



# Use of Tubo-Ovarian Ligation Via Colpotomy as A Potential Method for Sterilization in Mares

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## ABSTRACT

The goal of this study was to develop a safe, effective, and economical method for permanent sterilization of mares based upon tubo-ovarian ligation performed via colpotomy. In this study, we evaluated the application of a nylon cable tie (zip-tie) to the ovarian pedicle and oviduct of mares to induce ovarian ischemia and tubal ligation without removal of ovaries. Initially, efficiency of zip-ties on the ovarian pedicle was tested *in vitro* and *in vivo*. Based on the absence of leakage through the zip-tie ligated vessels in anatomic specimens, we confirmed the potential efficacy of the technique. Next, ligation of the ovarian pedicle via a standing colpotomy was conducted in five mares. Although the surgical procedure in these mares appeared to be quick and efficient, all five mares were noted to develop ovarian adhesions to surrounding abdominal viscera in either one or both ovaries postoperatively. Ovarian ischemia led to loss of ovarian activity based upon ultrasound examination, which was confirmed by a low plasma progesterone concentration in four of the five mares. During the postoperative period, four mares demonstrated clinical signs related to the ovarian adhesions and were euthanized. The postoperative complications associated with ovarian adhesions to abdominal viscera presented significant challenges, limiting the success of this study. While this technique resulted in ovarian ischemia and atrophy in four out of the five mares, we were unable to assess long-term effects on the health and reproduction of the mares due to the ovarian adhesions to the surrounding tissues and the potential for secondary complications. Although technically feasible, tubo-ovarian ligation via colpotomy does not appear to be a viable option for sterilization of mares using the described technique due to ovarian adhesions post procedure.

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## 1. Introduction

Several methods have been proposed for fertility control in equids, including surgical ovariectomy, immunocontraceptives directed against the zona pellucida or GnRH, and use of intrauterine devices, among others. For decades, these methods have aided

in behavioral modification, preparation of mount mares for semen collection, embryo transfer recipients, sterilization for breed registrations, research purposes and, infrequently, treatment of chronic colic associated with ovulation [1–5]. Several approaches have been described for ovariectomy, including vaginal or colpotomy, flank, oblique paramedian, ventral midline incisions, and laparoscopic technique [6]. Today, the colpotomy incision (which was introduced in 1903 by Williams) is one of the most common techniques, providing access to the ovary in the standing mare via an incision in the cranial vagina [6,7]. Ovaries are removed, and hemostasis is achieved via the use of a chain écraseur, which simultaneously cuts and crushes the ovarian pedicle [1–3,6,7]. Although effective, the ovariectomy procedure may be accompanied by a high rate of complications (approximately 4% in one study [5]) due primarily to excessive hemorrhage from the ovarian pedicle, limiting its application [8]. To address this common complication of excessive hemorrhage, Cokelaere et al. successfully demonstrated in 10 mares the

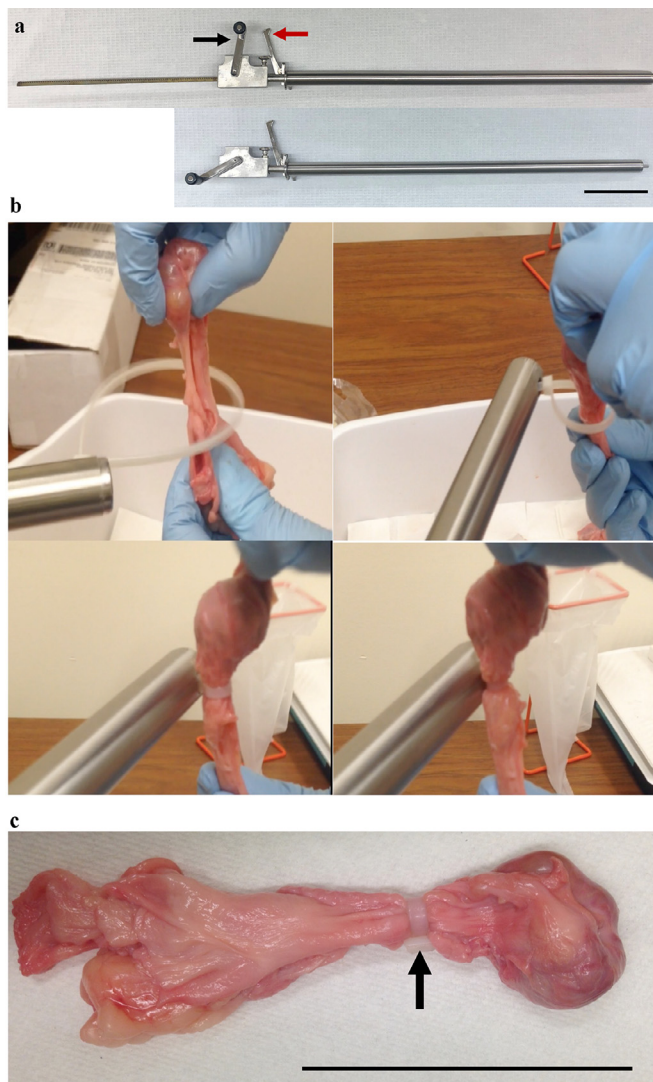
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**Fig. 1.** The designed instrument and application of zip-tie on the postmortem specimens.

a. A prototype instrument has been designed for this application by Aires Surgical Instruments, Davis, CA. After forming a loop, the end of the zip-tie was placed in the instrument. Turning the handle (black arrow) reduce the size of the loop. Once reached to the minimum size of the tie, the second handle (red arrow) can cut the zip-tie. The remaining portion of the zip-tie stays in the instrument. Bar = 10 cm  
 b. The loop of the cable tie was placed over the ovary to incorporate the mesovarium along with the oviduct. The loop was closed over the ovarian pedicle and oviduct. When the loop was closed snugly around the pedicle, the loop of the cable tie was tightened. The end of the cable tie was trimmed using the instrument.  
 c. A tightened zip-tie around the ovarian pedicle (arrow). Bar = 10 cm

application of a zip-tie to achieve hemostasis of the ovarian pedicle prior to ovarian excision by sharp dissection [9]. However, with all the modifications, upon the removal of ovaries, the likelihood of hemorrhage is not eliminated.

To further reduce the risk of significant hemorrhage from the ovarian pedicle, an alternative approach to ovariectomy in mares has been described involving the application of ligatures to the ovarian pedicle via laparoscopy without removal of the ovaries. Initially, coagulation of ovarian pedicles without removal of the ovaries was introduced in young female horses due to the smaller size/ underdeveloped ovarian pedicles [2,10]. With this procedure, the ovarian pedicle is ligated via flank laparoscopy, and the ovary undergoes ischemic necrosis or atrophy secondary to a loss of blood supply [2]. Recovery of mares subsequent to the procedure is

rapid, and loss of ovarian function is complete [2]. Furthermore, in a study by Rabelo et al., application of the zip-tie was performed through a surgical laparotomy in 12 mares [11]. On the left side, the ovary was excised after application of a zip-tie to the ovarian pedicle. On the right side, the zip-tie was placed on the ovarian pedicle without removal of the ovary. Mares were examined 15 to 45 days postoperatively for gross and histologic changes in the right ovary which had been ligated *in situ* with the zip-tie. The authors reported evidence of atrophy and ischemia of the right ovary subsequent to ligation without evidence of adhesion or abscess formation in the 12 mares examined [11]. Although long-term evaluation of the biocompatibility of these nylon zip-ties was not reported in these studies, there was complete encapsulation of the ovarian stump and zip-tie within three to four weeks after application [11].

Nylon (polyamide) zip-ties (cable ties or tie wraps) have been used in a number of experimental and clinical surgical applications [2,9,11–13]. Zip-ties have also been used in other species for ligation and hemostasis during ovariohysterectomy without the complications associated with the implanted foreign body [12,13]. Nylon cable ties are not approved medical devices for the horse; however, they can be heat sterilized and are available in a range of lengths and strengths that lend themselves to the proposed application in this study. As noted earlier, nylon cable ties have been used to ligate the ovarian pedicle in mares for ovariectomy as well as to induce ischemia and atrophy of the ovary *in situ* [2,9,11]. Hemostasis was reported to be effective in this method, thereby establishing the use of these devices to effectively control ovarian blood flow via laparoscopy.

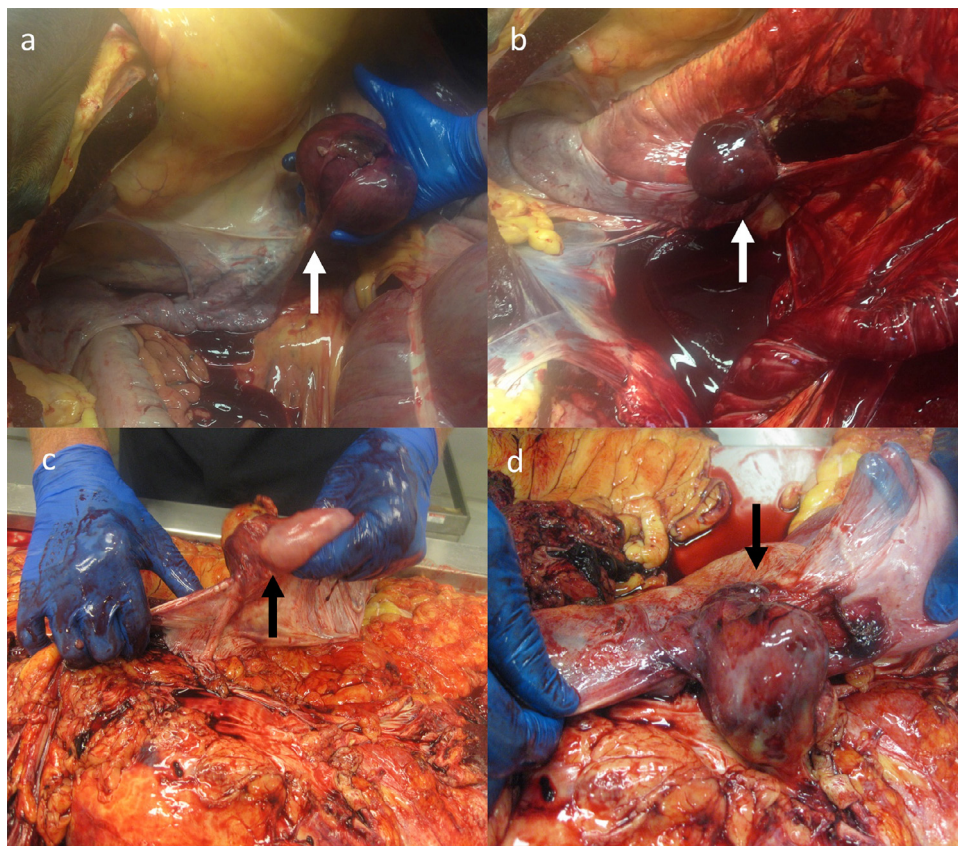
In the current study, prior to surgery on live mares, the zip-tie ligatures were tested for leakage on postmortem ovarian pedicle specimens. Using a custom-designed device for ligature application, tightening, and cutting, we then successfully attempted placement of the zip-tie ligatures via colpotomy technique (confirming placement and security on necropsy). Finally, we hypothesized that tubo-ovarian ligations via colpotomy using zip-ties (applied with our in-house device) is a viable alternative to existing techniques to induce ovarian necrosis/degeneration secondary to ischemia. We envisioned that this approach would provide a safe, economical, and effective method of sterilization of female equids while standing in a field setting.

## 2. Material and Method

### 2.1. Animal care and ethics

This study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky, Lexington, KY approved this protocol (number 2015-2119). Mares were transrectally scanned and their serum progesterone concentrations were analyzed prior to the study to assure their cyclicity. After the colpotomy, mares were humanely euthanized by pentobarbital overdose following the American Veterinary Medical Association (AVMA) guidelines for the euthanasia of animals.

Mares were restrained in stocks and sedation achieved using detomidine HCl (0.01–0.02 mg/kg; iv) and xylazine HCl (0.2 mg/kg, IV) followed by butorphanol tartrate (0.044–0.066 mg/kg; iv). Additionally, caudal epidural anesthesia was performed prior to the surgery, using 1.0–1.25 ml of 2% lidocaine/100 kg in combination with 0.17 mg/kg of xylazine. No local anesthesia was used in the cranial vagina. Lidocaine (2%) was applied topically to the ovarian pedicle prior to ligation. Adequacy of sedation and analgesia were assessed by the animal's response, as well as by determination of heart rate and respiratory rate. During surgery, heart rate and res-



**Fig. 2.** Ovarian adhesions to the surrounding tissues post-surgery  
a and b. the left ovary was adhered to mesentery of descending colon (arrow), Mare #2.  
c. The left ovary adhered to descending colon mesentery and left uterine horn (arrow), Mare #5.  
d. The right ovary adhered to the right uterine horn (arrow), Mare #5.

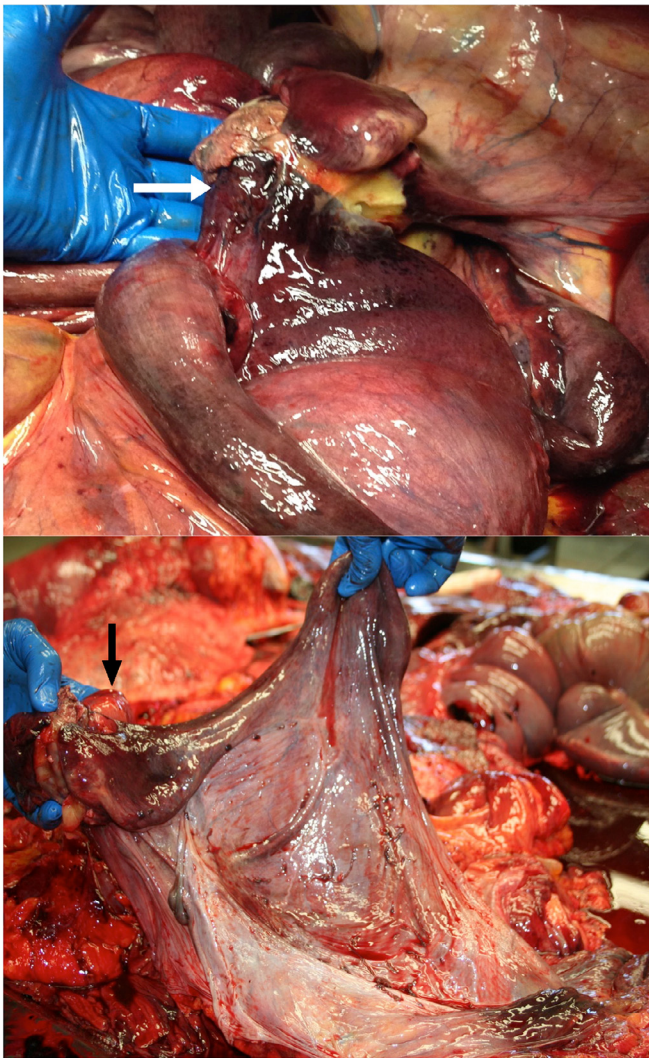
piratory rate were monitored. Surgical time (defined as the time from initiation of colpotomy incision to removal of the device from the mare's vagina), postoperative complications, postmortem and histologic findings were recorded. Mares received ceftiofur crystalline free acid (Excede – 6.6mg/kg; intramuscularly) as well as gentamicin (6.6 mg/kg; intramuscularly) and flunixin meglumine (1.1 mg/kg; intravenously).

Prior to surgery, food was withheld for 36 hours and mares returned to normal feeding after the immediate effects of sedation had subsided. Mares were held in box stalls for observation for a period of five days after surgery. Flunixin meglumine (1.1 mg/kg; PO bid) was given for three days postoperatively. All mares underwent a complete physical examination daily and were examined twice daily to determine temperature, pulse, respiratory rate as well as appetite, attitude, and pain. Preoperative and postoperative blood samples (EDTA and serum) were collected at 0, 3, and 10 days following the procedure for determination of complete blood count (CBC), serum amyloid A, and serum chemistry panel. Additionally, serum was collected, and progesterone concentration was measured before the study. Serum sample for progesterone assay was collected the day before surgery, on the day of surgery, and hourly for the first 12 hours post-operation, followed by a daily collection for seven days after the operation, and then proceed with weekly sampling until the mares were euthanized.

## 2.2. Surgical procedure

The vulva and perineum were cleaned and disinfected using chlorhexidine surgical scrub, and the vagina was flushed with di-

lute chlorhexidine solution to help dilate the vagina and decontaminate the vaginal tract. The surgeon entered the cranial vagina with a mosquito hemostat, creating a small, blunt puncture in the vaginal mucosa and submucosa at the 10 or 2 o'clock position relative to the cervix. The puncture was manually enlarged to allow insertion of one finger and then bluntly dissected to allow passage of the surgeon's hand into the abdomen. Once the abdomen was entered, the surgeon applied 2% lidocaine topically to the mesovarium and ovary by wrapping a lidocaine-saturated sterile gauze sponge (tethered with a long suture to avoid loss) around the ovarian pedicle. After anesthesia of the ovary and ovarian pedicle, the surgeon carried the preformed loop of the cable tie (<http://www.nelcoproducts.com>; length = 92 cm; width=7.62mm; minimum loop size = 4.7 mm and loop tensile strength = 54.5 kg), secured in an in-house designed instrument for application (Fig. 1; Aires Surgical, Davis CA), into the abdomen through the colpotomy incision. The ovary was identified based upon the presence of a ventral ovulation fossa, and the loop of the zip-tie was placed over the ovary to incorporate the mesovarium along with the oviduct (Fig. 1). The loop was closed over the ovarian pedicle and oviduct using care that no other viscera were entrapped. When the loop was closed snugly around the pedicle, the surgeon removed his/her arm from the abdomen, and the loop of the cable tie was tightened. The end of the cable tie was trimmed using the instrument. Both ovaries were accessed through the same colpotomy incision. The colpotomy incision was left open to heal by second intention. After application of tubo-ovarian ligatures, the dorsal lips of the vulva were anesthetized by injection of lidocaine (2%, SQ), and the vulvar lips were sutured to reduce the incidence of pneumovagina and irritation of the vagina postoperatively.



**Fig. 3.** Extensive ovarian adhesions to the surrounding tissues. Right ovary (on the top) fused to the base of cecum and mesentery of the descending colon (white arrow). Left ovary (bottom picture) developed into a large mass of connective tissue with adhesions to the colon and between spleen and stomach forming a pouch filled with serosanguineous fluid (Mare #3, arrow).

#### 2.3. Phase one: testing the strength of zip-tie for ligation of ovarian pedicle and testing the possibility of placing the ligatures via colpotomy in standing mares

A customized instrument for ligation of the ovarian pedicle via a standing colpotomy approach was designed and tested. *In vitro* testing was performed on postmortem specimens to determine ligation pressure (Fig 1). The strength of the ligature was tested by cannulating the ovarian artery, and the pressure required to induce leakage past the zip-tie ligature was determined by injecting air through the catheter by an air pump (Welch; Model #2534B-01).

Next, the efficacy of the custom-designed instrument was tested in its *in vivo* application. The tightening and trimming of zip-ties after ligation of the ovarian pedicle was performed in two mares under standing sedation via colpotomy. These two mares were euthanized immediately after surgery, the reproductive tracts were retrieved and evaluated postmortem. The ovarian artery was cannulated, and the absence of leakage through the zip-tie ligature under pressure was confirmed.

#### 2.4. Phase two: efficiency of tubo-ovarian ligations with zip-ties via colpotomy

Ligation of the ovarian pedicle via a standing colpotomy approach was conducted in five mares, and the presence of ligatures was evaluated after the surgery via ultrasound examination and at postmortem examination.

The first vaginal evaluation was performed five days post operation. After healing of the colpotomy incision, based on visual inspection by vaginoscopy, mares were examined by transectal palpation and ultrasonography to assess ovarian size, ovarian activity and blood flow. Ovarian blood flow was determined by color Doppler flow ultrasonography of the ovarian pedicle and compared to the baseline blood flow established during the preoperative period [14]. Mares were examined weekly after tubo-ovarian ligation by transectal ultrasound (SonoScape S8, SonoScape Co Ltd., Sonomed; Rome, Italy) and palpation to determine ovarian size, ovarian structures as well as blood flow. Additionally, jugular blood samples were taken for determination of serum progesterone concentrations. Serum progesterone concentration was measured by an in-house ELISA with a CV of 8.4%, a reportable range of 0.02–200 ng/mL and lower limit of detection of 0.02 ng/mL that has been previously validated for horses [15]. At the end of the observation period, mares were deemed reproductively inactive based upon the following criteria: (1) absence of follicles > 5 mm and absence of corpus luteum based on ultrasonography and (2) absence of an increase (> 1.0 ng/mL) in serum progesterone concentrations (during the breeding season).

Mares deemed to have no ovarian activity based on these criteria were euthanized and submitted for complete pathologic workup at the University of Kentucky Veterinary Diagnostic Laboratory. At necropsy, the colpotomy site, tubo-ovarian ligatures, ovary and abdominal cavity were examined for evidence of adhesions, abscesses or other pathology. The ovarian remnant was examined both grossly and by histopathology.

### 3. Results and Discussion

In the first phase of this study, we designed and constructed the required instrumentation for application of zip-tie ligatures to the ovarian pedicle of mares. The strength of the ligature was tested by cannulating the ovarian artery, and the pressure required to induce leakage through the zip-tie ligature was determined. Leakage of the ovarian artery occurred at ~630 mmHg pressure, suggesting that the ligation force obtained with this instrument should be more than adequate (normal systolic pressure/diastolic pressure <150 mmHg) to occlude ovarian blood flow using zip-tie ligatures [16]. After testing the instrument on postmortem specimens, ligation of the ovarian pedicle via a standing colpotomy approach was conducted in two mares. The surgical procedure in these mares appeared to be quick and efficient with a total operative time of  $8.0 \pm 2.6$  min (mean  $\pm$  std). The mares were euthanized immediately after surgery, and their reproductive tracts were retrieved postmortem. Zip-ties were found on the ovarian pedicles, the ovarian artery was cannulated, and absence of leakage through the zip-tie ligature under pressure was confirmed. Based upon the absence of leakage through the ligatures, we confirmed the potential effectiveness of the technique.

In the next phase, ligation of the ovarian pedicle via a standing colpotomy approach was conducted in five mares, and the presence of ligatures was evaluated after the surgery via ultrasound examination and at postmortem examination. Complete blood counts (CBC), serum amyloid A, and serum chemistry panels were performed for all the mares prior to surgery, at 3 and 10 days post-surgery and before euthanasia. The acute phase protein Serum Amyloid A was increased by at least 100-fold on day 3, but was

**Table 1**

Summary of the findings.

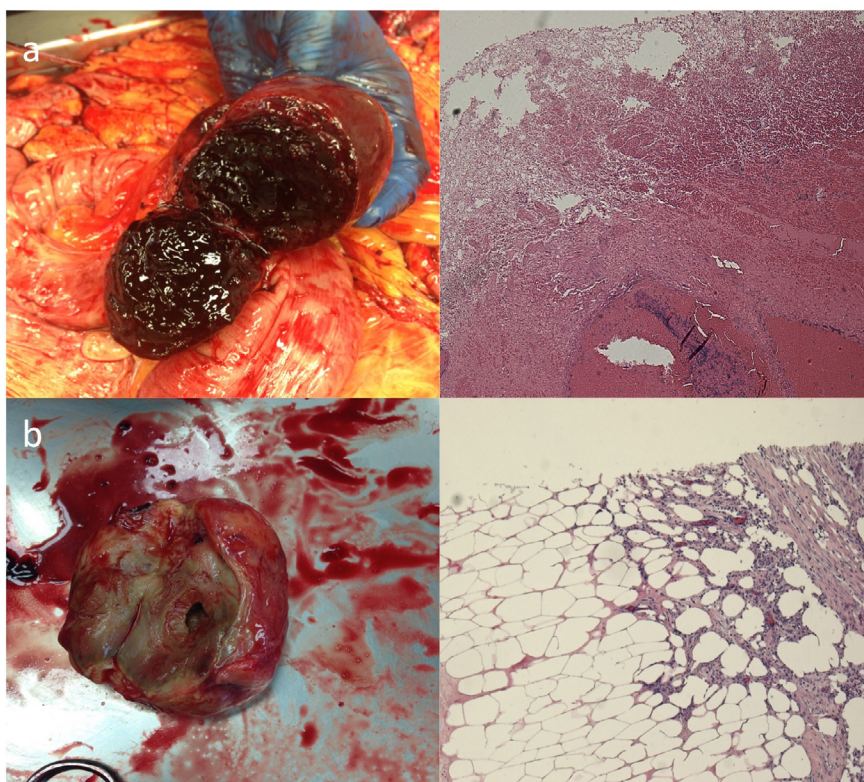
| Mare ID | Necropsy findings                                                                                                                                            |                                                                     | Interval – Surgery to necropsy | Histopathology                                                                                                                                                                                     |                       | Serum Progesterone                                                                                                            |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|-------------------------------------------------------------------------------------------------------------------------------|
|         | Left ovary                                                                                                                                                   | Right ovary                                                         |                                | Left ovary                                                                                                                                                                                         | Right ovary           |                                                                                                                               |
| Mare #1 | Focally adhered to a pedicle of mesentery / ovarian atrophy                                                                                                  | Focally adhered to a pedicle of mesentery / ovarian atrophy         | 60 days                        | Vacuolated                                                                                                                                                                                         | Non-functional tissue | 45ng/ml at T0. <1ng/ml after 96hrs post-surgery and stayed <1ng/ml until the time of euthanasia                               |
| Mare #2 | Diffusely enlarged by congestion/hemorrhage, fused to mesentery of descending colon                                                                          | Diffusely enlarged by congestion/hemorrhage.                        | 28 days                        | Infarcted ovaries characterized by subacute to chronic hemorrhage, mild suppurative inflammation, and organized fibrin clots, small foci of dystrophic mineralization, and superficial fibroplasia |                       | 55ng/ml at T0. <1ng/ml after 48hrs post-surgery and stayed <1ng/ml until the time of euthanasia                               |
| Mare #3 | Adhered to the spleen and left lateral abdomen peritoneum and contained central purulent material and fibrin (abscessation). Ovary contained a corpus luteum | Adhesion to cecum and mesentery of the descending colon             | 18 days                        | Corpus luteum was observed, non-functional granulosa cells                                                                                                                                         | Non-functional tissue | 20ng/ml at T0. <1ng/ml after 72hrs post-surgery until the time of euthanasia                                                  |
| Mare #4 | Adhesions / ovary infarcted. Moderately to markedly enlarged and infarcted. Red fibrous tissue was located around the ovary and extends to the left kidney.  | No adhesions. Ovary was mildly enlarged and had a dark red serosa   | 28 days                        | Infarcted ovary. Parenchyma was hemorrhagic and necrotic                                                                                                                                           | New vessel formation  | 12ng/ml at T0. ~5ng/ml after 48hrs post-operation, a rise around 10days, and then stayed <1ng/ml until the time of euthanasia |
| Mare #5 | Adhesions / some follicular development. Adhered to colon mesentery and left uterine horn                                                                    | Minimal adhesion / ovarian atrophy. Fused to the right uterine horn | 30 days                        | Atretic follicle                                                                                                                                                                                   | Corpus luteum         | 10ng/ml at T0. 40 ng/ml at 48hrs, <1ng/ml at 4 week, and 30ng/ml at the time of euthanasia                                    |

decreased on Day 10 to the pre-surgery level, suggesting an active inflammation after surgery that was quickly resolved. In the immediate postoperative period, the planned use of flunixin meglumine for control of postoperative pain proved inadequate, and the administration of either butorphanol tartrate (0.1 mg/kg) or a sustained-release formulation of buprenorphine (0.03–0.06 mg/kg) was required during the first 24 hours after surgery to control postoperative pain. Beyond 24 hours post-surgery, the need for narcotics to control pain was no longer required.

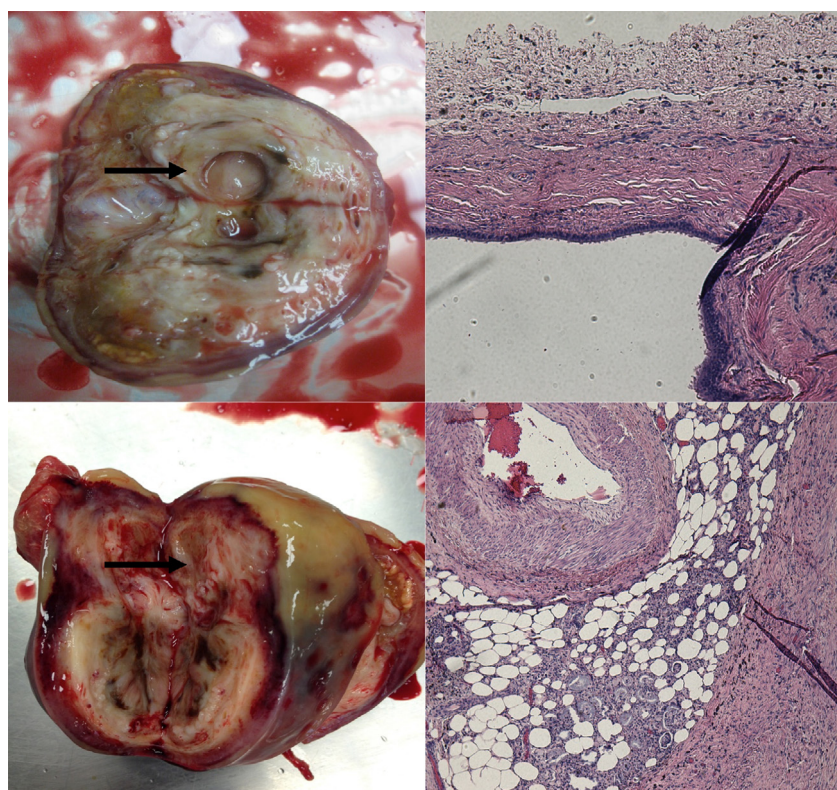
The effectiveness of the ligatures as a method for sterilization was investigated by ultrasonography of the ovary and determination of serum progesterone concentrations. Progesterone assay showed that four of the five mares had ablation of ovarian function by the time of euthanasia (Table 1). The serum progesterone concentration of three mares was <1ng/ml within 10 days after surgery (rapid drop within ~48 to 96 hours post-surgery) and remained undetectable thereafter. In one mare (Mare #4), the progesterone level dropped to 5ng/ml (initial progesterone concentration: 22ng/ml) in 48 hours after the surgery, followed by a rise around Day 10 after surgery, and then reach to <1ng/ml until the time of euthanasia. The last mare (Mare #5) showed an obvious fluctuation in the plasma progesterone concentration throughout the study, indicating an active ovary. We further monitored ovarian blood supply postoperatively in all the mares using color flow Doppler ultrasonography and ultrasound imaging. On transrectal palpation, the ovarian size was noticeably enlarged, estimated up to ~30cm. There were also signs of adhesions of the ovaries to the surrounding tissues. Due to the large size of the ovaries after the surgery and adhesion of the ovaries to the surrounding tissues, it was difficult, and in some cases impossible, to do a complete ultrasound examination of the ovaries. In the mares with fluctuating progesterone concentration (Mare #5), Doppler ultrasonography revealed evidence of ovarian blood flow after surgery. In the remaining mares, blood flow was recorded in the periphery of the ovaries, which could be due to new vessel formation or adhesion to the surrounding tissues.

We initially planned to assess the success rate of the surgery and postoperative well-being of the mares for at least two months. However, due to complications after the surgery, only one mare was maintained for 60 days after the surgery; the remaining mares were euthanized within one month of surgery due to concerns about the ovarian adhesions and secondary complications (Table 1). Necropsy findings confirmed the normal progression of healing at the colpotomy incision. As noted by transrectal palpation and ultrasound examination, ovarian adhesions to the surrounding tissues and organs were observed to some extent in all mares. Only one mare exhibited relatively minor adhesion formation to the mesentery of the descending colon (Mare #1). The remaining four mares demonstrated moderate to severe adhesions involving at least one ovary (Fig. 2 and 3). Two mares had adhesions around the left ovary; ovaries were adhered to the tip of the uterine horn, kidney or/and spleen. In a more severe case, the left ovary was enveloped in a large mass of connective tissue with adhesions to the colon, between the spleen and stomach, forming a pouch filled with serous fluid (Mare #3, Fig. 3). One mare developed adhesions on both ovaries; ovaries were adhered to the tip of the ipsilateral uterine horn and mesentery of the descending colon.

Necropsy and histopathology findings for four out of the five mares confirmed that the ovaries were non-functional (Fig. 4). Residual luteal tissue was noted on the ovaries of two mares (Fig. 5). In one of these mares (Mare #4), progesterone concentration decreased in the first 48 hours after the surgery but returned to its initial concentration in the next 6 days then reached a peak at 10 days post-surgery. As mentioned above, the progesterone concentration then started to decline, reaching <1 ng/ml at the time of euthanasia. Histopathology and necropsy findings for this mare indicate a completely infarcted left ovary and a non-functional right ovary with evidence of neovascularization. The mare with obvious fluctuations in plasma progesterone concentration (Mare #5), had viable ovaries at necropsy and on histopathological examination (Fig. 5).



**Fig. 4.** Gross and histopathology findings of ovaries after surgery. Gross and histopathology finding for these mares also confirmed that the ovaries were non-functional. a. macroscopic view of infarcted ovary (left). Microscopic finding of infarcted ovary X100 (Right), Mare #2. b. non-functional ovary (Left). Vacuolated ovarian tissue X100 (Right), Mare #1.



**Fig. 5.** Gross and histopathology findings of ovaries after the surgery. Necropsy and histopathological examination suggested the presence of viable ovaries (Mare #5). Top. Atretic follicle was noted on the left ovary (arrow). Microscopic view of the follicle X100 (Right). Bottom. Corpus luteum was noted on the right ovary (arrow). Start of vacuolation of interstitial cells in the ovary X50 (Left).



**Fig. 6.** Gross and histopathology finding of the retrieved zip-tie.

Two top pictures represent retrieve zip-tie around the ovarian pedicles. Bottom left picture represents the fixed tissue. Bottom right picture is histology representation of the tissue inside the zip-tie; thrombosis was observed inside the ligated vessels.

All applied ligatures and the surrounding tissues were retrieved after euthanasia for postmortem examination. On histological examination of the ovarian pedicles, **thrombosis was observed inside the ligated vessels** (Fig. 6). These histological findings are consistent with the placement of ligatures at the maximum tension allowed by the zip-tie to ensure complete closure of the pedicle. **On gross postmortem examination, however, some of these ligature loops appeared loose** (Fig. 6). This finding is attributed to **necrosis and shrinkage of tissues trapped inside the zip-tie**. It is possible that due to the atrophy of the pedicle and neovascularization, some degree of venous outflow and/or arterial inflow occurs, indicating by the fluctuation in the progesterone level in one of the mares.

In conclusion, although application of zip-tie ligatures to ovarian pedicles of mares via colpotomy proved a rapid, efficient, and technically simple procedure, **the post-operative complications associated with abdominal adhesions to the ovary presented significant challenges**, limiting the success of this study. Although this technique resulted in ovarian infarction or atrophy in four of the five mares, we were unable to assess long-term effects on the health and reproduction of the mares due to the frequency and extent of ovarian adhesions to the surrounding tissues and the potential for secondary complications.

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