm to aid with detection of knot

slippage during distraction. The knots were visually inspected for imperfections and re-tied using a new length of suture if visual inspection was unsatisfactory; the knots were retied if throws were asymmetrical or gaps were observed between the throws.

Knot size was measured using a digital micrometer (Olympus SZX7, Olympus America Inc. PA USA). Five knots out of ten in each group were randomly selected and the height and diameter of the knots were recorded to a resolution of 0.01 mm using a digital micrometer. A total of 480 knots were evaluated. The data was used to approximate knot volume for each group, using the formula $V = \pi r^2 h$ (Schaaf *et al.* 2010). An additional 480 knots (five additional knots in each group) were tied and weighed to a resolution of 0.1 mg using an electronic scale (Mettler AE 163, Mettler Direct, CA, USA). All ends were cut to 3 mm in length prior to weighing. The knots that were weighed were not tested under distraction.

The loading system for knot testing consisted of a metal rod fixed to the lower grip, with two metal inserts clamped in the upper grip. (Figure 3) The apparatus was mounted on a Universal testing machine (INSTRON® 5565, Norwood, MA), and coupled with a camera (Casio Exilim, Casio America Inc, Dover, NJ) recording at 60 frames per second to document the mode of failure. The initial distance between the metal rod and the upper grip was 10 cm. A preload of 21N was applied, to ensure uniformity and prevent slack in the suture, and the knots were tested by placing tension on the standing suture end representing the force originating along the

continuous suture line (Schaaf *et al.* 2010). The loading apparatus was distracted at a rate of 20 mm/min until knot slippage or suture breakage occurred.

Testing machine software (INSTRON Bluehill 2.24.787 software, Norwood, MA) recorded the instantaneous load versus distraction (elongation) until failure. The software detects the ultimate load to failure, known as the knot holding capacity (KHC), of the knot. The KHC is the maximum force that can be applied, in Newtons, before a knot breaks or slips. This ultimate force was recorded for each suture. Relative knot security (RKS) was calculated to describe a knot's holding capacity as a percentage of the suture's tensile strength (TS). This measurement allows comparisons between different suture types and sizes by standardizing the force applied. RKS was calculated by the formula (Schaaf *et al.* 2010):

$$\text{RKS\%} = \frac{KHC}{TS} \times 100.$$

Following testing, a value for knot efficiency was calculated by dividing the knot holding capacity by the volume of the knot for each possible combination of knot type, suture material and number of throws. This calculation enabled us to compare knots and determine the ideal knot, that is, the knot which had the highest strength while still maintaining a small volume.

$$Knot\ efficiency = \frac{KHC}{Volume}$$

Statistical analysis:

To establish the number of knots to be tied a power calculation was performed. Using this standard deviation from an equivalent study using 2-0 USP polydioxanone (Schaaf *et al.* 2010), a sample size of 10 knots for each combination of suture material, knot type and number of throws gave a power of 0.9 and a type I error rate of 0.05. Statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA) to collate the data, followed by analysis using commercial statistical software (Minitab version 16, Minitab Inc, State College, PA, and SAS 9.3 SAS Institute Inc. Cary NC). The normality of the data was evaluated using the Anderson Darling test, and parametric tests were selected for further statistical analysis of the data. A one-way analysis of variance (ANOVA) was used to compare the absolute difference in strength between the number of throws, the suture type, suture size and the type of knot. Post hoc testing using Bonferroni's analysis was used to further categorize significant differences. A Pearson's correlation was performed to identify the relation between knot size and weight. Data describing unraveling of knots was not normally distributed and was analyzed using a Fischer's exact test. Statistical significance was set at $p < 0.05$.

III. Results

Both the forwarder and the Aberdeen knot had a higher KHC (Figure 4,5) and RKS (Figure 6,7) for all suture types, numbers of throws, compared to all square and surgeon's knots tested ($p<0.001$). An exception to this was the Aberdeen knot using 2 USP polydioxanone with three throws and one turn, which did not have a significantly different KHC or RKS than square and surgeon's knots in polydioxanone. However, the Aberdeen knot with 3 throws and one turn in 2 and 3 USP polyglactin 910 had a higher KHC than all square and surgeon's knots tied (p<0.001). In comparing the forwarder knot to the Aberdeen knot, the forwarder knot had a higher KHC and RKS for all suture types and all number of throws ($p<0.001$). The strongest start knot evaluated was found to be a forwarder knot with four throws using 3 USP polyglactin 910. This knot had a significantly higher KHC (146.83N) than the strongest surgeon's (105.60N; $p<0.001$) and square (99.38N; $p<0.001$) knots using six throws in 3 USP polyglactin 910. The strongest end knot was an Aberdeen knot composed of five throws and one turn using 3 USP polyglactin 910. This knot had a significantly higher KHC (127.63N) than the strongest surgeon's (98.22N; $p<0.001$) and square (85.95N; $p<0.001$) knots using the highest number of throws tested (8 throws) in 3 USP polyglactin 910.

Unraveling or slippage did not occur in any forwarder or Aberdeen knot tested, but did occur in a percentage of the surgeon's and square knots with less than six throws at the start of a continuous suture line and less than seven throws at the end of a continuous suture line (Table 2).

Both the forwarder and Aberdeen knot had a larger KHC for all number of throws with both 2

65

USP and 3 USP polyglactin 910, compared to knots tied using 2 USP polydioxanone ($p<0.001$). Comparing forwarder knots with the same number of throws, the KHC increased as the strand diameter of polyglactin 910 increased from 2 USP to 3 USP ($p<0.001$). The same was true when Aberdeen knots with the same number of throws were compared (p<0.001). However, as the number of throws on the forwarder knots increased from three to six for 2 USP polydioxanone, 2 USP polyglactin 910 and 3 USP polyglactin 910, there was no significant increase in KHC as suture size increased (2 USP polydioxanone $p=0.850$; 2 USP polyglactin 910 $p=0.832$; 3 USP polyglactin 910 $p=0.520$). As the number of throws on the Aberdeen knots increased from three to six throws for either 2 USP or 3 USP polyglactin 910, there was also no significant increase in KHC ($p>0.078$). The number of throws and turns had no significant effect on the KHC of the Aberdeen knot for all numbers of throws tested within each type of suture material ($p>0.411$) with the exception of the Aberdeen knot using 2 USP polydioxanone comprising of three throws and 1 turn.

The number of throws placed on square and surgeon's knots did have a significant effect on KHC for the knots tested. The KHC of square and surgeon's knots increased as the number of throws increased, up to a maximum of six throws at the start of a continuous suture line ($p<0.031$) and up to seven throws at the end of a continuous suture line ($p<0.003$). However, the addition of a seventh and eighth throw did not significantly increase, and in fact slightly decreased, the KHC of square and surgeon's knots at the start of the suture line ($p>0.391$), compared to square and surgeon's knots with six throws for any suture type. The addition of an eighth throw at the end of a continuous suture line did not significantly increase the KHC of square and surgeon's knots at the end of a suture line ($p>0.309$), compared to square and

surgeon's knots with seven throws for any suture type.

Knot volume and knot weight increased as the number of throws increased in each size and type of suture tested, and for each style of knot. However, both the forwarder and Aberdeen knots had a smaller volume compared to surgeon's and square knots with the same number of throws ($p<0.001$). Overall, the forwarder knot was 30.03-63.72% smaller volume than square and surgeon's knots with the same number of throws. The forwarder knot also had a smaller weight compared to surgeon's and square knots with the same number of throws ($p<0.001$) (Figure 8). Forwarder knot's were 19.01-54.24% lighter than square and surgeon's knots with the same number of throws. The Aberdeen knot was 18.20-47.00% smaller than square and surgeon's knots with the same number of throws. When assessed by weight, the Aberdeen knot was 37.00-53.5% lighter than square and surgeon's knots with the same number of throws ($p<0.001$). Comparisons of measurement of knot size and weight showed good agreement in their results (Pearson correlation: $r=0.813$; $p<0.001$).

To determine the most efficient knot, the KHC, knot volume and weight were assessed and ranked concurrently. Regarding start knots, the forwarder knot with four throws using 3 USP polyglactin 910 provided the highest KHC (146.83N) and RKS (83.58%) with the smallest (23.91mm^3) and lightest knot (23.98mg) for all knot types tested. In comparison, a surgeon's knot with 6 throws, had a KHC of 105.58N, a RKS of 60.09%, a volume of 57.17mm^3 and a weight of 39.24mg. Regarding end knots, the Aberdeen knot with four throws and one turn using 3 USP polyglactin 910 provided the highest KHC (112.72 N) and RKS (72.63 %) with the smallest (29.02 mm^3) and lightest knot (20.82 mg) for all knot types tested. In comparison, a

surgeon's knot with 8 throws, had a KHC of 98.216N, a RKS of 63.37%, a volume of 81.24 mm^3 and a weight of 77.2 mg.

Batch testing to assess suture material uniformity was performed at the start of the investigation. In total, 3.8% of boxes were excluded from the study. One box of 3 polyglactin 910, one box of 2 polyglactin 910 and 2 boxes of 2 polydioxanone had a tensile strength outside of the acceptable range for this study (10% of the mean tensile strength); these boxes were discarded.

IV. Discussion

The KHC and RKS of both the forwarder and the Aberdeen knot was stronger than either surgeon's or square knots for all suture materials and number of throws, with the single exception of the Aberdeen knot with three throws and one turn in 2 USP polydioxanone. The higher KHC and RKS values demonstrated by the self-locking knots may be partially due to the fact that neither forwarder nor Aberdeen knots slipped or unraveled, whereas a percentage surgeon's and square knots did, especially if less than six throws were used at the start of a continuous suture line or seven throws were used at the end of a continuous suture line. In the knots tested, knots that failed due to slippage were weaker than those that did not. The increased security may be related to the fact that both the forwarder and the Aberdeen knot allows the suture to slide within the knot, absorbing energy without unraveling, whereas square and surgeon's knots do not have this property (Schaaf *et al.* 2009; Richey *et al.* 2005). An explanation for the observation of higher KHC in self-locking knots in 2 USP polyglactin 910 compared to 2 USP polydioxanone is likely due to the nature of the braided suture material compared to the monofilament suture. The combination of the multifilaments together with the coating (polyglactin 370 and calcium stearate) of the suture allowed for microscopic movement and sliding of the filaments within the Aberdeen knot (Ching *et al.* 2013). This absorbs energy and increases the force at which the suture breaks (Ching *et al.* 2013).

Conversely, polydioxanone is known to show increased plastic deformity (i.e. stretching) when placed under tension (Ching *et al.* 2013; Boland *et al.* 2005). It was noted during materials

testing in this study that when tension was placed on Aberdeen knots using polydioxanone, stretching of the suture was observed before suture breakage at the knot-suture junction. This stretching subjectively occurred to a greater extent than in knots performed in polyglactin 910. This may explain the decreased RKS when polydioxanone, compared to the same gauge polyglactin 910, was used, and may provide an explanation for the lower KHC noted in the Aberdeen knot composed of 3 throws and one turn (Kummerle 2012). This suggests that the Aberdeen knot may be less desirable in large gauge monofilament suture when compared to large gauge braided suture.

Increasing the number of throws had no effect on the KHC or RKS of forwarder or Aberdeen knots, with the exception of the Aberdeen knot using three throws and one turn in 2 USP polydioxanone. This findings in consistent with a previous study evaluating a single cycle to failure Aberdeen knot using 0 USP polydioxanone, where there was no significant difference in KHC, when three or more throws were used (Stott 2007). Our study did not test knots with fewer than three throws, due to the fact that previous studies using 2-0 and 0 polydioxanone found that fewer than three throws on an Aberdeen knot may result in unraveling of the knot when the turns unfolded themselves (Stott 2007; Schaaf *et al.* 2009). Given the data and efficiency scores obtained, it is recommended to place at least four throws and one turn when tying Aberdeen knots in large gauge (2 and 3 USP) polydioxanone and polyglactin 910, to ensure that the maximum KHC is achieved. This is especially true when 2 USP polydioxanone is used given the reduced KHC obtained when three throws were applied.

One description of self-locking knots states that the forwarder knot is performed using three throws; use of other numbers of throws was not discussed (Israelsson 2004). Given the data obtained in this study, it is recommended to place at least three throws when tying Forwarder knots in large gauge (2 and 3 USP), and a fourth throw can be placed to ensure that the maximum KHC and knot efficiency scores are achieved, although there was no significant difference between knot strength with three and four throws.

Self-locking knots tied during this study, using polyglactin 910 were significantly stronger than those tied using polydioxanone. While comparisons between suture materials tested in this way, have not been made using large gauge suture, previous work reported higher knot security comparing both 3-0 USP and 2 USP polyglactin 910 to polydioxanone (Boland *et al.* 2005; Fugazzi *et al.* 2013). It has been suggested that knots of larger diameter polydioxanone are more difficult to pull down into a tight knot than polyglactin 910, possibly due to increased suture memory expanding gaps in the knot.[11] It is understood that a loose knot has reduced knot security and is weaker than a tightly formed symmetrical knot (Ching *et al.* 2013). The surgeon's, square and self-locking knots formed from 2 USP polydioxanone in this study were all larger in size than knots formed from 2 USP polyglactin 910 for the same number of throws, supporting   this   hypothesis.

Based on the suture tested in this study, a braided large gauge suture would be recommended for all knot configurations tested to reduce knot volume and weight without compromising strength.

To the authors' knowledge, this is the first study to assess knot weight as a measure of knot size. The Pearson correlation performed shows that these methods show excellent correlation. Knot weight relates to the quantity of suture used per knot, rather than the distance between knot ends. This description is likely a more accurate evaluation of knot size, since it removes the variability caused by knot asymmetry and shape when measured in only two dimensions. Despite an improvement noted by a reduction in the variation of the measurements for weight versus volume, the values obtained for knot weight were consistent with the volume measurements, and both measurements demonstrate that a smaller quantity of suture material is used to tie Aberdeen knots with a similar KHC to square and surgeon's knots. The fact that the Aberdeen knot is smaller than square and surgeon's knots with the same KHC may be important in reducing local tissue reaction *in vivo* without sacrificing strength. Further testing will be required *in vivo* to determine if Aberdeen knots reduce tissue reactivity.

One drawback of this study is that the knots were tied on the loading device, then removed and measured by a digital micrometer, before being returned to the loading device to obtain the KHC. A previous investigation performed a pilot study concluding that when a knot is removed from the loading device and then replaced, it is more prone to slippage and required an increased number of throws to be secure (Schaaf *et al.* 2010). Therefore, it is possible that the knots were weakened by the protocol used in our study, and may in fact be stronger overall than the data shown if they had remained on the loading apparatus. However, all knots in the study were treated the same, therefore the results should be similar in magnitude across the comparisons.

The knots were also tested in a dry environment, which does not accurately mimic the *in vivo*

surgical environment where these knots will be placed. Previous studies have shown that certain biological substances, including fat, may weaken a knot, lowering the relative knot security (Schaaf *et al.* 2010). Further testing will be necessary to simulate biological situations and more accurately identify *in vivo* knot strength.

V. Conclusion

In conclusion, the biomechanical properties of the forwarder and Aberdeen knots provide advantages over surgeon's and square knots in overall strength, size and weight in large gauge suture materials. Strength advantages are due to the fact that the configuration of both forwarder and Aberdeen knots allow the suture strands to slide within the knot, absorbing energy and increasing the force require to break the suture material. In our *in vitro* investigation, the forwarder and Aberdeen knots were stronger than knots conventionally used in closure of the equine linea alba. Further investigation both *in vitro* and *in vivo* is warranted to determine the effects of biologic media on the strength and slippage of these knots before these self-locking knots are used in clinical cases. However, these knots shows promise as a possible method to increase knot security at the start and end of a continuous suture pattern, in suture sizes commonly used in equine ventral midline celiotomy closures.

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Figure 1. Formation of a forwarder knot

In figure 1a, a bite is taken through the tissue before holding the needle holders, the standing end (B), and the working end (A) of the suture adjacent to each other.

In figure 1b, the working end (A) of the suture is then turned around the needle holders and standing end (B) of the suture. The number of times this is performed depends on surgeon preference.

In figure 1c, the working end of the suture is then grasped by the needle holders through the placed loops.

In figure 1d, the needle holders are withdrawn, pulling the working end of the suture through the loops and locking the forwarder knot.
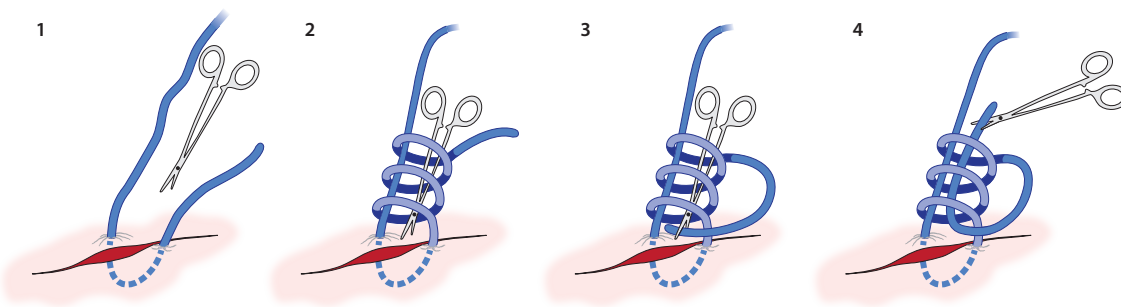
Figure 2. Formation of an Aberdeen knot

In figure 1a, a loop is formed in the suture (A) and passed through the tissue to the opposite side of the incision line.

In figure 1b, a second loop (B) is passed through loop (A).

In figure 1c, step 1b can be repeated any number of times, at the discretion of the surgeon.

In figure 1d, step 1b is repeated (placement of an additional throw)

In figure 1e, the end of the suture (C) is passed through loop (B) to form one turn. This can be repeated, again, depending on surgeon preference.

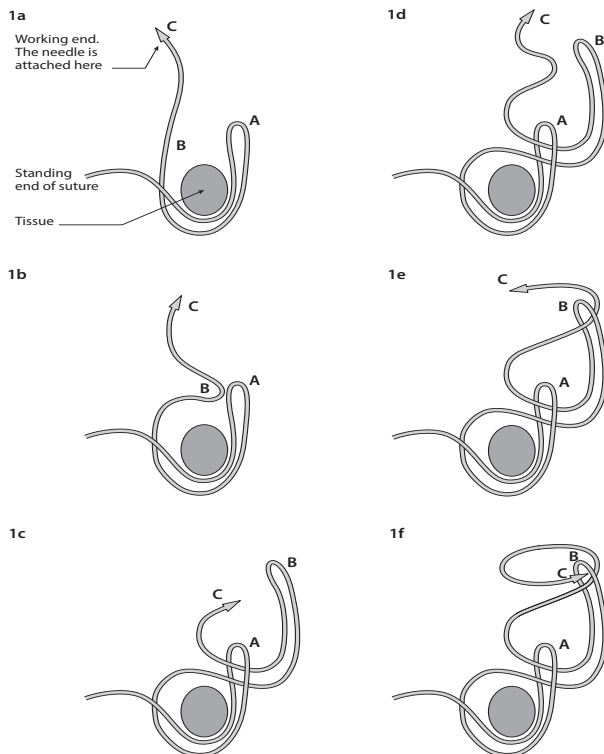In figure 1f, step 1e is repeated (placement of an additional turn)

Table 1. The number of forwarder, Aberdeen, square and surgeon's knots tied are shown. The knots were repeated in 2 USP polydioxanone, 2 USP polyglactin 910 and 3 USP polyglactin 910, giving a total of 960 knots.

Number of throws/Knot Type	Forwarder	Square Start	Surgeon's Start	Aberdeen 1 Turn	Aberdeen 2 Turns	Square End	Surgeon's End
3	10			10	10		
4	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10
7		10	10			10	10
8		10	10			10	10

Figure 3. The configuration of the loading apparatus used for testing the knots on a Universal

testing machine. The load cell is attached to the clamp at the top of the figure. A forwarder knot

is shown on the testing apparatus, tied to the bar. The free end of the suture, representing the

standing end of the continuous line, is held in the clamp and distracted to measure the load at
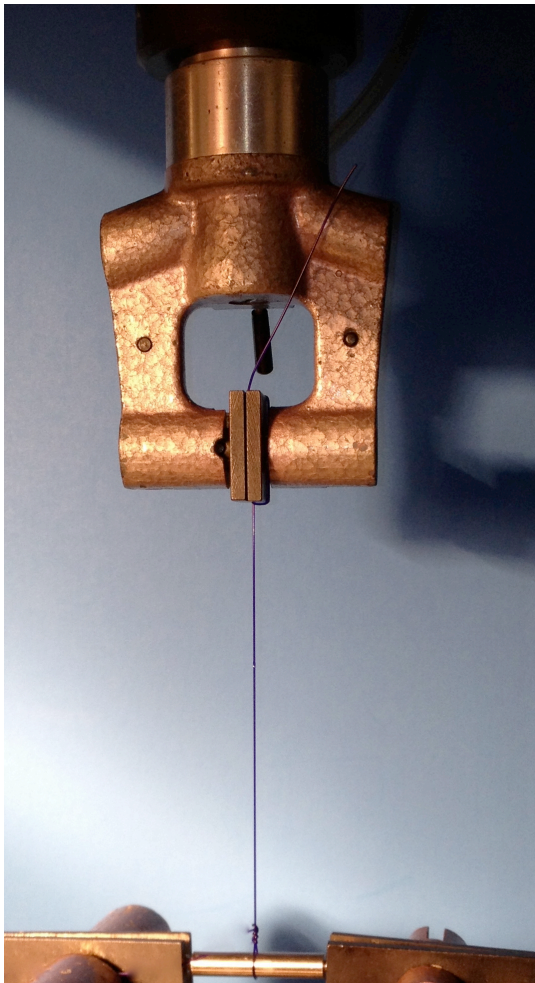
failure, or knot holding capacity, in Newtons.

Figure 4. Comparison of KHC for Forwarder, square and surgeon's knots. Graph A

represents knots in 2 USP polydioxanone; graph B represents knots in 2 USP polyglactin

910 and graph C represents knots is 3 USP polyglactin 910. The number of throws and

knot type are depicted on the x axis, and the KHC is shown in Newtons on the y axis. The

boxes represent the upper and lower quartiles; the whiskers represent the range; the

center line represents the median value. The Forwarder was noted to have a higher

average KHC than surgeon's or square knots with the same number of throws for all
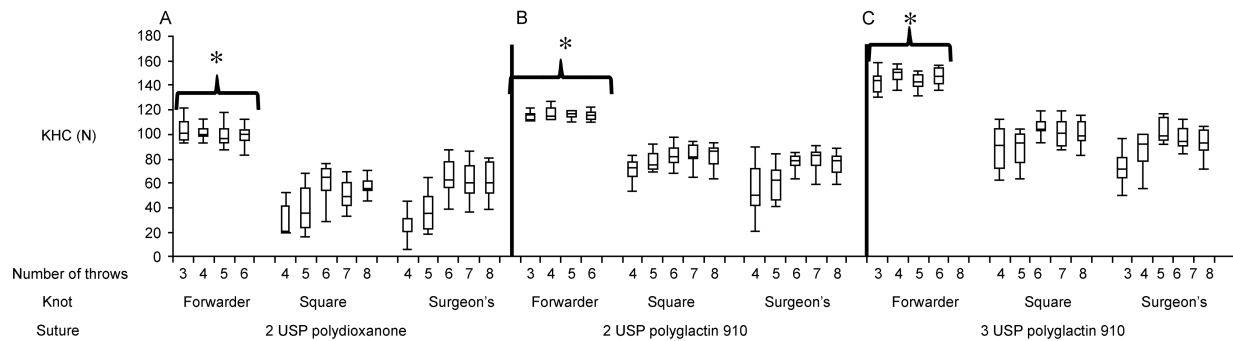
knots tested (p<0.001).

Figure 5. Comparison of KHC for Aberdeen, square and surgeon's knots. The number of throws, knot type and suture material is depicted on the x axis, and the KHC is shown in Newtons on the y axis. The Aberdeen was noted to have a higher average KHC than surgeon's or square knots for all knots tested, with the exception of the knot using 2 USP poldydioxanone with three throws and 1 turn (p<0.001).
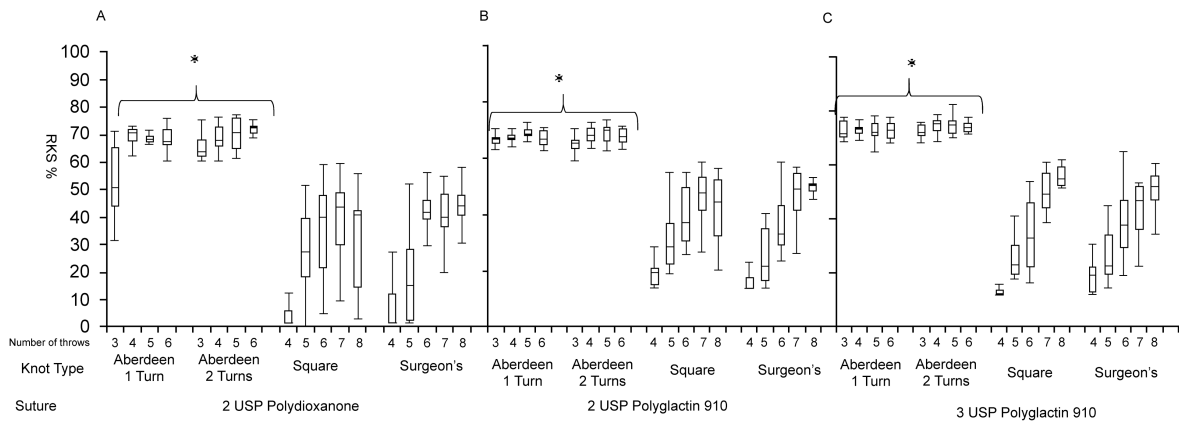
Figure 6. Box plot for comparison of values obtained for of relative knot security (RKS) for forwarder, square and surgeon's knots. The RKS standardizes the force based on suture's tensile strength. The boxes represent the upper and lower quartiles, the whiskers represent the range, and the center line represents the median value. Graph A represents knots in 2 USP polydioxanone; graph B represents knots in 2 USP polyglactin 910 and graph C represents knots is 3 USP polyglactin 910. The forwarder knot had a significantly higher RKS for all comparisons of knots with the same number of throws $(P<0.001)$.
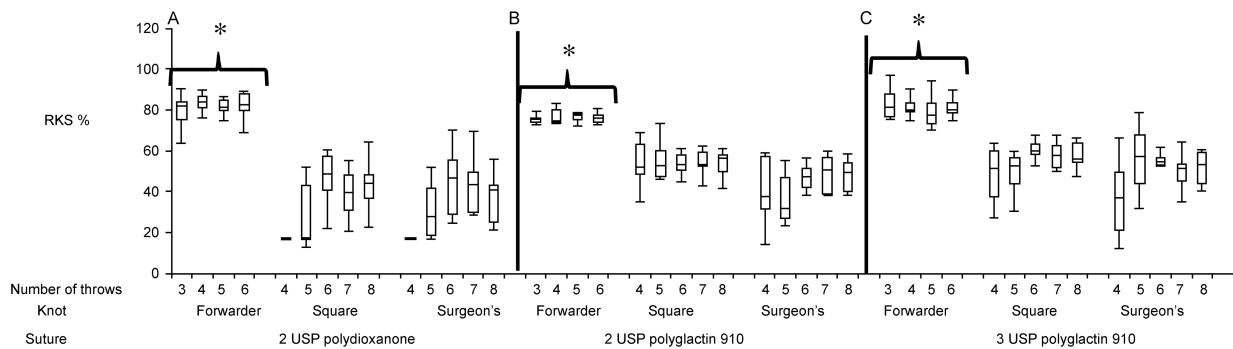
Figure 7. Comparison of RKS for Aberdeen, square and surgeon's knots, which standardizes the force based on suture's tensile strength. The number of throws knot type and suture material is depicted on the x axis, and the RKS is shown in Newtons on the y axis. The Aberdeen was noted to have a higher average RKS than surgeon's or square knots for all knots tested, except for the knot using 2 USP polydioxanone, with 3 throws and 1 turn (p<0.001).
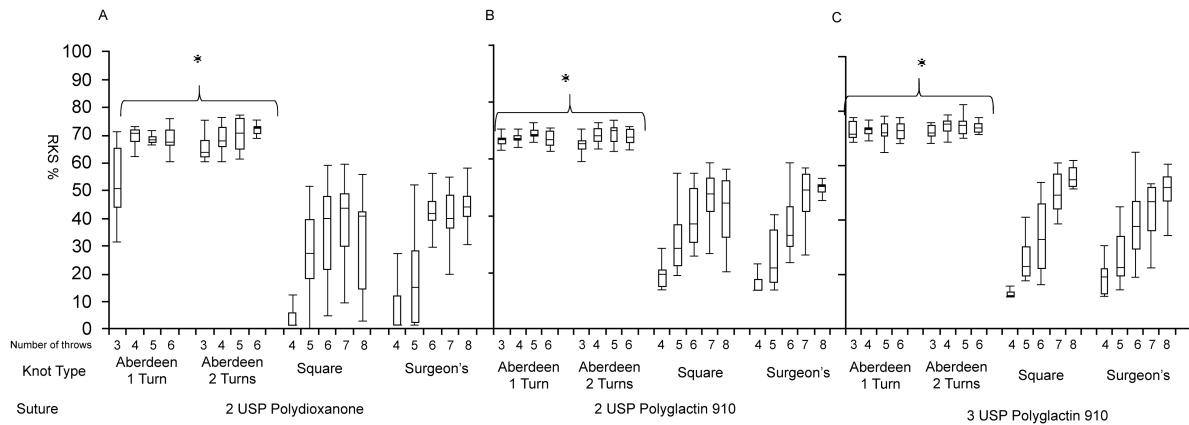
Table 2. Comparison of the percentage of each type of knot which unraveled or slipped during testing. No forwarder or Aberdeen knots unraveled during testing. Table A shows results for 2 USP polydioxnanone, Table B shows results for 2 USP polyglactin 910 and Table C shows results for 3 USP polyglactin 910

A) Knot Type/ Number of Throws	Forwarder	Square Start	Surgeon's Start	Aberdeen 1 Turn	Aberdeen 2 Turns	Square End	Surgeon's End
3	0			0	0		
4	0	90	0	0	0	100	40
5	0	20	0	0	0	60	20
6	0	0	0	0	0	10	10
7		0	0			10	0
8		0	0			0	0
B) Knot Type/ Number of Throws	Forwarder	Square Start	Surgeon's Start	Aberdeen 1 Turn	Aberdeen 2 Turns	Square End	Surgeon's End
3	0			0	0		
4	0	50	50	0	0	90	80
5	0	0	10	0	0	100	80
6	0	0	0	0	0	60	60
7		0	0			0	0
8		0	0			0	0
C) Knot Type/ Number of Throws	Forwarder	Square Start	Surgeon's Start	Aberdeen 1 Turn	Aberdeen 2 Turns	Square End	Surgeon's End
3	0			0	0		
4	0	40	30	0	0	100	100
5	0	20	30	0	0	80	70
6	0	0	0	0	0	60	20
7		0	0			0	0
8		0	0			0	0

Figure 8. Box plot of knot size represented by the weight of each type of knot. Graph A

represents knots in 2 USP polydioxanone; graph B represents knots in 2 USP polyglactin 910

and graph C represents knots is 3 USP polyglactin 910. The boxes represent the upper and lower

quartiles; the whiskers represent the range; the center line represents the median value. The

forwarder knot was noted to have a smaller average weight than surgeon's or square knots for all

knots tested, using the same number of throws *(P<0.001)*.

90