Evaluation of a Transvaginal Laparoscopic NOTES (Natural Orifice Transluminal Endoscopic Surgery) Approach to the Abdomen of Mares

by

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Abstract

The objectives of this study were to describe a natural orifice transluminal endoscopic surgery (NOTES) using laparoscopic and endoscopic instrumentation transvaginally into the standing mare's abdomen and identify structures that are visualized using this approach.

With the horse under sedation, a standing transvaginal approach was made in the cranial vaginal vault lateral to the cervix in each mare. A single approach through the vaginal wall was made on the left or on the right side of the cervix. The abdomen was explored and the abdominal viscera evaluated using a flexible endoscope followed by a rigid laparoscope. Incisional healing was monitored clinically by vaginoscopy post-operatively.

Exploration of the abdomen was sufficiently performed in all mares through a transvaginal approach using either a left or right sided approach. The endoscope allowed consistent visualization of the left kidney, spleen, nephrosplenic space, stomach, cecum, duodenum, left and right ovaries, diaphragm, and caudal peritoneal reflection. The liver was observed with somewhat less consistency on either the left or right side. The laparoscope provided similar views of the caudal abdomen but was limited in both the cranial advancement and in its lateral mobility due to the confines of the pelvis. Healing

of the vaginal incision occurred rapidly with apparent closure of the incision by 3 days.

NOTES techniques through a transvaginal approach may be a useful tool in the diagnosis of intra-abdominal disorders encountered in the mare.

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Introduction

Natural orifice transluminal endoscopic surgery or NOTES is a technique that as the name implies involves the introduction of an endoscope into the abdomen through a natural orifice such as a portion of the gastrointestinal or urogenital tracts. A traditional skin incision is not required; rather an endoscopist or surgeon perforates the luminal wall to allow for access to the abdominal cavity. Much of the development of this technique has been driven by the advancement of human medicine but has been performed in animal models.

Until recently, visual evaluation of the abdomen was limited to non-invasive intraluminal examination through an endoscope or to the more invasive transcutaneous laparoscopy or laparotomy approaches. Advanced endoscopic techniques and equipment were developed for intraluminal use and, as such, the capabilities of the endoscopist were advanced. For the years leading up to the reports of NOTES development it was generally considered to be a mistake to perforate the lumen of the organ being examined and, as such, great care was taken to avoid this complication.

The first NOTES procedure was described in 2004 as an experimental procedure for swine.[1] Since that time the field of NOTES has been the source of much research and development as it pertains to human medicine, however limited work has been done to date to bring this advancement to veterinary medicine and the few reports are limited to small animal medicine. A similar technique, if proven safe and effective for use in the equine patient, would serve as a model in the development of a wide array of new endoscopic procedures and treatments in the wake of this emerging technology.

Natural orifice incisions are not entirely new to the equine species. The colpotomy approach for hand assisted ovariectomy is a well established,[2-4] but arguably outdated procedure that involves a transluminal approach to the abdomen. This technique, however, is not minimally invasive as it requires the blind insertion of the surgeon's entire hand and instrumentation through the incision into the abdomen. Due to the limitations and risks of such an approach, along with the advancement and availability of laparoscopes and descriptions of laparoscopic ovariectomy techniques,[5-7] this type of approach is less commonly performed. The major risks, though not extremely common but which are often fatal, associated specifically with the traditional colpotomy approach are hemorrhage from the uterine branch of the urogenital artery or eventration of the bowel through the incision post operatively.[2-4]

Laparoscopic examination of the equine abdomen provides a minimally invasive means of exploring the abdomen and has evolved greatly since the original descriptions of its use in the horse.[8-10] Diagnostic laparoscopy has become common place in equine surgery and medicine and has been documented to be beneficial in providing a diagnosis for causes of chronic colic,[11-15] obtaining an intra-abdominal biopsy,[16] and to confirm neoplasia, peritonitis, or visceral rupture.[17-18]

The design of this study is intended to modify the previously described colpotomy approach not for the purpose of an ovariectomy, but rather to introduce endoscopic and laparoscopic instrumentation for visualization of the abdomen. This will be performed in much the same way as one would perform a traditional laparoscopic evaluation to examine the abdomen thus developing a minimally invasive NOTES procedure for use in the horse that the researchers theorize will be both safe and effective at abdominal exploration.

Validation of this technique could provide equine practitioners and researchers a method of diagnosing various intra-abdominal disorders. Disorders that could potentially be detected through such an approach includes, but is not limited to, intestinal entrapments, adhesion formation, abdominal abscesses, inflammatory bowel diseases, diaphragmatic hernias, or peritonitis. Additionally, other intra-abdominal disorders not pertaining to the gastrointestinal tract, such as reproductive disorders and neoplastic processes, may be evaluated through this modality. Visualization of the abdominal compartment, specifically the gastrointestinal system, is of particular significance in horses due to these types of disorders being a leading cause of referral to surgical centers.

This technique would provide the ability to explore, visualize, diagnose, and potentially treat these various conditions. Additional theorized benefits of NOTES procedures over standard laparoscopic procedures in humans include shorter recovery times, reduced pain, and no visible scarring. In the equine patient, it is reasonable to believe that some of these benefits may transfer across species lines and hold true for the mare as well. Shorter recovery times and reduced pain would likely result in decreased hospitalization times and aftercare required by the owner and veterinarian and could potentially allow the mare to return to athletic performance sooner. While the majority of mares are unaffected by the prospect of a scar in the flank, some mares, particularly those used as show horses, may benefit from this approach. These benefits would be in addition to those seen in the use of minimally invasive laparoscopy over that of traditional laparotomies.

The purpose of this study is to evaluate the technical feasibility of exploring the mare's abdomen through a transvaginal approach, to compare a right and left sided portal placement, to compare the visualization obtained with a laparoscope to that of the endoscope, and to evaluate a healthy mare's intra-operative and post-operative tolerance of the procedure. By showing that visualization is adequate and that the mares tolerate a transvaginal incision as a portal for visualization, we hope to open this field of surgery to the equine patient.

Literature Review

The first NOTES procedures described were actually in the animal model. In 2004 the first published reports of this technique outlined a model for a transgastric approach to the swine abdomen. Since this time, numerous other reports of use in humans and further development in animal models have been published, however, to date few purely veterinary applications have been described.

Multiple "natural orifices" lend to multiple approaches that all fall under the heading of being NOTES procedures. The most commonly used are transvaginal and transgastric routes. Human endoscopists have become adept at gastric endoscopy for extraluminal procedures, such as peg tube placement or drainage of pseudocysts and, as such, this has become a natural route for the development of the first NOTES procedures.[19] In addition to the transvaginal and transgastric routes, other endoscopic approaches to the abdomen that have been described are transrectal and transurethral. Combination techniques using multiple NOTES portals[20] or hybrid techniques that combine NOTES with traditional laparoscopy[21] have also been detailed.

To date there are no universally accepted optimal or standard NOTES routes for any given procedure in humans. Furthermore, it may be important to realize that in the development of these techniques the transgastric route is the only route that has direct access to the upper or cranial abdomen in comparison with the other routes that all enter the abdomen caudally.[19] Descriptions of human and porcine anatomy and accessibility through these portals are readily available, however, one must be careful not to extrapolate too much from the human literature in relation to other species. The horse in particular has obvious significant differences in regards to size and anatomy but consideration must also be given to differences in patient positioning for surgery (recumbent vs. standing) as well as differences in their systemic response and acceptance.

Only within the last few years have NOTES applications been applied to the veterinary patient, specifically to the canine model. Transgastric ovariectomy in the dog has been determined to be a technically challenging though still feasible procedure resulting in less post-operative pain in comparison to laparoscopic techniques[22,23] and may be beneficial in select cases.

Another study evaluating the feasibility of a hybrid laparoscopic and NOTES cholecystectomy in dogs through transcolonic, transgastric, and transvaginal routes was recently published. This study found that the procedure was viable and apparently safe through all the examined routes and resulted in no manifestation of post-operative pain.[24]

The researchers of this project believe that with the continued expansion and evaluation of these clinical and diagnostic techniques in other species in conjunction with the ever improving technology and capabilities of available endoscopes,[25,26] advanced techniques for the treatment and diagnosis of intra-abdominal disorders specific to the equine patient will become available. The first step in this process is to determine the practicality of such an approach, and evaluate the visualization and accessibility of the abdominal compartments. By modifying the traditional colpotomy approach to reduce the size and placement of the portal, the researchers believe that complications traditionally associated with the placement of the incision can be avoided. This modified incision in combination with NOTES techniques that are being described in other species a safe and effective technique for evaluating the equine abdomen can be developed. Thus, acting as a stepping stone in the development of even more advanced minimally invasive procedures as an alternative to traditional laparoscopy in the horse.

Although, in human medicine NOTES techniques have not yet become universally accepted or routine in recent years cases of abdominal exploration, liver biopsy, appendectomy, cholecystectomy, gastrojejunostomy, fallopian tubal ligation, oophorectomy, hysterectomy, splenectomy, and herniorrhaphy have all been reported.[1,27-35] Application and success of NOTES in these various swine and human models and in some specific cases where traditional techniques were precluded is leading not only to the development of additional procedures and techniques but is also changing attitudes and perception. These procedures are becoming more accepted and will likely one day lead to more routine use.

Objectives

The objective of this study is intended to demonstrate that a standing vaginal laparoscopic approach to the abdomen in a mare is an acceptable method of visualizing the abdominal compartment that does not result in any significant post-operative complications, pain, or illness in a healthy patient that would preclude its use in the clinical evaluation or treatment. Additionally, the study is intended to describe the normal anatomy that is visible using a traditional laparoscope compared to that of a flexible endoscope. The design of this study should demonstrate both the safety and the utility of the emerging technology of NOTES procedures, specifically that of a standing transvaginal laparoscopic approach, in the equine patient allowing for further development of diagnostic and treatment techniques for use through this approach.

Materials and Methods

Animals

The Institutional Animal Care and Use Committee approved the following protocol used in the study. Transvaginal laparoscopy was evaluated in 8 healthy adult mares between the ages of 6 and 22 years (mean age = 14 years). All mares were of a stock horse type breeding weighing between 400 and 550 kgs with a mean weight of 464 kgs. Each mare was determined to be healthy on the basis of a physical exam and pre-operative CBC. All mares were maintained in a teaching herd and their immediate history was well known.

Pre-operative Treatments

To ensure optimal visualization, the mares were held off feed for 48 hours prior to evaluation. Procaine penicillin G (22,000 IU/kg of body weight, intramuscularly), gentamicin (6.6 mg/kg of body weight, intravenously), and flunixin meglumine (1.1 mg/kg of bodyweight, intravenously) were administered 30 minutes prior to surgery. Routine abdominocentesis was performed to obtain a pre-operative baseline of peritoneal fluid total nucleated cell count and total protein as well as cytology. Each mare was restrained in stocks and sedated using detomidine hydrochloride (0.01 mg/kg of body weight, intravenously) and butorphanol tartrate (0.01 mg/kg of body weight, intravenously). The sedation was re-dosed in increments of half the initial dose to desired effect throughout the procedure. After the sedation took effect a colpotomy surgical preparation was performed as follows.

Surgical Preparation

The mares were palpated per rectum and evacuated of all manure. A routine aseptic preparation of the perineal region was performed utilizing a povidone iodine scrub of the perineal region for five minutes and a dilute povidone-iodine solution rinse of the vaginal vault. A 28-French Foley catheter was placed into the bladder to help maintain a dry operating field and sterility during the procedure in the event that the mare attempted to urinate. Each mare had a single approach made into the abdomen, 4 mares from the right side and 4 mares from the left side of the cervix. The side of the incision was randomly assigned for each mare. A lidocaine soaked gauze sponge was placed over the predetermined location of the approach, right or left side, of the cranial vaginal vault. After 5 minutes of contact time with the lidocaine sponge, the approach was made through the vaginal wall into the into the peritoneal cavity.

Right Sided Colpotomy Approach

In 4 mares, the approach was made on the right side of the cervix. To minimize the risk of hemorrhage in making the approach to the abdomen, sharp dissection was avoided. Instead, a pair of curved mosquito hemostats was placed in the left hand of the surgeon (Figure 1). Upon entry into the vaginal vault the hemostats were palmed to prevent inadvertent damage to the vaginal wall. The tip of the instrument was directed laterally and placed approximately 3 to 4 cm lateral to the cervix between the 1 and 2 o'clock position. The hemostats were inserted bluntly through all layers of the vaginal wall and the underlying peritoneum. The hemostats were opened within the abdomen and retracted back into the vaginal vault while still in the open position to create an approximately 2 cm incision into the abdomen. The surgeon at this point placed a single finger into the opening to ensure that all layers had been penetrated and communicated with the abdomen. A cold sterilized two meter flexible endoscope[a] was introduced into the incision by the surgeon, an assistant controlled the scope and a systematic evaluation of the abdomen was performed (Figure 2). The endoscope was then removed and replaced with a 62 cm zero degree rigid laparoscope[b] and a systematic evaluation of the abdomen was again performed. The abdomen was primarily examined on the same side as the incision, however, using a hand intravaginally the surgeon guided the laparoscope ventral to the rectum to visualize the opposite, or left side. After

[a] Fujinon, Inc. Wayne, NJ 07470
[b] Richard Wolf Medical Instruments Corp. Vernon Hills, IL 60061

completion of laparoscopy the scope was removed and the incision was allowed to close by second intention. Digital videos and images of the abdominal compartment were obtained and saved using both the endoscope and laparoscope.

Left Sided Colpotomy Approach

In 4 mares, the approach was made on the left side. A pair of curved mosquito hemostats was placed in the right hand of the surgeon. The tip of the instrument was directed laterally and placed approximately 3-4 cm lateral to the cervix between the 10 and the 11 o'clock position. Entry into the abdomen was performed similar to the right side and the mares were systematically evaluated using the previously described procedure using the endoscope and laparoscope. Positive pressure insufflation was not used for evaluation of either the left or right sides.

Post-operative Care

After surgery, physical examinations were performed on each mare daily for a total of 7 days. The mares were continued on procaine penicillin G (22,000 IU/kg of body weight, IM, BID), gentamicin (6.6 mg/kg of body weight, IV, Q24H), and flunixin meglumine (1.1 mg/kg of bodyweight, IV, BID) for a total of 3 days.

The localized inflammatory response to surgery was measured by performing serial abdominocentesis in the mares. In addition to the sample taken pre-operatively abdominocentesis was performed at 1, 2, 3, and 7 days post-operatively.

The mares were confined to a stall without being tied or forced to stand for 7 days, after which time they were allowed to return to pasture turnout with unrestricted activity. The surgical incisions were examined by video endoscopic vaginoscopy on days 3 and 7 post-operatively.

Results

All transvaginal approaches were performed without complication. No apparent injury to any of the internal organs was detected and no excessive bleeding or inadvertent puncture of the uterine branch of the urogenital artery was observed.

Of the four mares that received a right sided cervical approach to the abdomen, the endoscope was easily passed in all cases. On the right side the endoscope allowed consistent viewing of the right ovary and uterine horn (Figure 3), base of the cecum and the duodenum (Figure 4), the caudal peritoneal reflection (Figure 5), and the caudal dorsal aspect of the diaphragm in all mares. In two of the four mares the caudal aspect of the right lobe of the liver located just cranial to the base of the cecum was also readily observed (Figure 6).

The laparoscope provided similar views of the cecum and duodenum (Figure 7), right ovary (Figure 8), and caudal dorsal diaphragm in all cases. The right liver lobe was evident in only one of four cases (Figure 9). The right caudal peritoneal reflection could not be seen with a rigid laparoscope.

Of the four mares that received a left sided cervical approach to the abdomen, the endoscope was easily passed in three of the four cases. In one case the incision was made too dorsal, at the 12 o'clock position, which was believed to have penetrated the medial fold of the fornix and directed the scope back toward the right side of the abdomen. In the three cases where the approach was made closer to 10 o'clock position the endoscope allowed consistent viewing of the left ovary and uterine horn (Figure 10), the caudal dorsal aspect of the diaphragm, the spleen, left kidney and nephrosplenic ligament (Figures 11-13), the caudal aspect of the left lateral lobe of the liver (Figure 14), the left lateral aspect of the stomach (Figure 15), and the caudal peritoneal reflection on the left side (Figure 16). In the case with the dorsal approach the endoscope was directed to the right side of the abdomen allowing for viewing of the right ovary, cecum, duodenum, and the medial aspect of the left kidney.

The laparoscope provided similar views of the spleen, kidney, left ovary, and caudal dorsal diaphragm in all cases. In the horse with a more dorsal approach the cecum and duodenum were identified. The left lateral liver lobe and the left caudal peritoneal reflection could not be clearly visualized in any horse with a laparoscope. The laparoscope was able to be passed under the rectum using a hand intravaginally in an attempt to view the contralateral side of the abdomen in relation to the approach, however, the views obtained were often obscured by what appeared to be mesentery or ventral abdominal contents and in all cases the views were inferior to the ones obtained when the incision was on the same side of the abdomen.

For all mares, regardless of left or right sided approach, visualization of the ventral contents of the abdomen were variable. Various segments of jejunum, large colon, and small colon were consistently present with both the endoscope and laparoscope on both sides. The bladder was occasionally visible but was not consistently present due to its small size caused by the placement of an indwelling catheter. Additionally, the abdominal viscera contralateral to the side of the vaginal approach were not consistently viewed with either instrument.

Intra-operatively 2 mares developed mild subcutaneous emphysema in the perineal region that resolved spontaneously within 12 hours of surgery. The mares were confined to stalls for observation for seven days post-operatively. Appetite, attitude and water intake remained normal for all but one of the mares during this time. One mare showed signs of mild abdominal pain on day 5 post-operatively. The mare was observed to paw and lie down intermittently and overall seemed mildly uncomfortable. This mare had been off all medications for 48 hours at this point. She received one additional dose of flunixin meglumine (1.1 mg/kg of bodyweight, IV) and was given four liters of mineral oil and four liters of water via a nasogastric tube. Signs of colic persisted for a total of 4 hours and then subsided. No further signs of abdominal pain were observed.

The mares all underwent endoscopic vaginoscopy on days 3 and 7 post-operatively. By day three all of the mares' incisions were closed and visually appeared to be covered by mucosa with no communication remaining between the vaginal vault and the abdomen (Figure 17). The incision site was further contracted and less apparent by day 7 (Figure 18).

The results of the analysis of the peritoneal fluid obtained by serial abdominocentesis are summarized in Appendices C and D. Using a paired t-Test (assuming unequal variances) analysis, the peritoneal fluid obtained each day was analyzed. In comparison of the left and right sided approaches, there were no significant differences in either the total nucleated cell counts (TNCC) or in the total protein (TP) of the fluid on any day. The pre-operative (day 0) TNCC and TP levels was also compared to days 1, 2, 3, and 7. When compared to day 0, the TNCC on days 1, 2, and 3 were significantly different (based on a 95% confidence interval). The TNCC on day 7, however, was not significantly different from the pre-operative day 0 level. The TP was significantly different from day 0 on all subsequent days.

Discussion

Exploration and visualization of the left or right compartments of the dorsal abdomen were successfully performed in all eight mares. Ventral exploration was limited but is to be expected with a standing procedure. In seven of the eight mares visualization within the abdomen was as expected. In the remaining case a left sided approach was intended, however, due to the dorsal location of the incision, the scope was directed into the right side of the abdomen.

The abdomen was divided into left and right sides due to the nature of the caudal mesenteric origins of the bowel. This mesentery seemed to prohibit the movement of either scope medially, or across midline, from the entrance into the abdomen. Using a hand for intra-vaginal guidance the rigid laparoscope could be passed into the abdomen then guided under the rectum to visualize the opposite side. In general, however, the abdominal contents on the side of the approach were much easier to visualize.

The use of a laparoscope through a transvaginal approach was limited in several dimensions. The overall length of the laparoscope did not allow it to be passed cranially past the nephrosplenic space or the base of the cecum, thus it did not allow for clear imaging of the cranial dorsal abdomen on either side. Medial to lateral movement was also limited, which was attributed to the confinements of the transvaginal approach cranially and the vestibular opening on midline caudally. Both these confinements are close in proximity to the midline of the mare, the cranial to caudal distance between the two thus allowed for minimal movement in a medial to lateral direction.

The use of a forward looking flexible endoscope offered a surprising amount of maneuverability within the abdomen. As with most standing laparoscopic techniques, we were limited to viewing the dorsal compartment of the abdomen as well as the right or left half on which the approach was made. Medial or ventral deviation directed the endoscope into the mesentery of the small intestine or small colon which resulted in limited to no visibility.

The most significant problem encountered using the endoscope was that it could not maintain rigidity over the length of the shaft. Once passed through the incision the endoscope would sag in the middle of the abdomen in a cranial to caudal direction thus resulting in poor control over the position of the distal end of the scope. This was able to be counteracted by allowing the midsection of the scope to be supported by the abdominal viscera. A hand placed in the vagina could be used to guide the scope dorsally through the caudal portion of the abdomen. On the left side of the mare the scope could be maneuvered between the left kidney and splenic space to rest on the nephrosplenic ligament. This allowed the operator to maintain dorsal placement of the scope into the cranial portion of the abdomen, resulting in consistent visualization of the stomach and left lateral lobe of the liver. To reach the more ventral aspects of the cranial abdomen the scope could be passed along the body wall with some consistency, but little control in a dorsal to ventral direction could be maintained.

One of the objectives of this study was to examine each mare through a single transvaginal incision which largely limited viewing of both the left and right halves in the same mare. The question is then raised, is it possible to fully explore a mare's abdomen using a NOTES technique? Given the relative ease of the procedure and subsequent healing seen in these mares, we believe that making both a left and right sided transvaginal approach to fully explore a single abdomen would be possible. However, further use of the technique and application to clinical patients will be needed to confirm these observations. One argument against this would be the increased risk of potentially fatal hemorrhage from the uterine branch of the urogenital artery associated with another approach. Though this complication is well known as a result of this type of approach,[2-4] the author believes that this can be minimized by the use of blunt dissection with hemostats or closed scissors into the abdomen rather than sharp dissection with a blade.

Visualization of the left and right dorsal quadrants of the abdomen through a single incision would be a major advantage of this procedure; however, in this study the researchers were unable to consistently accomplish this task. The main limitation of the laparoscope was that medial to lateral mobility was insufficient to allow for adequate maneuverability and visualization within the abdomen as previously discussed. In addition to this, the overall length of the laparoscope appeared insufficient to be passed under the small colon to the contralateral side far enough to prevent the small colon from falling back over the laparoscope and obscuring visualization. The flexible endoscope was limited, not by medial to lateral mobility or length, but by flexibility. When passing the endoscope under the small colon to the contralateral side the weight of the small colon appeared to force the scope ventrally into the abdomen and visualization was inadequate. Unfortunately, this was not overcome at the time of the study, however in retrospect the researchers feel that this may be able to be overcome with modifications to the equipment. Possible modifications to the flexible endoscope could include the use of a rigid sleeve or the insertion of a stiffening wire into the instrument portal. Additionally, a hand placed in the rectum may be able to elevate the small colon dorsally to allow ventral passage of the endoscope, however it is likely that when the hand is removed the scope would again displace ventrally. Though it was not evaluated in the study, rectal palpation would likely need to be done by the same person that was passing the scope due to the limited space behind the mare. Furthermore, due to the close proximity of the rectum to the scope portal there would potentially be an increased risk of septic peritonitis as it would be difficult to keep the scope from carrying contaminates from rectal palpation into the abdomen when advanced through the vaginal wall.

Intra-operatively two mares developed perineal emphysema. One mare had a left sided approach and one was on the right. Nothing in the procedure of these two mares was unusual in relation to the other cases. No treatment was given for this condition and from the time of initial onset the emphysema had completely resolved within 12 hours. Though no positive pressure insufflation of the abdomen was used, or deemed necessary to obtain adequate visualization, room air entered the abdomen to equilibrate the normal negative intra-abdominal pressure. This natural insufflation allowed for visualization within the abdomen, but may have also caused the subcutaneous emphysema noted. It is also the author's belief that the emphysema was likely the result of the air that remained in the abdomen dissecting caudally from the wall of the vagina through the perineal tissues.

In seven of eight mares the procedure was well tolerated with no apparent post-operative complications. The remaining mare's vital signs remained within normal range for her entire recovery; she began to show mild signs of abdominal discomfort (pawing and laying down) on day five post-operatively. The origin of colic signs in this mare is not certain. Likely causes could include the common causes such as gas, enteric spasm, or a mild impaction. Other possible causes due to the surgical procedure such as pain at the incision site, peritonitis, or even adhesions, however, cannot be ruled out. Treatment of the mare with intravenous flunixin meglumine, in combination with mineral oil and water via a nasogastric tube resulted in resolution of colic behavior. This mare was monitored for the remaining two days with no additional signs of discomfort. The mare was turned back out into a pasture at that time and continued to do well with no additional signs of colic observed over the following 30 days.

Intra-abdominal disorders, specifically those of a gastrointestinal origin, are a leading cause of referral to a specialty center. Advances in laparoscopic techniques employed over the last couple of decades have provided the equine surgeon a minimally invasive way to visualize many of these conditions. Laparoscopy, as previously described, can be performed in a standing sedated animal thus bypassing the necessity for general anesthesia and also avoiding the potential complications of a large incision required for a ventral midline celiotomy. NOTES procedures have been developed as a means of further minimizing the invasiveness of abdominal exploration.

A single portal entry into the abdomen allows for instrumentation and visualization through the same portal and therefore permits minor procedures, such as biopsies to be performed without the need for multiple incisions. This results in minimal scar formation and has been theorized in humans to result in less post-operative pain and reduced hospitalization times thus resulting in a faster return to normal activity.[36] While a single portal technique can be accomplished using an operating laparoscope, an endoscope also lends itself particularly well, is readily available in longer lengths, and does not rely on a direct or straight path as would a rigid laparoscope. As endoscopes and instruments continue to become more sophisticated in their capabilities and they become more accessible to the veterinarian it is reasonable to expect that the technical level and operative capabilities of NOTES procedures will also advance, just as it has in the human realm. While a standard single portal scope may not be able to perform much more than a small biopsy, simply the addition of a second portal has the potential to allow for much larger sections of tissue to be removed, whether that be for the purpose of diagnosis or treatment. These benefits are all in reference to a pure NOTES technique. That is to say that the only portal created is through a natural orifice, or in this study through the vaginal wall.

Not addressed in the design of this study however, is the possibility of a combination or modified NOTES approach. Further studies and techniques could be developed to include combining this approach with a traditional flank approach. This would provide the surgeon with an orthogonal view of the abdomen as well as give a more three dimensional appreciation of the visceral anatomy for various procedures. Though this would require a team approach, it may prove useful in mares where complex instrumentation is required through a narrow paralumbar fossa.

One concern of using a NOTES approach in horses is the post-operative formation of adhesions at the entry site. NOTES has been theorized to reduce the incidence of adhesion formation in humans, but has been reported with varying frequency in experimental swine models.[33,34] This is of particular importance to equine patients and until further studies are performed any claims of increased, or decreased frequency of adhesion formation in relation to other abdominal approaches can not be made. Other potential disadvantages include the technical difficulty in performing this procedure. Though we did not use total operating time as an evaluation of the procedure in this study, it was noted that the first several cases took longer than the last procedure. This is attributed to learning to maneuver the scope throughout the abdomen in an efficient manner. A surgical team greatly improves the efficiency of the procedure by having one person pass the scope and guide it intra-vaginally when needed with a second person controlling the scope's visual angle. This has been reported in experimental swine models in which total exploration time needed to identify all pertinent abdominal viscera was less than three minutes.[36]

It is the author's opinion that the overall visualization within the abdomen using a transvaginal approach is satisfactory and that this study validates such an approach for the diagnosis of numerous disorders using standard equipment available in most referral hospitals. Furthermore, with continued innovations and with the introduction of more advanced operating endoscopes,[26] the possibility of further developing a transvaginal approach to the abdomen for therapeutic applications should be considered.

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Appendix A: Equipment Manufacturers

a. Endoscope

   Manufacturer: Fujinon, Inc.

      10 High Pointe Drive

      Wayne, NJ 07470

   Model: EC-450HL5

   Working length: 169 cm

   Diameter: 12.8 mm

b. Laparoscope

   Manufacturer: Richard Wolf Medical Instruments Corp.

      353 Corporate Woods Pkwy

      Instruments Corp. Vernon Hills, IL 60061

   Working length: 62 cm

   Diameter: 10 mm

Appendix B: Summary of Visualization

Frequency of observed abdominal viscera with the use of a left of right sided transvaginal approach using an endoscope or laparoscope in mares

Viscera	Approach and scope			
	Left sided endoscope (n=4)	Right sided endoscope (n=4)	Left sided laparoscope (n=4)	Right sided laparoscope (n=4)
Left ovary & uterine horn	3	0	3	0
Right ovary & uterine horn	1	4	0	4
Liver	3	2	0	1
Diaphragm	4	4	4	4
Greater curvature of the stomach	3	0	0	0
Spleen	3	0	4	0
Small colon	4	4	4	4
Left Kidney	4	0	4	0
Right Kidney	0	0	0	0
Cecum	1	4	1	4
Duodenum	1	4	1	4
Bladder	4	4	4	4
Jejunum	4	4	4	4
Caudal peritoneal reflection (left)	3	0	0	0
Caudal peritoneal reflection (right)	1	4	0	0

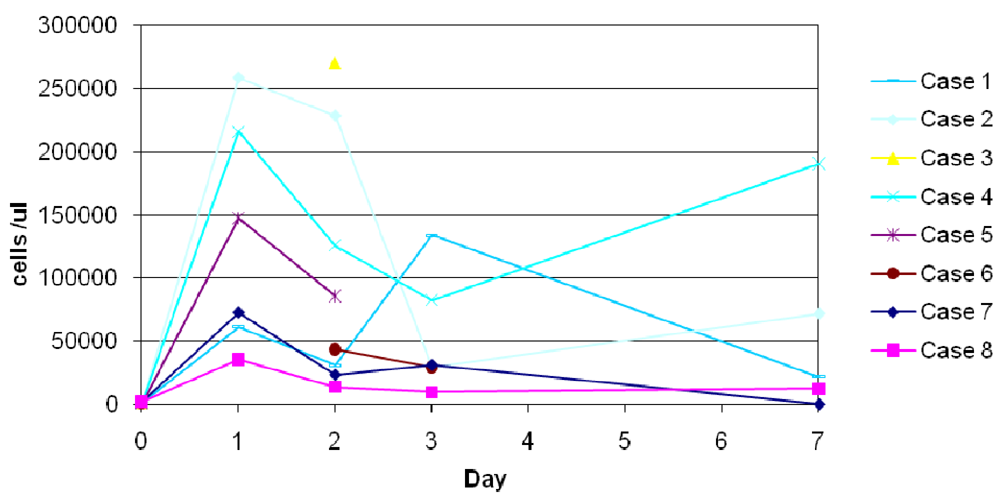
Appendix C: Peritoneal Fluid Analysis (Total Nucleated Cell Count)

A. Total nucleated Cell Count (TNCC) per µL

Horse Number	1	2	3	4	5	6	7	8
Side of Approach	right	left	right	left	right	left	right	left
TNCC Day 0	1260	1620	1400	1360	620	1210	2020	2170
TNCC Day 1	61200	258380		215940	147560		35470	73000
TNCC Day 2	30820	228370	270000	125710	85790	43430	13940	23570
TNCC Day 3	133950	29100	*	82480	*	29610	9850	31320
TNCC Day 7	21840	71750	*	190630	*	*	12360	112.98

*Unable to obtain abdominal fluid

B. Graph of total nucleated Cell Count (TNCC) per µL



C. Statistical analysis of left and right side approach (TNCC)

t-Test: Two-sample assuming unequal variances

Abdominocentesis Total Nucleated Cell Count, Day 0**

	left	*right*
Mean	1590/ul	1325/ul
Variance	178200	329966.6667
Observations	4	4
Hypothesized Mean Difference	0	
df	6	
t Stat	0.743485979	
P(T<=t) two-tail	0.485271162	
t Critical two-tail	2.446911846	

Abdominocentesis Total Nucleated Cell Count, Day 1**

	left	*right*
Mean	182440/ul	81410/ul
Variance	9433123600	3447375100
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	1.541858933	
P(T<=t) two-tail	0.220773119	
t Critical two-tail	3.182446305	

Abdominocentesis Total Nucleated Cell Count, Day 2**

	left	*right*
Mean	105270/ul	100137.5/ul
Variance	8690159733	13764681492
Observations	4	4
Hypothesized Mean Difference	0	
df	6	
t Stat	0.068502112	
P(T<=t) two-tail	0.947611788	
t Critical two-tail	2.446911846	

Abdominocentesis Total Nucleated Cell Count, Day 3**

	left	*right*
Mean	43127.5 /ul	71900 /ul
Variance	689176625	7700405000
Observations	4	2
Hypothesized Mean Difference	0	
df	1	
t Stat	-0.453659232	
P(T<=t) two-tail	0.728868238	
t Critical two-tail	12.70620473	

Abdominocentesis Total Nucleated Cell Count, Day 7**

	left	*right*
Mean	87497.66 /ul	17100 /ul
Variance	9260175324	44935200
Observations	3	2
Hypothesized Mean Difference	0	
df	2	
t Stat	1.262508769	
P(T<=t) two-tail	0.334037623	
t Critical two-tail	4.30265273	

**No signficant difference in results
*** Indicates a significant difference

D. Statistical analysis of days one thru seven (TNCC)

t-Test: Two-sample assuming unequal variances

Day 0 to Day 1 TNCC***

	Day 0	*Day 1*
Mean	1457.5 /ul	131925 /ul
Variance	237850	8214317750
Observations	8	6
Hypothesized Mean Difference	0	
df	5	
t Stat	-3.526042161	
P(T<=t) one-tail	0.008405245	
t Critical one-tail	2.015048372	

Day 0 to Day 2 TNCC***

	Day 0	*Day 2*
Mean	1457.5 /ul	102703.75 /ul
Variance	237850	9631029827
Observations	8	8
Hypothesized Mean Difference	0	
df	7	
t Stat	-2.917979346	
P(T<=t) one-tail	0.011202199	
t Critical one-tail	1.894578604	

Day 0 to Day 3 TNCC***

	Day 0	*Day 3*
Mean	1457.5 /ul	52718.33333 /ul
Variance	237850	2174348777
Observations	8	6
Hypothesized Mean Difference	0	
df	5	
t Stat	-2.692643569	
P(T<=t) one-tail	0.021580989	
t Critical one-tail	2.015048372	

Day 0 to Day 7 TNCC**

	Day 0	*Day 7*
Mean	1457.5/ul	59338.596 /ul
Variance	237850	6128070622
Observations	8	5
Hypothesized Mean Difference	0	
df	4	
t Stat	-1.65331117	
P(T<=t) one-tail	0.086805477	
t Critical one-tail	2.131846782	

**No signficant difference in results
*** Indicates a significant difference

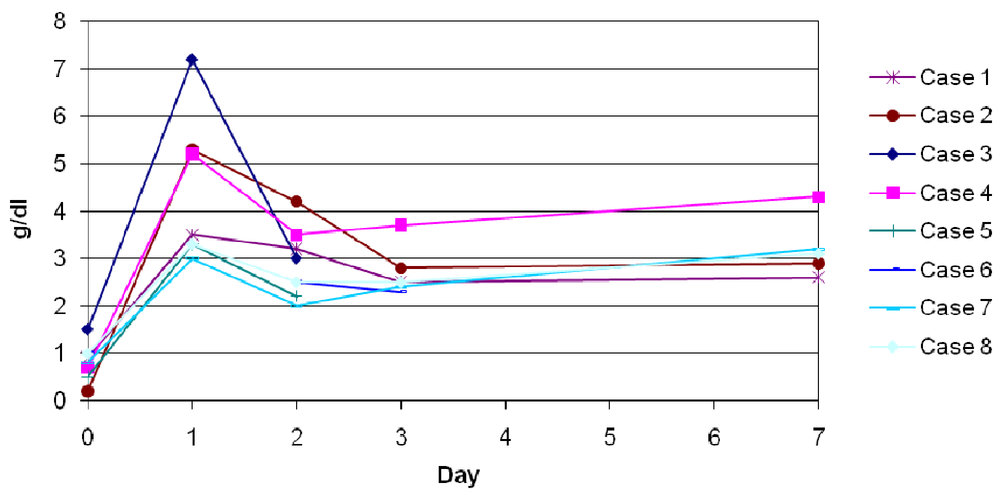
Appendix D: Peritoneal Fluid Analysis (Total Protein)

A. Total protein (TP) in grams per dL

Horse Number	1	2	3	4	5	6	7	8
Side of Approach	right	left	right	left	right	left	right	left
TP Day 0	0.9	0.2	1.5	0.7	0.5	1	1	0.8
TP Day 1	3.5	5.3	7.2	5.2	3.3		3.3	3
TP Day 2	3.2	4.2	3	3.5	2.2	2.5	2.5	2
TP Day 3	2.5	2.8	*	3.7	*	2.3	2.5	2.4
TP Day 7	2.6	2.9	*	4.3	*	*	3.1	3.2

*Unable to obtain abdominal fluid

B. Graph of total protein (TP) in grams per dL



C. Statistical analysis of left and right side approach (TP)

t-Test: Two-sample assuming unequal variances

Abdominocentesis Total Protein, Day 0**

	left	*right*
Mean	0.675 g/dl	0.975 g/dl
Variance	0.115833333	0.169166667
Observations	4	4
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.123902974	
P(T<=t) two-tail	0.303999383	
t Critical two-tail	2.446911846	

Abdominocentesis Total Protein, Day 1**

	left	*right*
Mean	4.5 g/dl	4.325 g/dl
Variance	1.69	3.6825
Observations	3	4
Hypothesized Mean Difference	0	
df	5	
t Stat	0.143657133	
P(T<=t) two-tail	0.891381533	
t Critical two-tail	2.570581835	

Abdominocentesis Total Protein, Day 2**

	left	*right*
Mean	3.05 g/dl	2.725 g/dl
Variance	0.976666667	0.209166667
Observations	4	4
Hypothesized Mean Difference	0	
df	4	
t Stat	0.596899932	
P(T<=t) two-tail	0.582717572	
t Critical two-tail	2.776445105	

Abdominocentesis Total Protein, Day 3**

	left	*right*
Mean	2.8 g/dl	2.5 g/dl
Variance	0.406666667	0
Observations	4	2
Hypothesized Mean Difference	0	
df	3	
t Stat	0.940875072	
P(T<=t) two-tail	0.416179713	
t Critical two-tail	3.182446305	

Abdominocentesis Total Protein, Day 4**

	left	*right*
Mean	3.466666667 g/dl	2.85 g/dl
Variance	0.543333333	0.125
Observations	3	2
Hypothesized Mean Difference	0	
df	3	
t Stat	1.249401225	
P(T<=t) two-tail	0.300119835	
t Critical two-tail	3.182446305	

**No signficant difference in results
*** Indicates a significant difference

D. Statistical analysis of days one thru seven (TP)

t-Test: Two-sample assuming unequal variances

Day 0 to Day 1 TP**

	Day 0	*Day 1*
Mean	0.825 g/dl	4.4 g/dl
Variance	0.147857143	2.413333333
Observations	8	7
Hypothesized Mean Difference	0	
df	7	
t Stat	-5.931667422	
P(T<=t) one-tail	0.00029033	
t Critical one-tail	1.894578604	

Day 0 to Day 2 TP**

	Day 0	*Day 2*
Mean	0.825 g/dl	2.8875 g/dl
Variance	0.147857143	0.538392857
Observations	8	8
Hypothesized Mean Difference	0	
df	11	
t Stat	-7.042028396	
P(T<=t) one-tail	0.0000107436	
t Critical one-tail	1.795884814	

Day 0 to Day 3 TP**

	Day 0	*Day 3*
Mean	0.825 g/dl	2.7 g/dl
Variance	0.147857143	0.268
Observations	8	6
Hypothesized Mean Difference	0	
df	9	
t Stat	-7.461371917	
P(T<=t) one-tail	0.0000192289	
t Critical one-tail	1.833112923	

Day 0 to Day 7 TP**

	Day 0	*Day 7*
Mean	0.825 g/dl	3.22 g/dl
Variance	0.147857143	0.417
Observations	8	5
Hypothesized Mean Difference	0	
df	6	
t Stat	-7.50337207	
P(T<=t) one-tail	0.000144925	
t Critical one-tail	1.943180274	

**No signficant difference in results
*** Indicates a significant difference

Appendix E (Ancillary Data)

*This table contains additional pre and post operative data obtained but not included in the results of the study.

Case Number	1	2	3	4	5	6	7	8
Weight (lbs)	**1100**	**1075**	**900**	**900**	**1200**	**1100**	**1000**	**900**
Weight (kgs)	**500**	**489**	**409**	**409**	**545**	**500**	**455**	**409**
Age (years)	**22**	**15**	**13**	**13**	**11**	**21**	**11**	**6**
Surgical side (R/L)	right	left	right	left	right	left	right	left
CBC								
PCV Day 0	42.5%	48.1%	44.6%	38.5%	43.7%	37.7%	47.6%	40.7%
PCV Day 1	39.2%	49.9%	42.6%	39.9%	39.1%	34.8%	42.9%	38.0%
PCV Day 2	39.1%	47.7%	40.8%	39.8%	40.9%	35.7%	45.8%	38.4%
PCV Day 3	40.3%	45.2%	41.6%	42.5%	41.2%	31.7%	41.1%	34.0%
PCV Day 7	41.3%	41.9%	39.1%	36.8%	37.3%	35.2%	44.0%	37.6%
WBC Day 0 ($x10^3$/ul)	9.46	8.24	5.87	7.29	10.35	6.12	7.31	7.62
WBC Day 1 ($x10^3$/ul)	3.69	12.59	8.44	13.02	9.62	8.77	8.32	7.5
WBC Day 2 ($x10^3$/ul)	4.17	13.19	6.01	12.01	5.99	7.77	10.22	6.56
WBC Day 3 ($x10^3$/ul)	6.86	10.9	5.81	10.3	7.94	8.14	7.42	6.25
WBC Day 7 ($x10^3$/ul)	10.75	11.5	10.36	12.13	17.88	10.25	9.08	7.53
Fibrinogen Day 0 (mg/dL)	200	100	100	200	100	400	200	400
Fibrinogen Day 1 (mg/dL)	200	400	300	500	300	400	200	200
Fibrinogen Day 2 (mg/dL)	300	500	500	500	400	400	300	300
Fibrinogen Day 3 (mg/dL)	400	400	400	400	300	500	300	300
Fibrinogen Day 7 (mg/dL)	200	400	400	500	500	500	400	300

Figures

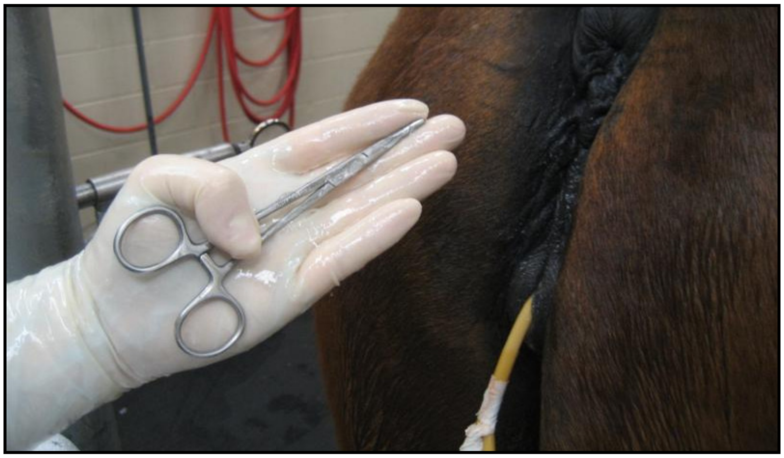


Figure 1: The colpotomy approach for the insertion of the endoscope and laparoscope is made bluntly using the tips of mosquito hemostats grasped in the palm of the surgeon’s hand.

Figure 2: The procedure is performed with the surgeon (left) guiding the scope vaginally through the incision while an assistant (right) operates the viewing angle of the flexible endoscope.

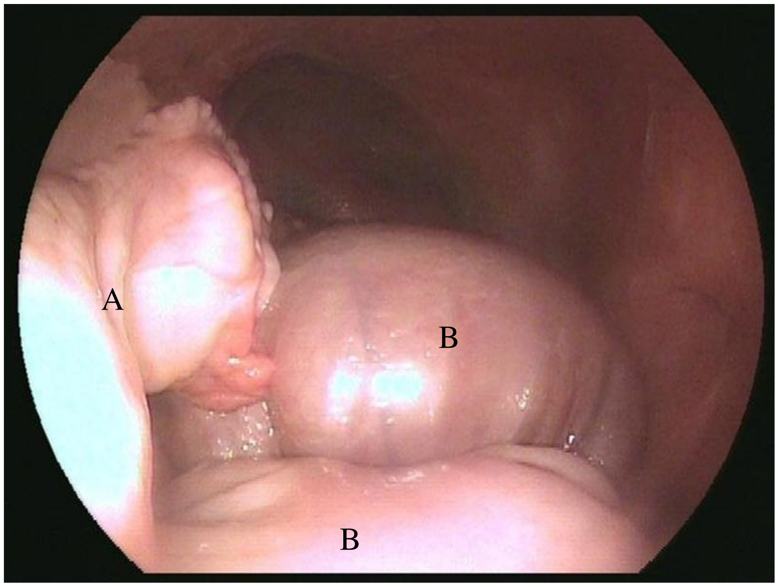


Figure 3: Caudal to cranial view of the right ovary (A) with large bowel lying both ventral and cranial (B).

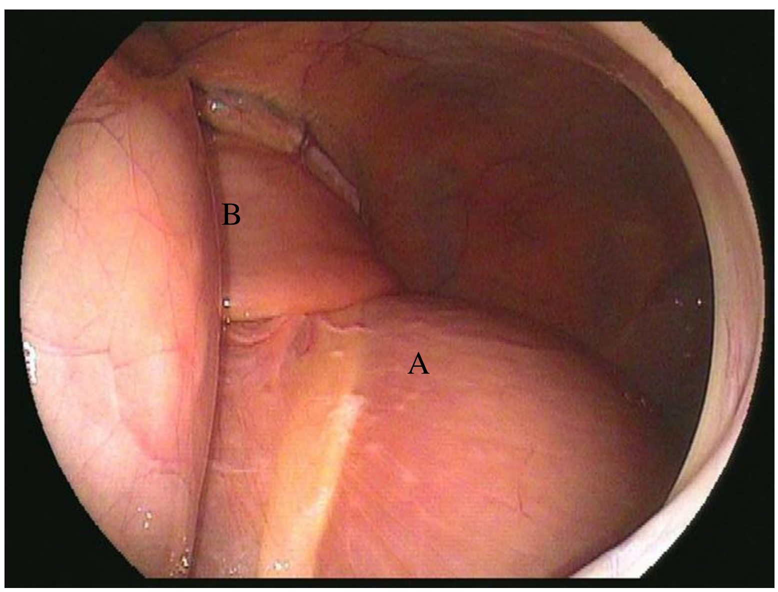


Figure 4: Caudal to cranial view of the base of the cecum (A) and the duodenum (B) as it courses from a cranial to caudal direction. A section of large colon is seen on the left of the image.

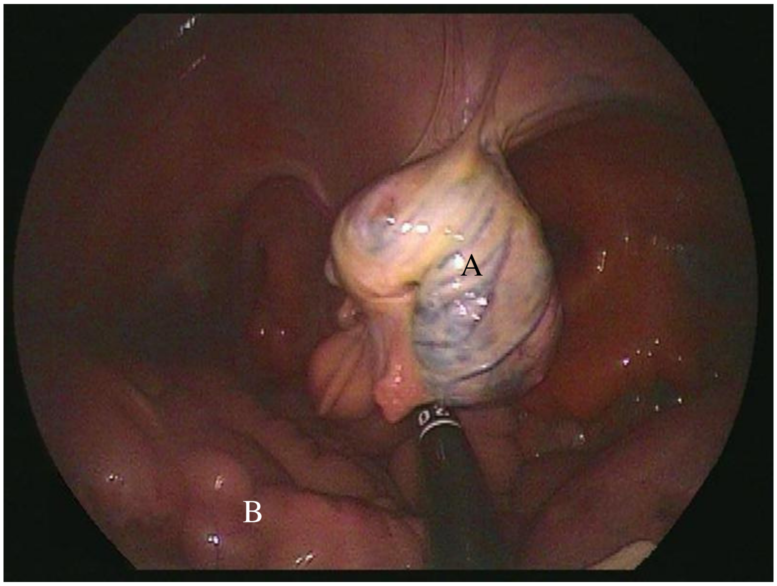


Figure 5: Cranial to caudal view of the right caudal peritoneal reflection obtained by retroflexing the scope. The right ovary is again seen (A) with small colon (B) ocated ventrally.

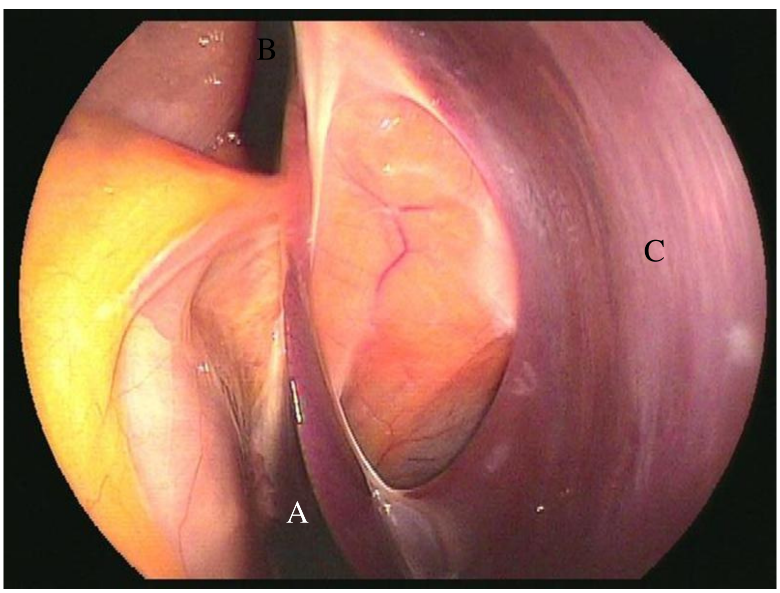


Figure 6: Caudal to cranial view of the caudal aspect of the right lobe of the liver (A) suspended by the triangular ligament (B). The diaphragm (C) is seen along the entire right aspect of the image.

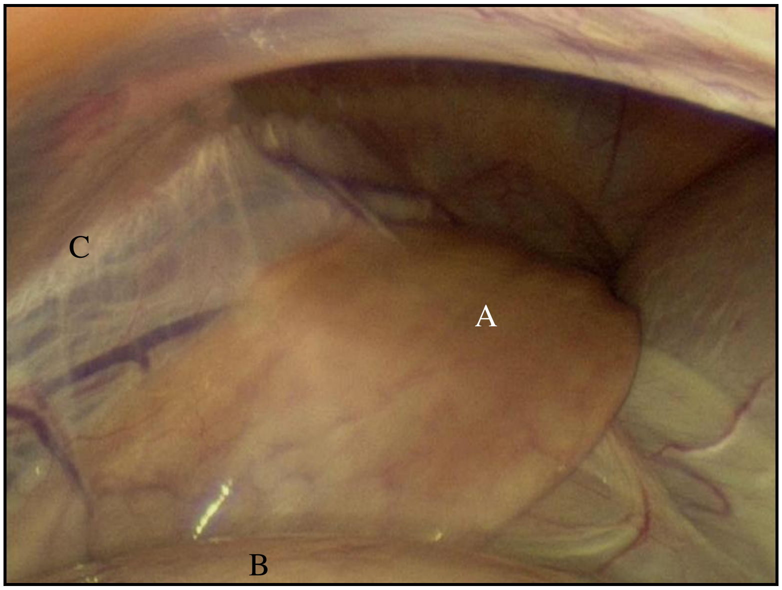


Figure 7: View of the duodenum (A) as it courses over the base of the cecum (B), note the short mesenteric attachment of the duodenum at this level (C).

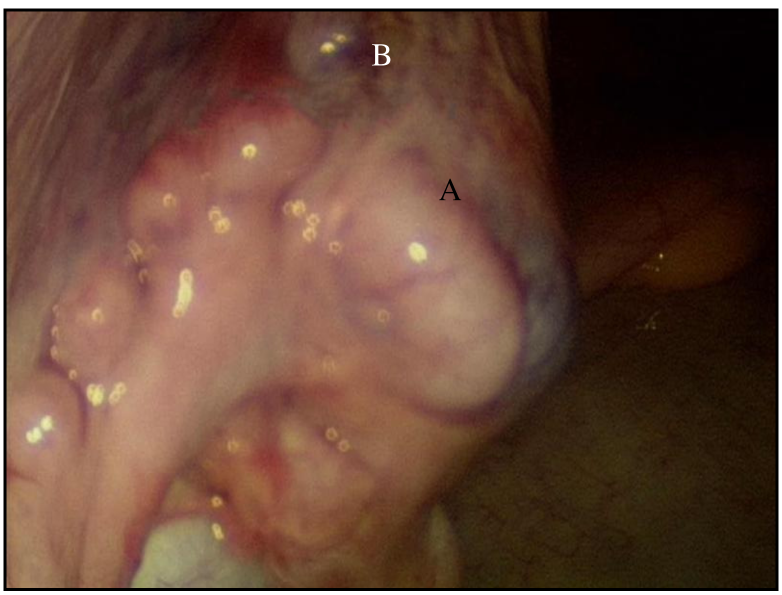


Figure 8: Caudal to cranial view of the right ovary (A) and mesovarium (B).

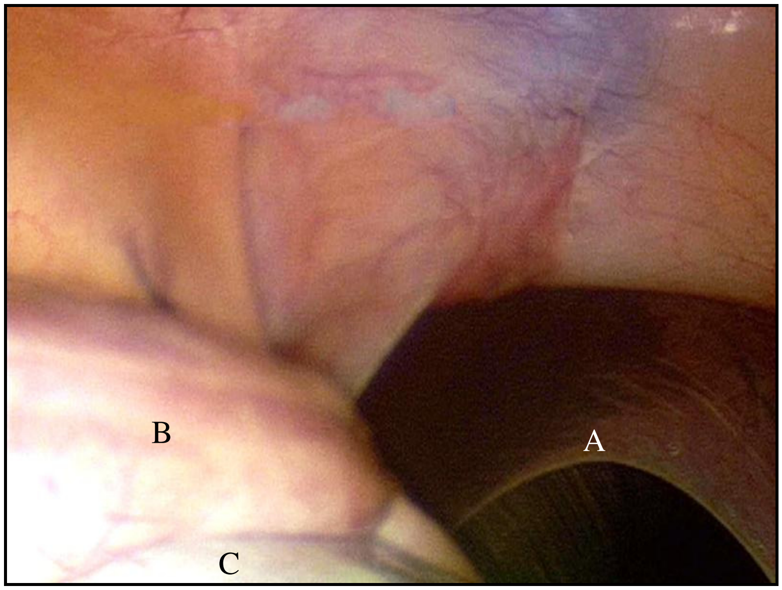


Figure 9: View of the caudal aspect of the right lobe of the liver (A), the duodenum (B) is seen suspended from the dorsal body wall as it courses around the base of the cecum (C).

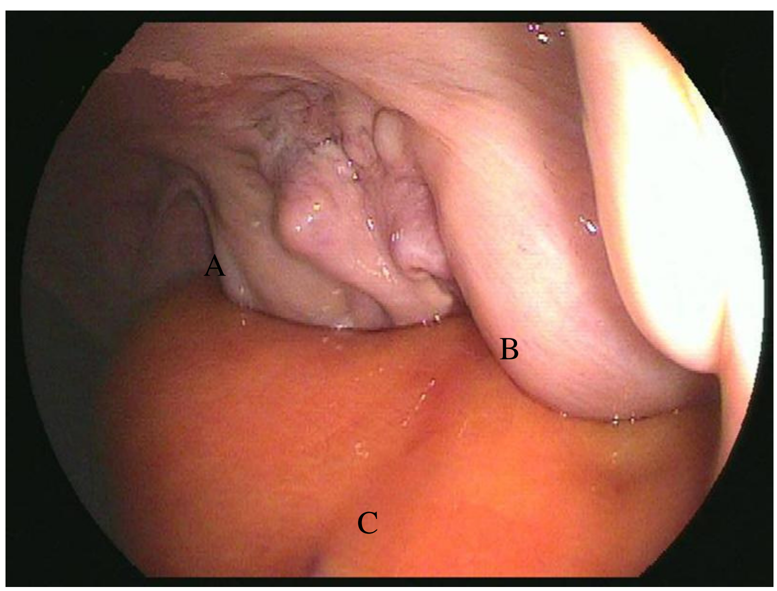


Figure 10: Caudal to cranial view of the left ovary (A) and uterine horn (B). Yellow mesenteric fat is observed in the ventral portion of the abdomen in this image (C).

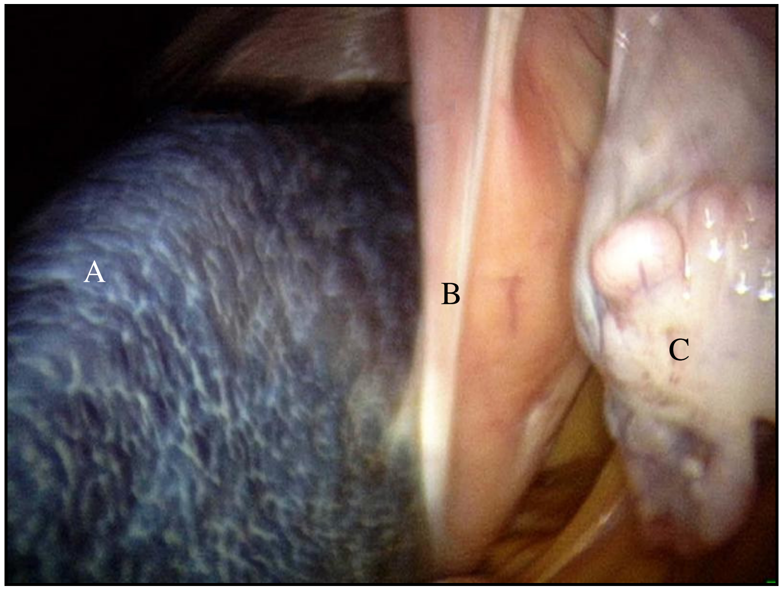


Figure 11: Caudal to cranial view of the left abdomen. The spleen (A), left kidney (B), and left ovary (C) can all be visualized in this image.

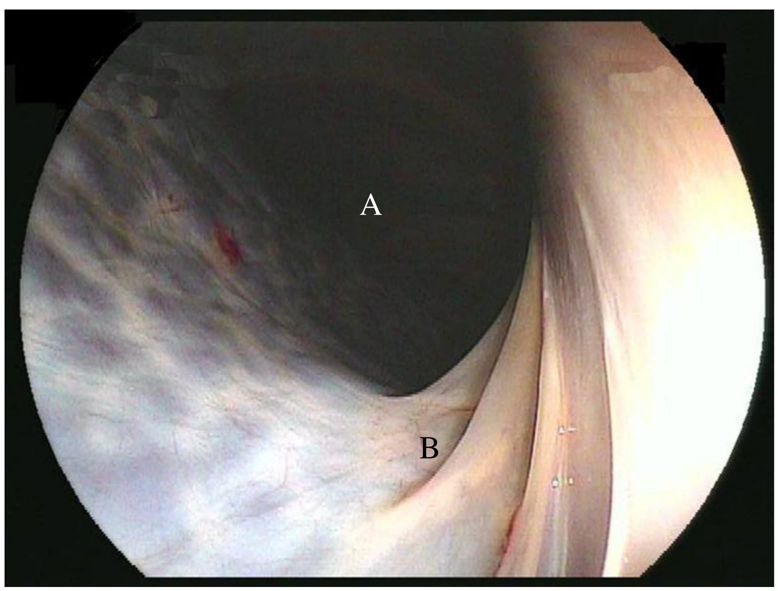


Figure 12: Caudal to cranial view of the nephrosplenic space (A) and nephrosplenic ligament (B).

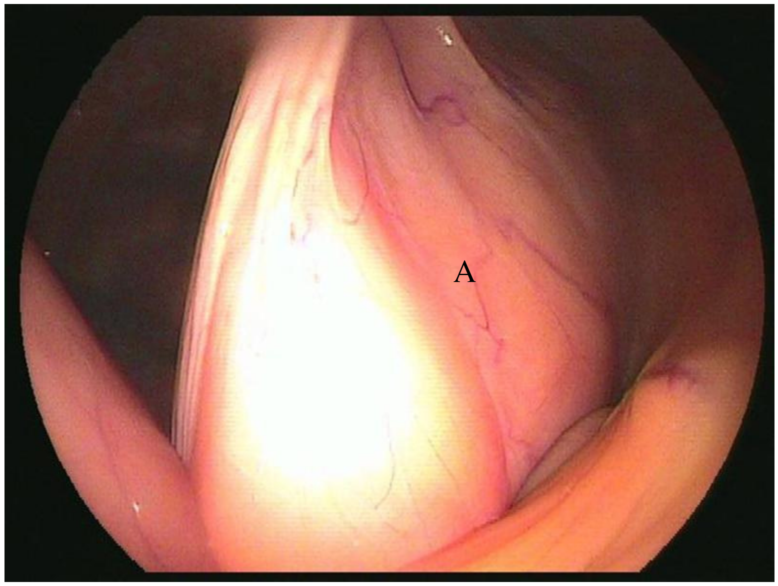


Figure 13: Caudal to cranial view of the caudal medial aspect of the left kidney (A).

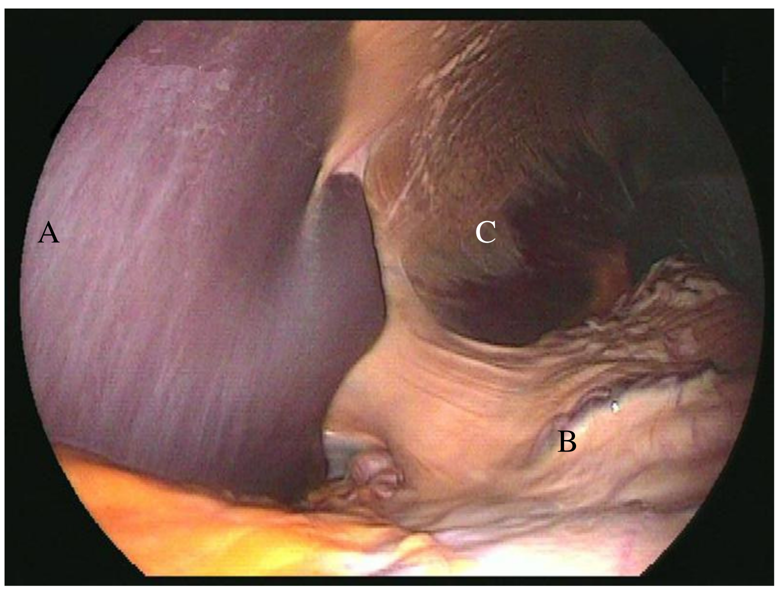


Figure 14: Caudal to cranial view of the caudal aspect of the left liver lobe (A) and stomach (B). The diaphragm is seen in the background dorsally (C).

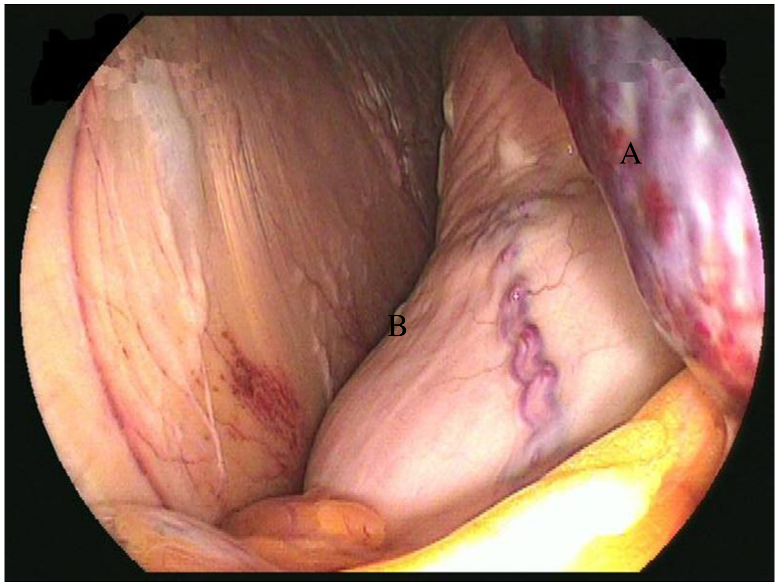


Figure 15: Caudal to cranial view of the cranial abaxial border of the spleen (A) and the underlying greater curvature of the stomach (B).

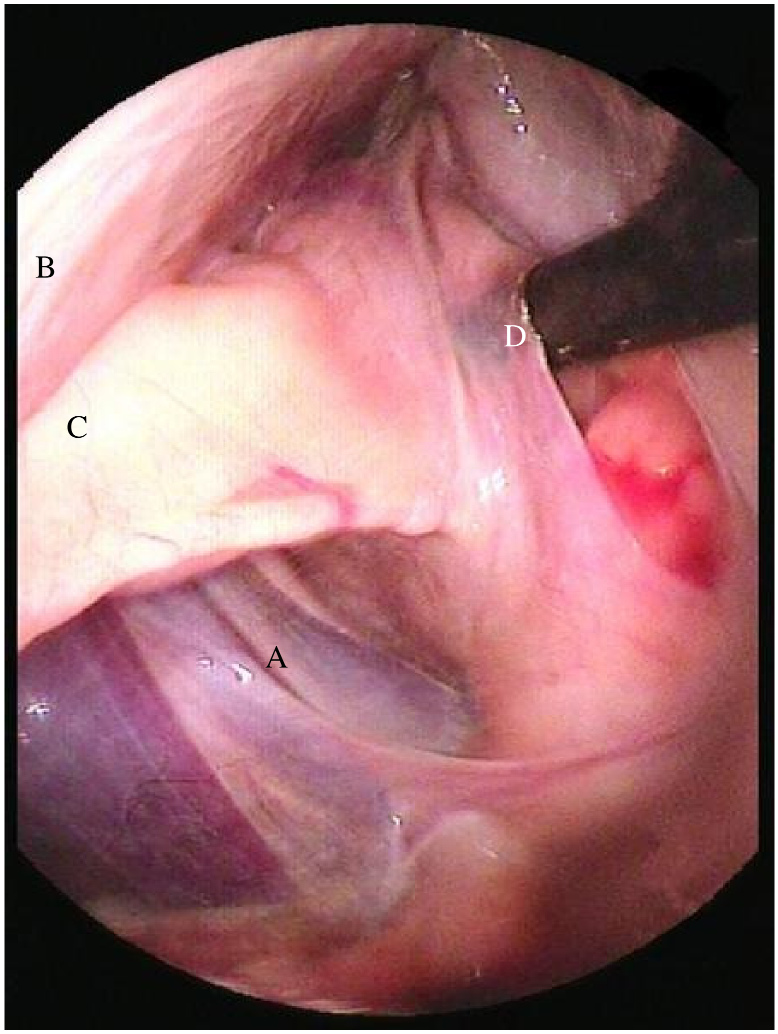


Figure 16: Cranial to caudal view of the left caudal peritoneal reflection (A) obtained by retroflexing the scope. The rectum (B) and the left uterine horn (C) are seen. The endoscope can be seen as it passes through the peritoneal opening of the approach (D).

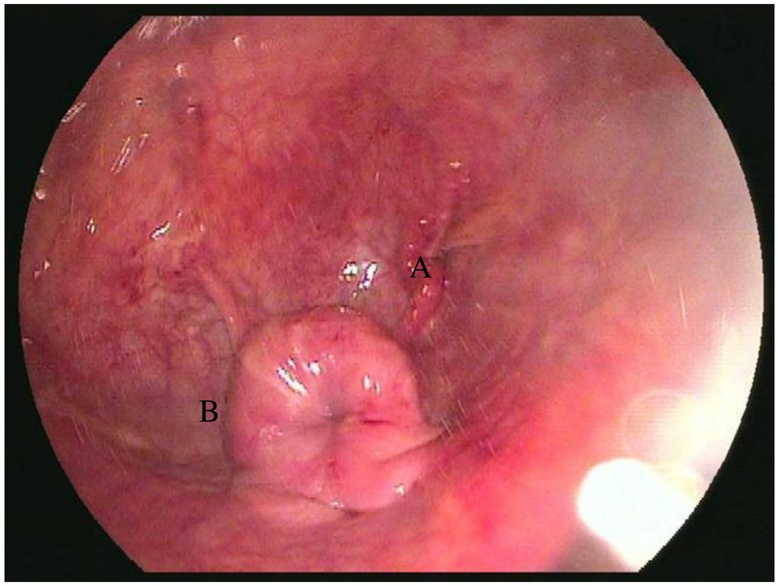


Figure 17: View of a 3 day post-operative surgical incision (A) made at the 2 o'clock position lateral to the cervix (B).

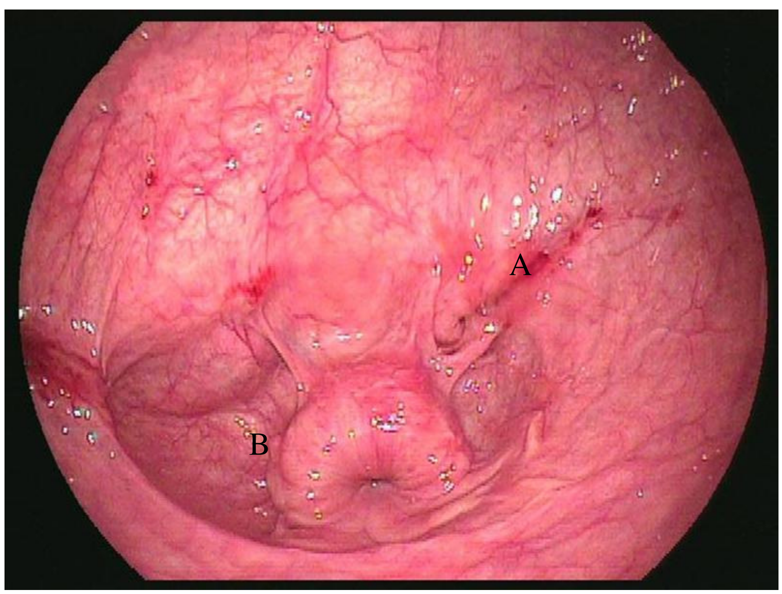


Figure 18: View of a 7 day post-operative surgical incision (A) made at the 2 o'clock position lateral to the cervix (B).