FACULTY OF VETERINARY MEDICINE
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Vaginectomy in dogs

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Preface:

Writing a thesis combining a cadaver study and a retrospective case study was a challenging, but nonetheless a very interesting task. During this period I had the opportunity to closely work with an ECVS Diplomate and last year veterinary surgery resident. This not only helped tremendously with writing this thesis, but it also further increased my interest in the field of veterinary surgery.
It is certain the final result wouldn't have been the same without their guidance and assistance.
I would therefore like to make use of this preface to thank a couple of people in particular.

Firstly I would like to sincerely thank my supervisors Prof. dr. Hilde de Rooster and veterinarian Steven Pil. Prof. dr. Hilde de Rooster helped me understand the vaginectomy technique, guided me through the process of writing this thesis and was quick in providing constructive feedback. Veterinarian Steven Pil guided me through the cadaver study portion of this thesis and helped me interpret the results.

Secondly, I would also like to thank my parents for always supporting me throughout this long and difficult study. My friends and colleagues also deserve to be mentioned here; they helped make the last couple of years an enjoyable and rewarding experience.

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Summary:
Vaginectomy is an infrequently performed invasive surgery for, according to literature, a mostly benign vaginal process. Indications for vaginectomy include vaginal tumors (leiomyoma, fibroleiomyoma and leiomyosarcoma), polyps, vaginal prolapses etc. The surgical technique is two-folded and consists of an extended laparotomy approach with caudal ovariohysterectomy followed by an episiotomy approach with removal and reconstruction of the vaginal wall. Although the surgery itself is invasive, the combined approach results in a favourable outcome with an apparently low risk of intra- and post-operative complications.

This thesis entails a description of how vaginectomy is performed, a cadaver study with vessel mapping by using a methylene blue latex mixture and a retrospective case study of patients presented at the Ghent University Small Animal Clinic from January 2009 to December 2018 in which partial or total vaginectomy was performed.
In literature vaginal masses are described to be mostly benign leiomyomas (80%) whereas our retrospective case study hints towards a more frequent malignant leiomyosarcoma (63%) presentation. If leiomyosarcomas are more frequent compared to leiomyomas, a wide surgical resection is warranted and partial vaginectomy should be avoided. During vaginectomy, traction is placed on the vagina and its supplying blood vessels via the episiotomy site. With the cadaver study, we investigated if this traction resulted in damage or rupture of the supplying vessels. Our study demonstrated that the uterine branch of the vaginal artery and the caudal vesical artery do rupture during the traction phase of the vaginectomy via the episiotomy site. The uterine branch of the vaginal artery and the caudal vesical artery are deeply located within the pelvic cavity and thus cannot be ligated during the laparotomy approach of the surgery. Nevertheless, in none of the 32 dogs retrospectively reviewed clinically relevant postoperative haemorrhage has been reported.

Samenvatting:
Vaginectomie is een weinig frequent uitgevoerde operatietechniek die voornamelijk wordt uitgevoerd omwille van, volgens de beschikbare literatuur, een goedaardig vaginaal gezwel. Indicaties voor vaginectomie omvatten vaginale tumoren (leiomyoma's, fibroleiomyoma's en leiomyosarcoma's), poliepen, vaginale prolapsen etc. De chirurgische techniek is tweeledig en bestaat uit een uitgebreide celiotomie-benadering met caudale ovariohysterectomie, gevolgd door een episiotomiebenadering met verwijdering en reconstructie van de vaginale wand. Hoewel de chirurgische ingreep zeer invasief is, resulteert de gecombineerde benadering in een gunstige uitkomst met een ogenschijnlijk laag risico op intra- en postoperatieve complicaties.

Deze verhandeling omvat een beschrijving van de chirurgische techniek gehanteerd tijdens vaginectomie, een kadaverstudie met focus op het in beeld brengen van de omgevende bloedvaten met behulp van een methyleenblauw-latex mengsel en een retrospectieve gevallenstudie van patiënten aangeboden voor partiële of totale vaginectomie tussen januari 2009 en december 2018 op de Kliniek Kleine Huisdieren van de Universiteit Gent.
In de literatuur worden vaginale massa's beschreven als meestal goedaardige leiomyoma's (80%), maar onze retrospectieve studie wijst eerder naar een frequenter voorkomen van kwaadaardige leiomyosarcoma's (63%). In het geval van leiomyosarcoma's is een uitgebreide excisie aangewezen en zou partiële vaginectomie vermeden moeten worden.
Tijdens vaginectomie wordt manuele tractie via de episiotomie geplaatst op de vagina en de aanvoerende bloedvaten. Door middel van de kadaverstudie onderzoeken we of deze manuele tractie uiteindelijk resulteert in beschadiging of het doorscheuren van deze aanvoerende bloedvaten. Onze kadaverstudie toont aan dat de uteriene tak van de vaginale arterie en de caudale vesicale arterie scheuren tijdens de tractiefase van de vaginectomie via de episiotomie benadering. De uteriene tak van de vaginale arterie en de caudale vesicale arterie liggen zeer diep in de bekkenholte en kunnen bijgevolg niet geligeerd worden tijdens de laparotomie benadering van de chirurgische ingreep. Ondanks deze bevindingen werd in geen enkele van de 32 honden opgenomen in de retrospectieve studie klinisch relevante postoperatieve bloeding vastgesteld.

I. Introduction:

Despite the fact that our ancestors started domestication of dogs over 10,000 years ago, the genetic diversity within dog breeds has only been under severe pressure since the last few centuries. The strong and focused selection pressure inherent in the development of domestic breeds has yielded over 350 different dog breeds, ranging from the giant Great Dane to the tiny Chihuahua, in this relatively short period of time. This decline in genetic diversity unfortunately also has negative consequences such as an elevated risk for a large amount of canine tumors (Nelson, 2014). A recent study showed the incidence rate of malignant and benign tumors in dogs was approximately 800 and 900 cases per 100,000 dog-years at risk, with higher rates observed in purebred dogs and bitches (Dobson et al., 2002; Baioni et al., 2017). Vaginal and vulvar tumors represent only 0.85 to 3% of all canine tumors (Brodey and Roszel, 1967; Thacher and Bradley, 1983; Nelissen and White, 2012; Gruntzig et al., 2015).

Over 80% of the reported vaginal tumors are benign smooth muscle tumors. Most are leiomyomas and fibroleiomyomas but also fibromas and polyp formations can occur (Brodey and Roszel, 1967; Thacher and Bradley, 1983). Malignant tumors are less frequently observed and include the leiomyosarcomas, carcinomas and transmissible venereal tumors (Hill et al., 2000; Nelissen and White, 2012). Transmissible venereal tumors occur worldwide, but are mostly diagnosed in tropic and subtropical regions where they represent 37 to 60% of the malignant tumors (Nelissen and White, 2012). In our part of the world, leiomyosarcoma is the most common malignant vaginal neoplasia (Hill et al., 2000; Chiers, 2017).

Benign vaginal tumors are most often intraluminal and pedunculated, originating from the vaginal wall. They progressively grow towards the vestibular region or towards the cervix (Nelissen and White, 2012). Their size can therefore hinder the function of other organs. Benign vaginal tumors can, besides the intraluminal and pedunculated presentation, present as non-pedunculated masses with a broad base or have an irregular shape. However, these latter presentations are far less frequent compared to the pedunculated tumors. Also, extraluminal benign tumors are rare presentations (Salomon et al., 2004; Nelissen and White, 2012). Most malignant tumors have, in contrast to benign tumors, a rather broad base and show an infiltrative growth (Farese et al., 2008; Tivers and Baines, 2010; Nelissen and White, 2012).

The most frequent clinical symptom of vaginal neoplasia, besides stranguria, constipation and tenesmus due to the intrapelvic mass effect and pressure closing of the urethra, colon and rectum, is vulvar discharge, often combined with the sudden appearance of a mass bulging between the labia (Salomon et al., 2004; Purswell, 2010). Other clinical symptoms include perineal swelling, haematuria, dystocia, dyschezia, vaginal prolapse, excessive licking of the vulvar region or rather vague complaints like lethargia, pyrexia, abdominal pain, vomiting, weight loss, anorexia and exercise intolerance (Crawford et al., 2002; Nelissen and White, 2012; Weissman et al., 2013; Yap et al., 2017). Although rapid growth of a vaginal mass may hint towards a malignant process, differentiation between a benign and malignant vaginal neoplastic process based solely on the presented clinical symptoms is not possible and a further diagnostic work-up is needed.

Valuable information, besides the clinical symptoms and the suspicion of neoplasia from the anamnesis, can be obtained by performing digital vaginal and rectal palpation (Igna et al., 2016). This technique helps to differentiate between neoplasia, oedema and a prolapse. Furthermore, it additionally provides a more correct idea of the neoplasia's size, mobility and localisation (Tivers and Baines, 2010).

A tentative diagnosis of vaginal neoplasia can be made based on the signalment of the patient and the location of the tumor. However, definitive diagnosis with tumor typing can only be made after histopathological examination. Although for some tumor types, such as the transmissible venereal tumor, cytology can be diagnostic, histologic interpretation of tissue biopsies is the only possibility to diagnose leiomyosarcomas (Saba and Lawrence, 2012).

Further work-up of the patient includes a complete blood count and serum biochemistry tests to obtain information concerning the general health condition of the animal, if there is neutrophilia, thrombocytopenia or if a paraneoplastic syndrome is present. This blood work also allows detection of anaemia and clotting disorders, which is of utmost importance when proceeding with surgery later on (Nelissen and White, 2012).

To visualize the extent of the vaginal neoplasia, medical imaging techniques such as ultrasound, native radiographs, cystoscopy, contrast vaginourethrography, CT and MRI can be used (Viehoff and Sjollema, 2003; Nelissen and White, 2012; Weissman et al., 2013; Igna et al., 2016). Due to the intrapelvic localisation of the vaginal mass, native radiographs and ultrasound are of limited value and only useful as a screening tool. More information about the localisation of the vaginal mass can be obtained by cystoscopy and vaginourethrography. Additionally, vaginourethrography can help differentiation between stenosis and strictures (Weismann et al., 2013). Unfortunately, these techniques are unlikely to provide sufficient information, such as the origin of the mass and the distance from the urethral orifice, required for surgical planning. The position of the urethral orifice relative to the vaginal mass could be assessed via contrast radiography. A more exact localisation of the anatomic site of origin, delineation of the attachment to the vaginal wall, and the exact distance from the urethral orifice can be obtained with a CT scan. Furthermore, the CT scan can result in improved contrast resolution compared to radiographs. MRI is currently rarely used for vaginal neoplasia, but might become the gold standard for imaging, as leiomyomas can be differentiated from malignant vaginal tumors such as leiomyosarcomas, based solely on their MRI characteristics (Weissman et al., 2013).

Although most vaginal tumors are benign, complete staging with thoracic radiographs and thorough abdominal ultrasound should ideally be completed before surgery. Suspicion of malignancy can be based on rapid growth of the neoplasia or with nonpedunculated tumors (Salomon et al., 2004; Tivers and Baines, 2010; Saba and Lawrence, 2012). Cytological examination of fine needle aspirates (FNA) may be used to determine the biological nature of the mass, but is generally not advised due to the deep localisation of the mass and its peripheral hypervascularisation (Salomon et al., 2004). Furthermore, fine needle aspirates (FNA) have a limited role in differentiation of malignant and benign vaginal neoplasia. Definitive differentiation of leiomyomas and leiomyosarcomas is based on the histopathological appearance and the mitotic count and/or index obtained during histopathological examination (Venkateswaran, 2008).

Leiomyosarcomas are described to metastasis to the spleen, regional lymph nodes, lungs and spinal cord (Brodey and Roszel, 1967; Salomon et al., 2004; Bacci et al., 2010; Liptak and Forrest, 2012). In general, metastases are present in 50% of the leiomyosarcoma cases, but this percentage is much lower when speaking about genital leiomyosarcomas (Salomon et al., 2004).

The treatment of vaginal neoplasia consists of a (sub)total vaginectomy. This technique includes the removal of the complete vagina or a part of the vaginal wall surrounding the vaginal mass. In the intact bitch, a complete removal of the female genital tract is performed prior to the vaginectomy (Nelissen and White, 2012). This additional surgical intervention is advised due to the oestrogen-dependent characteristics of vaginal leiomyomas. Compared to the 15% recurrence rate in intact bitches, a zero-recurrence rate of leiomyomas is observed if a simultaneous ovariohysterectomy had been performed at the time of the tumor removal (Nelissen and White, 2012; Saba and Lawrence, 2012; Weissman et al., 2013). Most benign tumors are, given their biological behaviour and because they are mostly pedunculated, good candidates for local resection via episiotomy. Malignant tumors, on the other hand, require a more extensive surgical resection as a consequence of their broad base and strong infiltrative nature (Kapatkin et al., 1992; Nelissen and White, 2012). The treatment of malignant vaginal neoplasia requires total vaginectomy; techniques combining an episiotomy approach with laparotomy, if necessary even further augmented by ostectomy or pubic osteotomy (Igna et al., 2016).

Vaginectomy performed by laparotomy combined with episiotomy results in few intra- and postoperative complications (Kyles et al., 1996; Gower et al., 2008; Nelissen and White, 2012; Alonge et al., 2015; Igna et al., 2016). As to intraoperative complications, haemorrhage is most frequent and might result in substantial blood loss (Prassinos et al., 2010). However, this complication is most of the time promptly managed and resolved by coagulation of the damaged vessel through the episiotomy approach (Nelissen and White, 2012). The most frequently observed postoperative complication is serohemorrhagic discharge. Furthermore, incontinence is possible when the neurovascular supply to the bladder, urethra and/or rectum is compromised by not carefully dissecting the vagina (Salomon et al., 2004). Other possible postoperative complications include bacterial infection, perineal swelling, irritation of the episiotomy incision, bleeding, dysuria, stranguria, pollakiuria, haematuria and perineal hernia formation (Viehoff and Sjollema, 2003; Salomon et al., 2004; Connery and Spotswood, 2012).

The long-term prognosis of vaginal leiomyomas after surgical excision is, due to the very low probability of recurrence, favourable (Salomon et al., 2004). Median survival time after surgical removal is difficult to determine since the patient profile consists of bitches of median to old ages who frequently suffer of age-related problems, not associated with the vaginal neoplasia and vaginectomy (Nelissen and White, 2012). Vaginal leiomyosarcomas, in contrast to leiomyomas, have a reserved to bad prognosis as a result of their strong infiltrative growth, which increases the risk of recurrence (Tsioli et al., 2011; Igna et al., 2016). The average postoperative survival time varies between 5 months up to 2 years (Salomon et al., 2004; Nelissen and White, 2012; Weissman et al., 2013; Yap et al., 2017).

Although vaginectomy is an infrequently performed invasive surgery for, according to literature, a mostly benign process, intra- and postoperative complications seem rare. The surgical technique combining a laparotomy and episiotomy approach yields a favourable outcome. However, when looking into the technique a few major questions still remain. Wouldn’t we expect more complications due to the manual traction placed onto the vagina, and more precisely the supplying blood vessels, via the episiotomy site? Do these blood vessels get ruptured during surgery? And if so, why is haemorrhage not a major complication?
The goal of this dissertation is therefore to map the supplying vaginal blood vessels within the pelvic cavity, describe the full procedure of vaginectomy and to compare the clinical data of patients presented at the Ghent University Small Animal Clinic from January 2009 to December 2018 in which partial or total vaginectomy was performed.

II. Material & Methods:

A. Cadaver Study:

Five cadavers of different dog breeds, age and neuter status were used. All animals had been euthanized for reasons unrelated to this study. Latex injection with minimal vessel dissection was performed in all dogs.

Abdominal procedure:
The dogs were positioned in dorsal recumbency and the abdominal cavity was opened by performing a caudal ventral midline celiotomy approach. Careful dissection and organ displacement were performed to visualise the abdominal aorta just caudal to the origin of the renal arteries.

Through a small incision in the aortic wall, a feeding-tube (6F Nutrisafe Sterile Feeding Tube 10 mL Syringe, Abnoba Pet Store, Brierley Hill West Midlands, United Kingdom) was placed in the abdominal aorta just caudal to the renal arteries (Fig. 1) and secured by an encircling ligature (polydioxanone). To reduce the total amount of latex needed to achieve filling of the vascular structures of interest, both femoral arteries were ligated through a small skin incision at the location of the femoral triangle (Fig. 1), bounded by the sartorius muscle and the pectineus muscle. A 20 mL syringe was used to inject an aqueous latex solution (V-Sure Eco Latex Natuurrubber, Vosschemie Benelux, Lier, Belgium) mixed with methylene blue (Methyleenblauw 25 mL, Euromex, Edegem, België) as a colouring agent into the abdominal aorta. Adequate contrast was achieved by mixing 5 mL methylene blue per 100 mL Latex. The total amount injected into the feeding tube was deemed sufficient when counterpressure on the 20 mL syringe plunger was felt.

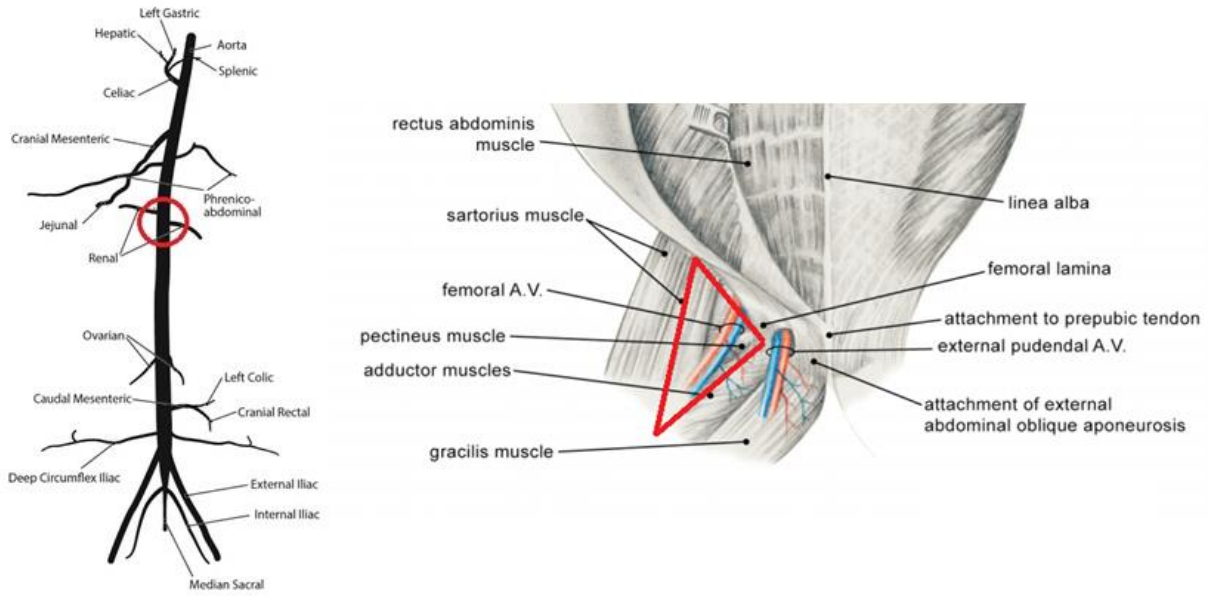

Figure 1 – Feeding-tube placement localisation at the height of the renal arteries (adapted from Culp et al., 2015) and localisation of the femoral artery ligation within the femoral triangle (adapted from Constantinescu et al., 2007)

After curing of the latex for at least 12 hours, the celiotomy incision was enlarged until it reached from the xiphoid cartilage to the pecten of the pubic bone.

In intact dogs, the ovarian pedicles were double ligated (polydioxanone) and cut as during a standard ovariohysterectomy (Fig. 2A). The bladder was retroflexed to gain exposure to the vagina and the surrounding vessels leading to the female genital tract (vaginal artery, internal pudendal artery, caudal vesical artery, uterine branch of the vaginal artery, …). A double encircling ligature (polydioxanone) was placed just caudal to the cervix that was subsequently transsected cranial to those ligatures. The female genital tract was then removed (Fig. 2B).

Next, the muscles located on the ventral aspect of the ischiatic spine and pubic ramen were removed. A handsaw was used to remove an ischiopubic flap which allowed maximal intra-pelvic visibility and accessibility of the female genital tract (Fig. 7A). Hereupon, the dogs were repositioned and placed in a perineal position in order to perform a standard total vaginectomy.

Vaginal procedure:
Dogs were positioned in sternal recumbency with the hindquarters slightly elevated (Trendelenburg position) and the pelvic limbs extended caudally over the edge of the surgical table (Fig. 3A). The tail was restrained cranially in the dorsal midline and a purse string suture was placed around the anus. Doyen clamps were positioned with one blade in the vestibular lumen and the other blade on the skin surface. A midline episiotomy incision was then made until visibility of the urinary papilla was achieved (Fig. 3B). As soon as the urinary papilla was visible, a urinary catheter (e.g., Foley catheter, Pediatric (2-way), 5 cc balloon, A.M. Bickford, New York, United States) was placed to highlight the papilla (Fig. 3C) as to prevent inadvertent damage during surgery. Immediately cranial to the urinary papilla a full thickness circular incision of the vaginal wall was made. The vagina was then, by a combination of blunt and sharp dissection, lifted from its intrapelvic attachments. The combination of dissection and manual traction on the vagina resulted in total removal of the vagina (total vaginectomy).

Post-vaginectomy:
After total vaginectomy the dogs were again positioned in dorsal recumbency and intra-abdominal and intra-pelvic damage to supplying blood vessels was mapped by taking pictures and comparing these to what is found in a leading textbook on veterinary anatomy (Barone, 2001). Vessel damage was then compared to pictures taken before vaginectomy.

B. Ovariohysterectovaginectomy technique in bitches with vaginal neoplasia:

Preoperative procedure:

Preoperatively the bladder is expressed and a purse string suture is placed around the anus after insertion of a compress. The perivulvar and perineal area are widely clipped in preparation for surgery. In intact female dogs, the ventral abdomen is clipped from xiphoid to pubis in preparation of the ovariohysterectomy. The width of the clipping should be to the level of the mammary chain or beyond. If possible, a urinary Foley catheter is introduced preoperatively. First, the dog is positioned in dorsal recumbency and the abdominal site is surgically prepared for the caudal ovariohysterectomy procedure. The perineal site is surgically prepared after the dog has been repositioned in a perineal position for vaginectomy through episiotomy.

Abdominal procedure: Caudal ovariohysterectomy

The dog is positioned in dorsal recumbency and the abdominal cavity is opened by performing a ventral midline celiotomy approach extending from umbilicus to os pubis. An incision through the skin and subcutaneous tissues is made to expose the linea alba. The linea alba is then lifted and a stab incision into the abdominal cavity is made. This stab incision is extended by usage of surgical scissors or a scalpel blade towards the cranial xiphoid and caudal pubis. The uterine horns are identified and followed cranially to locate the ovaries. A blunt opening is made in the mesovarium to allow ligation of the ovarian vessels and suspensory ligament. The ovarian pedicles are transsected after double ligation. Next, a second opening is made in the mesometrium, close to the cervix and adjacent to the uterine body, uterine artery and vein. The mesometrium is then ligated and cut.
After performing the standard steps for ovariohysterectomy, the bladder is retroflexed through the incision to gain exposure to cervix, vagina and associated structures.
The attachments between the vagina, rectum and urethra get carefully dissected and resected using a combination of blunt and sharp dissection. After visualisation, the vaginal arteries and vessels are ligated. Furthermore, the perivaginal tissues get bluntly dissected as far caudally as possible. Finally, sutures are placed, as caudal as possible but avoiding the urethra, caudally of the cervix, which is subsequently resected (Fig. 2). The female genital tract, including cervix, is removed and the standard celiotomy closure is performed in three layers (fascia/linea alba, subcutaneous tissue and skin). The fascia/linea alba and subcutaneous tissue are appositionally closed in a continuous pattern. The skin is reapposed by placing an intradermal suture.

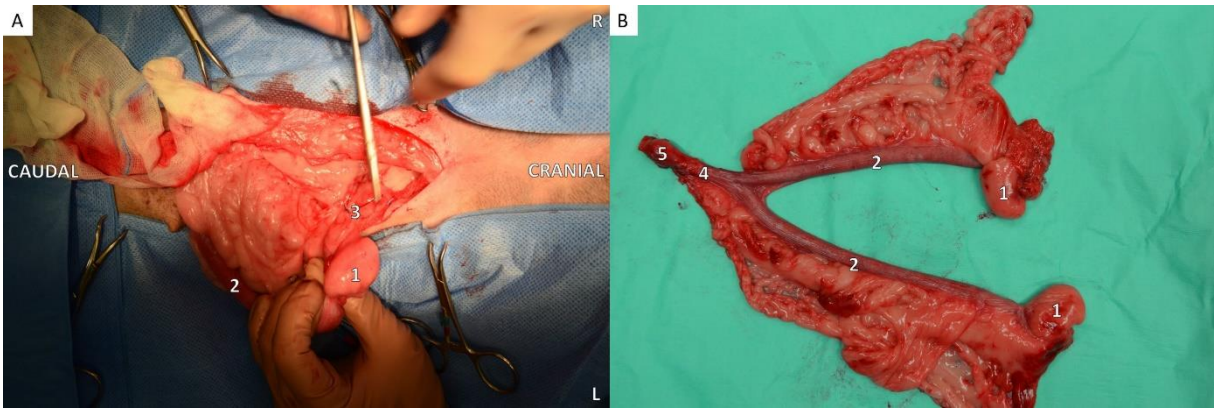

Figure 2 - Caudal ovariohysterectomy
A: Abdominal presentation and ligation of the female reproductive tract after ventral median celiotomy approach.
B: Female genital tract, including cervix, post caudal ovariohysterectomy.
1) Ovarian bursa 2) Uterine horn 3) Ovarian pedicle 4) Uterine body 5) Cervix

Vaginal procedure: Episiotomy and urinary papilla localisation

The dog is positioned in sternal recumbency with the pelvic limbs slightly elevated (Trendelenburg position) and extended over the edge of the surgical table. A towel or soft padding is placed underneath the pelvic limbs to prevent ischemic or neurologic injury (Fig. 3A). The tail is restrained cranially in the dorsal midline. The perineal site is surgically prepared and a purse string suture is placed if this wasn't already done preoperatively. Before surgical drape placement, the perineal site is scrubbed thoroughly once more. Surgical drapes are then placed so that the vulvar cleft and dorsal commissure are exposed and the anus is excluded from the surgical field. Doyen clamps, or other noncrushing clamps, are positioned with one blade in the vestibular lumen and the other blade on the skin surface. An episiotomy is performed to allow access to the vestibule, urinary papilla and vagina (Fig. 3B). Episiotomy consists of a midline skin incision through the dorsal commissure of the vulvar opening to just distal of the external anal sphincter. The episiotomy is completed by using Metzenbaum scissors to incise the muscular layer and the mucosa in the same plane as the skin incision. When visibility of the urinary papilla is achieved, a Foley catheter is placed, if this was not already done preoperatively, to prevent unwanted iatrogenic damage to the urinary papilla during surgery (Fig. 3C). During surgery, haemorrhage can be managed with haemostats and electrocoagulation.

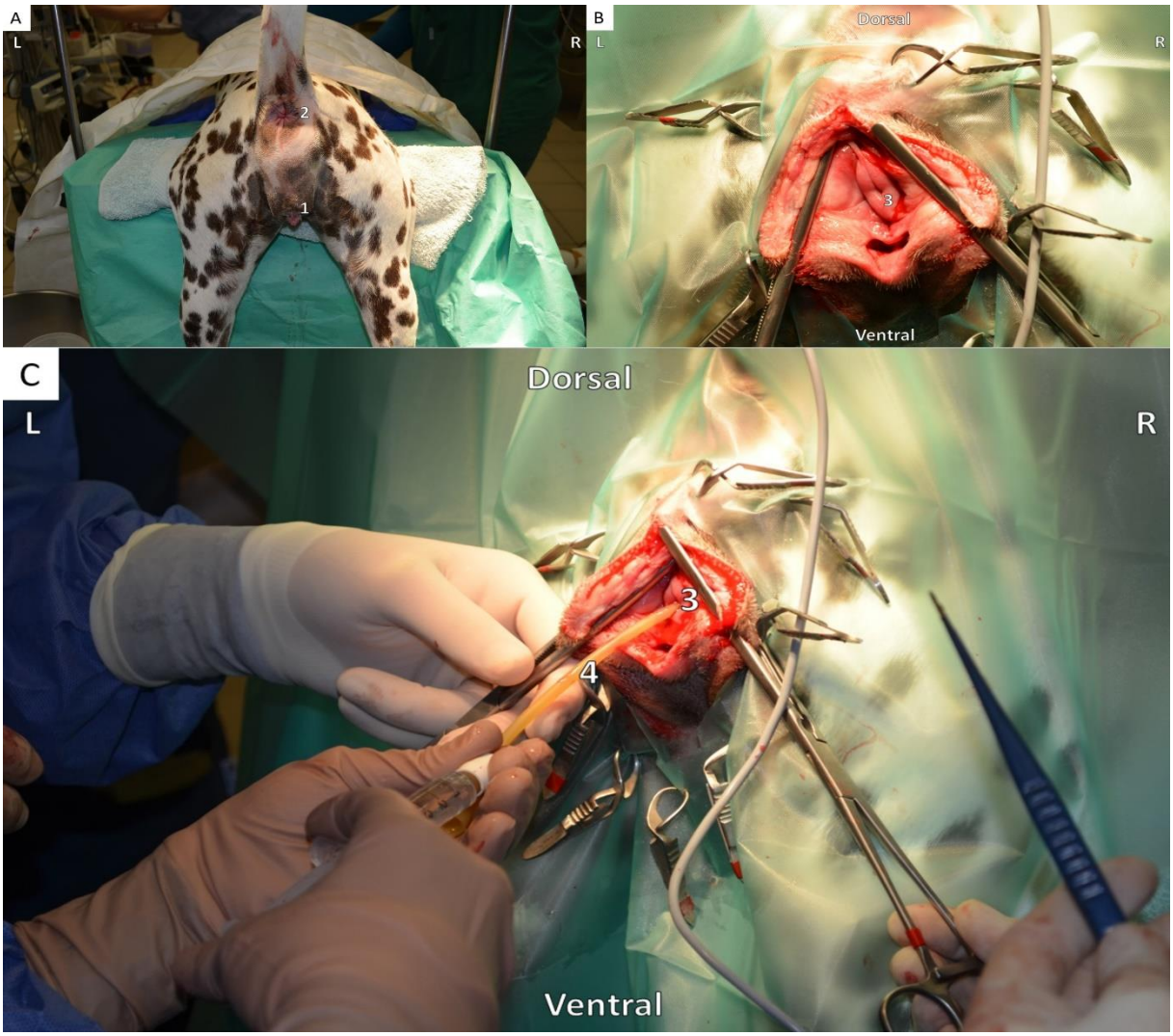

Figure 3 - Vaginal procedure: Positioning, episiotomy and placement of a urinary catheter
A: Positioning of the dog for episiotomy.
B: Midline episiotomy incision with urinary papilla visualisation.
C: Urinary catheter insertion.
1) Vulva 2) Anus purse string 3) Urinary papilla 4) Urinary catheter

Vaginal procedure:

Stay sutures are placed on the ventral vaginal wall, cranially to the urinary papilla, to assist in further visualisation and positioning during surgery (Fig. 4A). A full thickness circular incision of the vaginal wall is made immediately cranial to the urinary papilla (Fig. 4B). By a combination of blunt and sharp dissection, the vagina and tumor are lifted from their intrapelvic attachments (Fig. 4C). Intraoperative bleeding are quickly controlled by usage of electrocautery and haemostats. The combination of dissection and manual traction on the vagina results in complete removal of the vagina (total vaginectomy) and associated tumor (Fig. 4D and 4E).

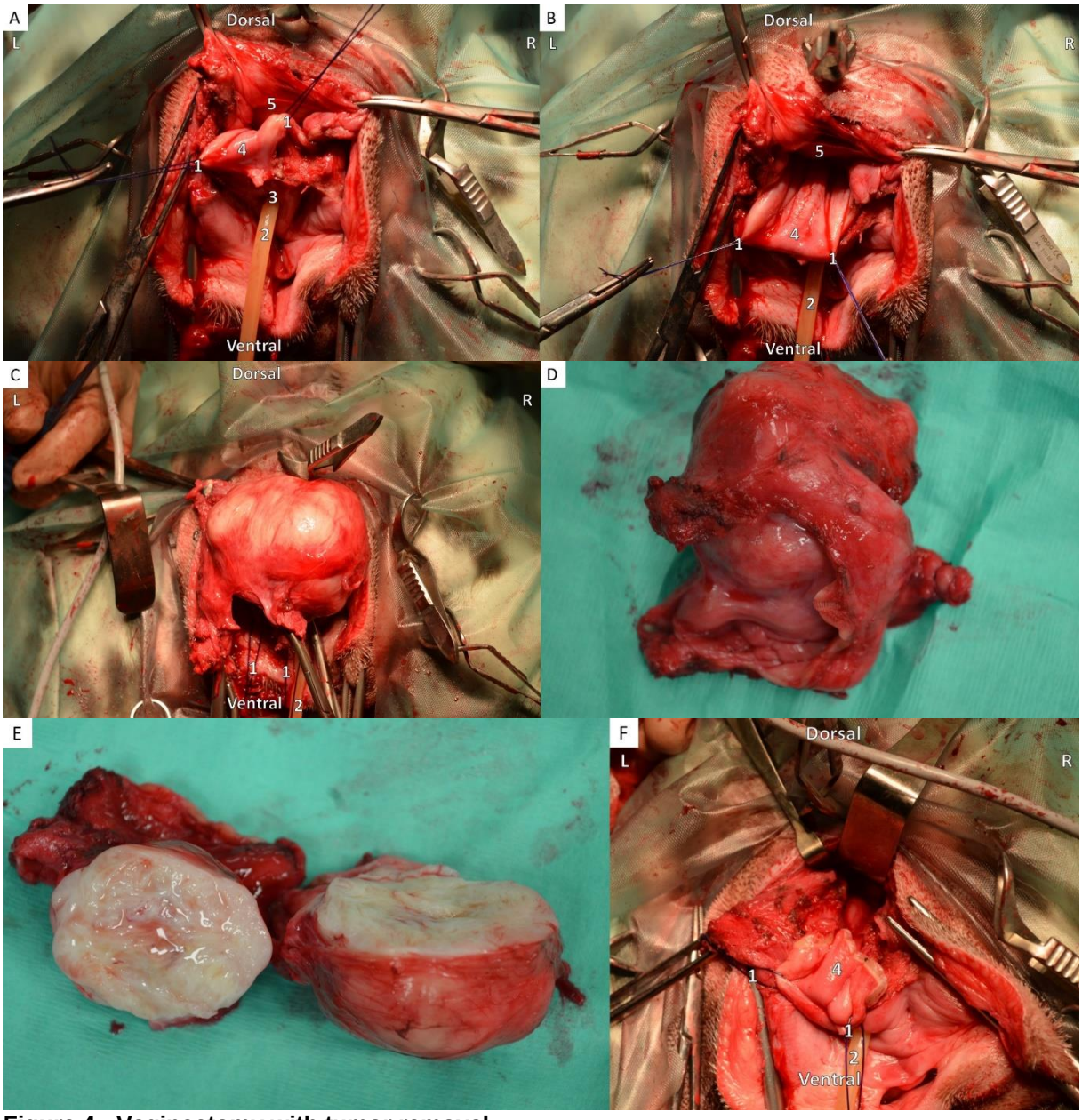

Figure 4 - Vaginectomy with tumor removal
A: Placement of stay sutures on the ventral vaginal wall.
B: Inverting the cranial aspect of the vagina.
C: Externalised vaginal tumor by inverting the cranial aspect of the vagina through the episiotomy.
D: Tumor after excision (low grade leiomyosarcoma).
E: Cross-sectional view of the tumor (low grade leiomyosarcoma).
F: Ventral vaginal wall after tumor excision.
1) Stay suture 2) Urinary catheter 3) Urinary papilla 4) Ventral vaginal wall 5) Dorsal vaginal wall

Closure of the intrapelvic opening of the vagina is achieved by appositionally suturing the remaining ventral vaginal wall to the remaining dorsal vaginal wall (Fig. 5A). Handheld retractors can be used to achieve a better visualisation. The episiotomy is closed in 3 layers. The vaginal mucosa is appositionally closed with continuous sutures (poliglecaprone (Monocryl®)), or polydioxanone). Then the muscularis and subcutaneous tissue are appositionally closed in a continuous pattern (poliglecaprone). The skin is reapposed by usage of an intradermal suture (poliglecaprone) (Fig. 5B and 5C). The purse string suture around the anus and inserted compresses are removed.

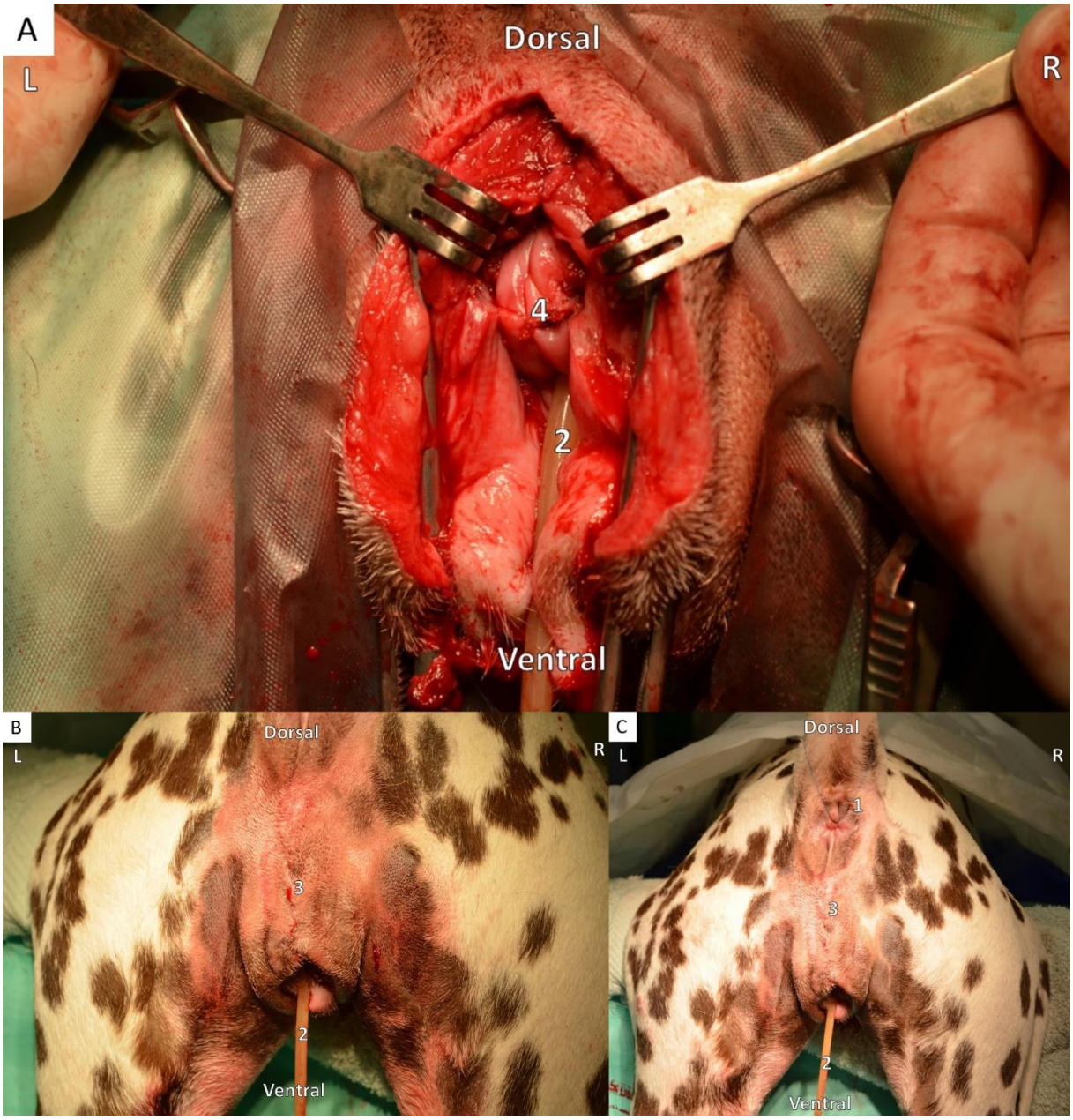

Figure 5 - Creation of a 'new' dorsal vaginal wall and closing of the episiotomy site in 3 layers. A: Creation of a 'new' dorsal vaginal wall by appositionally suturing the ventral vaginal wall to the remnants of the dorsal vaginal wall.
B: Episiotomy site after closure in 3 layers.
C: Overview of the episiotomy site after surgery.
1) Purse string 2) Urinary catheter 3) Episiotomy site 4) 'New' dorsal vaginal wall

C. Retrospective Case Study:

Inclusion criteria:
The medical records of all female dogs from January 2009 to December 2018 in which partial or total vaginectomy was performed at the Ghent University Small Animal Clinic were reviewed. The following search criteria were used in the medical record database (FileMaker®): Vaginectomy, episiotomy and vagina. Patient files matching the search criteria were reviewed for their relevance to the subject "vaginectomy in dogs". Patients were included if a vaginal mass was diagnosed and/or if vaginectomy was performed. All available cases (n=32) were included in this study.

Data pertaining to age, breed, neuter status, clinical signs noted by the owner, duration of signs before vaginectomy, indication for vaginectomy, surgery type (partial vs total vaginectomy), intraoperative complications, duration of hospitalisation, tumor type and histologic findings were recorded. Histologic descriptions of the tumor, including assessment of resection margins, were obtained from the original histopathology reports. Histologic characteristics measured included differentiation, necrosis, mitotic count and grade. The histological assessment was performed by a Diplomate in veterinary pathology.
Complications that occurred during hospitalisation were defined as major if they required surgical revision, and minor in case of events that resolved spontaneously or promptly after medical intervention.

III. Results:

A. Cadaver Study:

The size of the cadavers used, ranged from a small Yorkshire terrier to a big Great Dane. Other breeds used included a rough haired Teckel, a Labrador retriever and a Boxer. Both the Great Dane and the rough haired Teckel were intact. The other presented breeds were already castrated. Age, clinical history, weight and body condition score (BCS) were not available for the presented cadavers.

Although a larger dog resulted in easier visualisation, we were able to achieve sufficient visualisation of the vaginal supplying vessels by combining a caudal laparotomy approach, ischiopubic flap removal and retroflexion of the bladder (Fig. 7, 8 and 9). The removal of an ischiopubic flap was crucial to achieving visualisation of the supplying vessels located deeply within the pelvic cavity. Visualisation of the supplying blood vessels was further improved by vascular casting with latex mixed with methylene blue as a colouring agent. Curing of the latex for 12-24 hours resulted in strong and firm coloured vessels. The latex solution was able to reach even the smallest vesical vessels. Ligation of the abdominal aorta at the height of the renal arteries and at both femoral arteries at the height of the trigonum femorale mediale (Scarpa's triangle) resulted in adequate filling of the supplying female genital tract vessels (Fig. 1).

The major blood supply of the female genital tract is derived from the vaginal artery (Fig. 6). Other supplying vessels include the ovarian artery and the internal pudendal artery. The vaginal artery continues as the uterine branch of the vaginal artery, the median rectal artery and the caudal vesical artery.

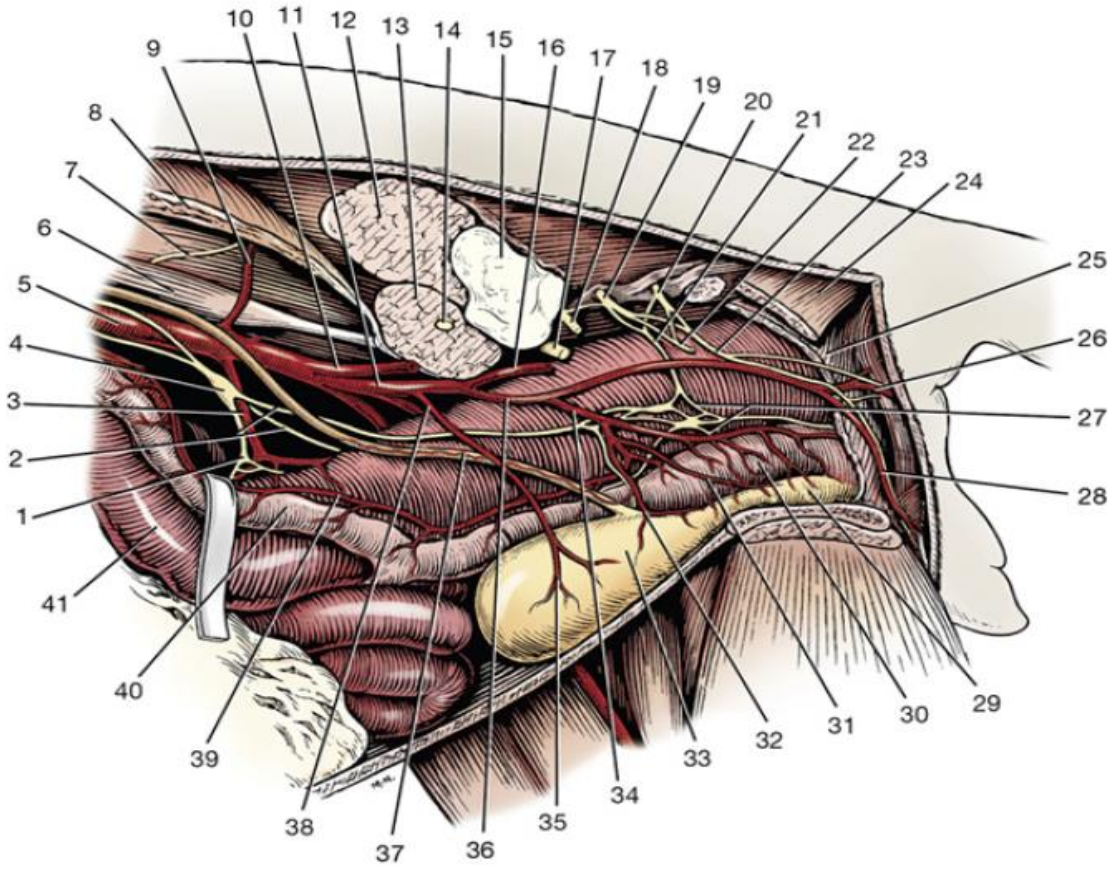

Figure 6 – Autonomic nerves and vessels of the pelvic region, left lateral view (Guide to the Dissection of the Dog, 8th Edition).
29) Urethra 30) Vagina 31) Urethral branch of the vaginal artery 32) Caudal vesical artery 33) Bladder 34) Vaginal artery 35) Cranial vesical artery

Figure 7 – Ventrodorsal and lateral view of the abdomen of a spayed female dog one day after latex injection. An ischiopubic flap has been removed.
A: Ventrodorsal overview after ischiopubic flap removal.
B: Ventrodorsal overview of the vaginal artery and its target organs.
C: Ventrodorsal close-up view of the vaginal artery and its target organs.
D: Lateral view of the vaginal artery and its target organs.
1) Vaginal artery 2) Caudal vesical artery 3) Urethral branch of the vaginal artery 4) Internal pudendal artery 5) Uterine branch of the vaginal artery a) Bladder b) Urethra c) Ureter and ureteral branch of the vaginal artery d) Descending colon e) Uterine stump

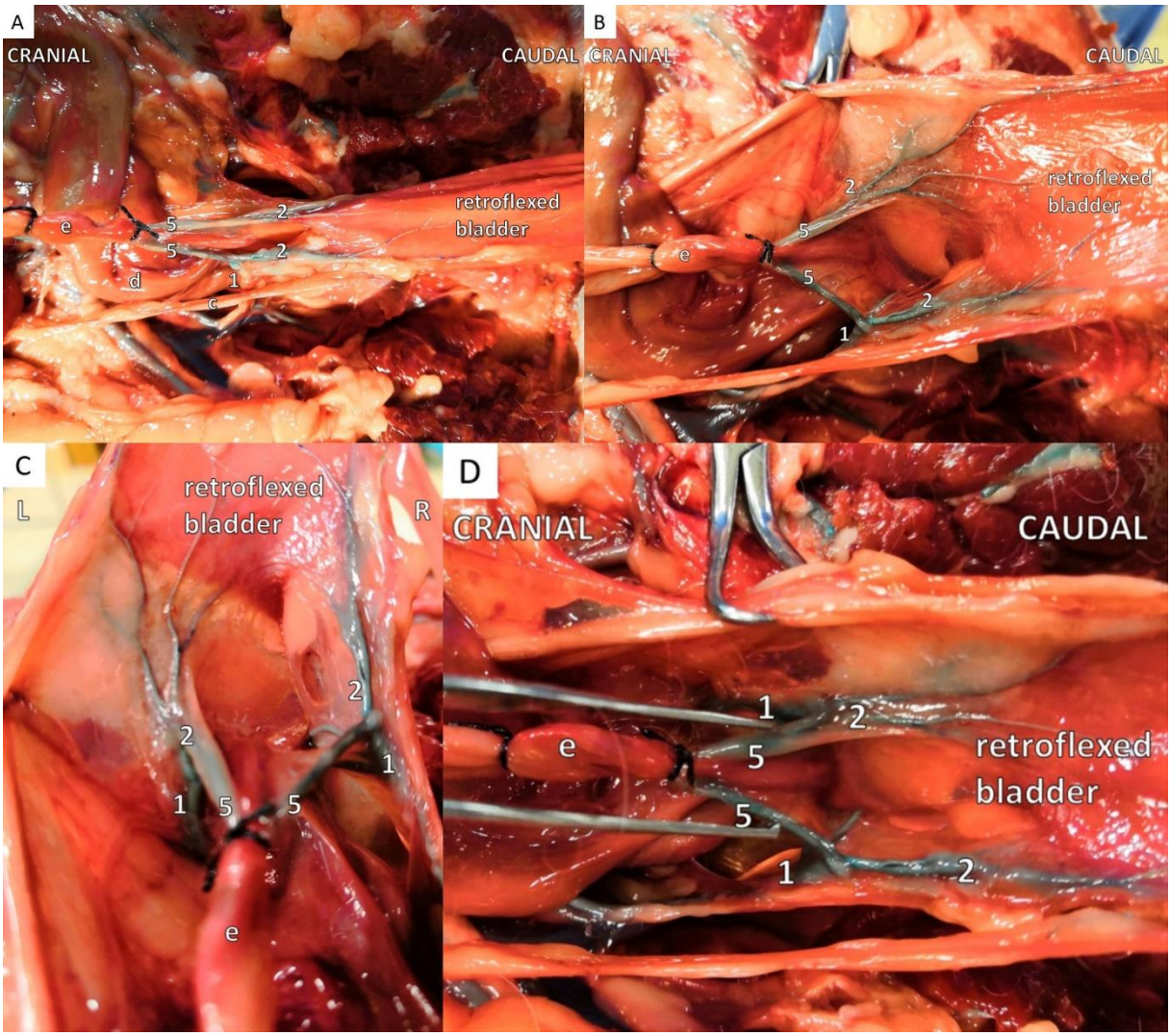

Figure 8 – Blood vessel distribution between vagina, bladder and urethra visualised by latex injection.
A: Ventrodorsal overview of the vaginal artery with retroflexed bladder.
B: Ventrodorsal overview of the caudal vesical arteries with retroflexed bladder.
C: Craniocaudal overview of the caudal vesical arteries with retroflexed bladder.
D: Detailed ventrodorsal view of the vaginal artery and its target organs.
1) Vaginal artery 2) Caudal vesical artery 3) Urethral branch of the vaginal artery 4) Internal pudendal artery 5) Uterine branch of the vaginal artery a) Bladder b) Urethra c) Ureter and ureteral branch of the vaginal artery d) Descending colon e) Uterine stump

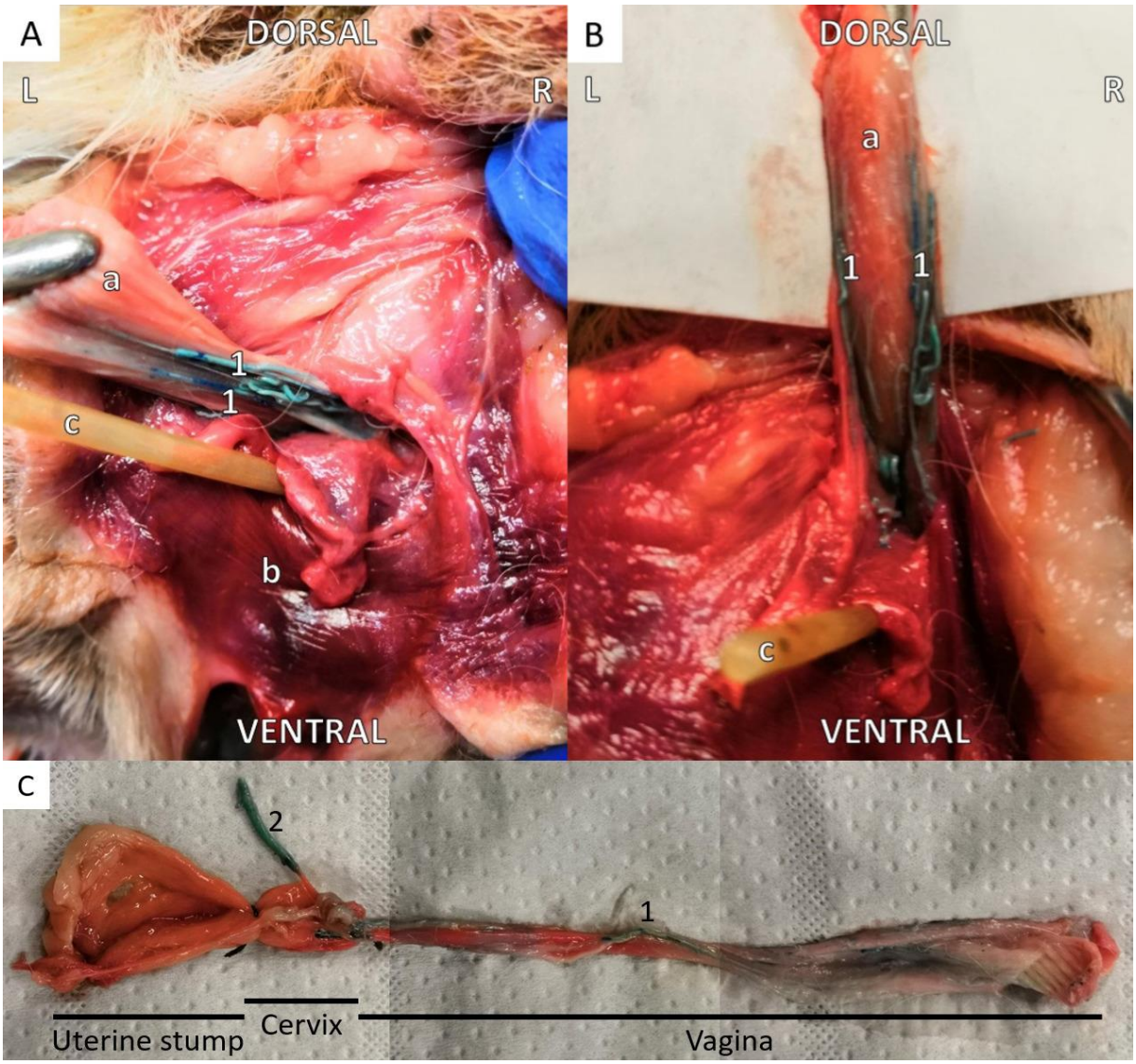

Figure 9 – Images obtained during and post vaginectomy via episiotomy.
A: Caudal traction on the caudal aspect of the vagina after circular dissection cranial to the urethral orifice.
B: Dorsal traction on the vagina and vaginal artery during vaginectomy.
C: Specimen consisting of vagina, cervix and uterine stump post vaginectomy.
1) Vaginal artery 2) Uterine branch of the vaginal artery a) Vagina b) Vestibule of vagina c) Urinary catheter

The caudal traction on the vagina and its supplying blood vessels (vaginal artery, caudal vesical artery, uterine branch of the vaginal artery, …) via the episiotomy site resulted in ruptured vessels. In particular the caudal vesical artery and the uterine branch of the vaginal artery (Fig. 10) do rupture during the vaginectomy via episiotomy. No damage was observed to other blood vessels. Damage to these vessels was consistent in all cadavers when following the standard vaginectomy procedure via episiotomy.

In all available cadavers (n=5) similar damage to the caudal vesical artery and the uterine branch of the vaginal artery was observed. Although neutered female dogs had a less prominent genital tract vascularisation, vessel damage was comparable to those found in the intact female dogs.

Figure 10 – View of the latex-casted vaginal, urethral and vesical blood vessels post-vaginectomy. Visualisation of surgery-related damage to the caudal vesical artery and uterine branch of the vaginal artery.
A: Ventrodorsal overview of the vaginal artery with retroflexed bladder post vaginectomy.
B: Lateral view of the vaginal artery post vaginectomy.
C: Detailed lateral view of damaged blood vessels (caudal vesical artery and uterine branch of the vaginal artery) post vaginectomy.
1) Vaginal artery 2) Caudal vesical artery 3) Urethral branch of the vaginal artery 5) Uterine branch of the vaginal artery a) Bladder b) Urethra d) Descending colon

B. Retrospective Case Study:

Demographics:
During the past 10 years, 32 dogs underwent vaginectomy at the Ghent University Small Animal Clinic of the Faculty of Veterinary Medicine.
Of those 32 dogs, 17 (53.1%) were already spayed before being presented for tumor removal. Unfortunately, the time interval between gonadectomy and presentation could not consistently be retrieved from the files. Median age at time of surgery was 9 years and 10 months (range: 2 years 7 months - 14 years 7 months). Affected dog breeds included Beagle (n=4), Labrador Retriever (n=4), Canis Vulgaris (n=3), Cavalier King Charles Spaniel (n=2), English Cocker Spaniel (n=2), Jack Russel Terrier (n=2), Leonberger (n=2), Pug (n=2) and one of each of the following: American Cocker Spaniel, Dalmatian, English Bulldog, German Pointer, German Shepperd, Malinois, Maltese, Scottish Terrier, Shar-Pei, Welsh Corgi and a Yorkshire Terrier.

Clinical findings:
The clinical signs that prompted referral for vaginectomy included the presence of an undefined vaginal mass hindering urination and/or defecation in 28 dogs. Out of these 28 dogs, 3 had a previous history of vaginal mass removal that was not evaluated by histopathology at that time and were suspected for a vaginal mass relapse. The remaining 4 dogs presented with vaginal prolapse (1), vestibulovaginale stenosis (1), extensive urethral neoplasia (1) or chronic peritonitis (1).

Staging:
Ten out of 32 patients underwent the staging process of determining the extent of a local neoplasia and the presence or absence of distant or regional metastases.

Thoracic radiographs were taken in 9 patients, with following results: no radiographic signs of thoracic metastases (6), suspicion of lung metastases (1), bronchial pattern (1) and multiple dense nodules throughout the lung parenchyma (1).

Abdominal radiographs were taken in 1 patient, showing a perianal mass, vaginal and distal ureteral enlargement and radiopaque uroliths.

An abdominal ultrasound was obtained for 10 patients, with following results: no echogenic signs of lymphadenopathy (6), liver nodules (1), splenic nodules (1), hypoechogenic lesions in the spleen (1), thickened irregular bladder wall with a mass-like lesion at the dorsal wall (1).
Ultrasound guided fine needle aspirates (FNA's) were obtained from 1 patient. The FNA was not diagnostic and showed red blood cells, few neutrophils and some epithelial cells.

A cystogram and vaginourethrogram was performed on 1 patient: no abnormalities were detected cranial to the pedunculated vaginal mass.

An abdominal and thoracic CT-scan was taken on 1 patient showing a large cyst-like or necrotic dorsal vaginal mass with extension caudally in the vagina and vulva and a single nodule in the caudal right lung lobe.

Surgery:
Complications that occurred during hospitalisation were defined as major if they required surgical revision, and minor in case of events that resolved spontaneously or promptly after medical intervention. None of the patients presented for vaginectomy (n=32) had major complications.

Histopathological findings:
Histopathology records were available for 25 out of the 32 patients and following tumor types were observed (Fig. 11): Leiomyosarcoma (14), Leiomyoma (7), Fibro-leiomyoma (1), Inflammatory non-neoplastic granulation tissue (1), Transitional cell carcinoma (1), Lymphangiosarcoma (1).

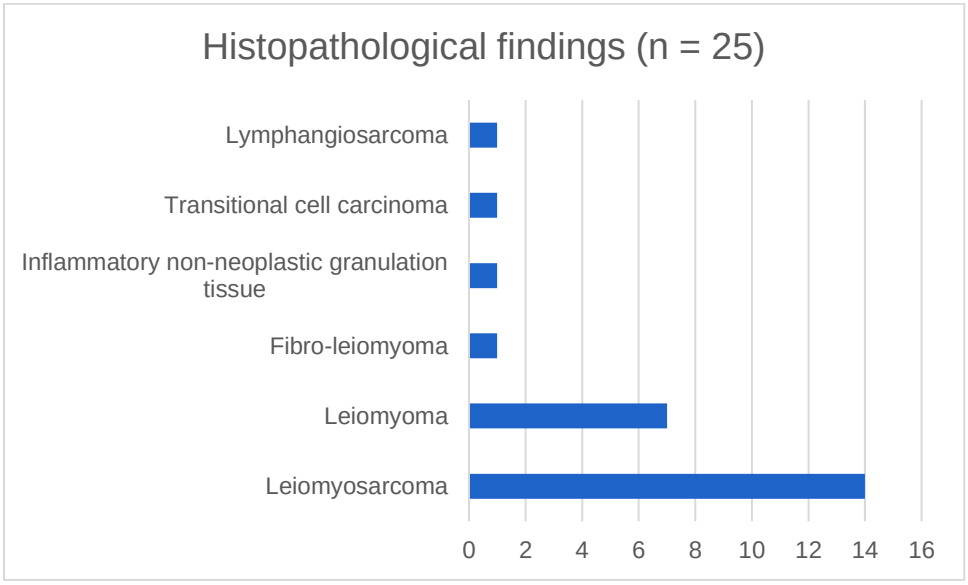

Figure 11 – Histopathological findings of patients presented at the Ghent University Small Animal Clinic (January 2009 – December 2018) for vaginectomy.

Histopathological tumor differentiation:
Leiomyosarcoma:
General leiomyosarcoma tumor differentiation consisted of narrow to broad interlacing fascicles of densely packed moderately to strongly pleiomorphic oval or spindle cells with oval to elongated nucleus and fine stippled chromatin with a small eosinophilic nucleolus and a mild amount of, sometimes vacuolised, eosinophilic cytoplasm surrounded by a small to mild amount of collagen connective tissue and fibrovascular stroma.

Leiomyoma:
Leiomyomas presented, on histology, more as broad interlacing fascicles of slightly pleiomorphic oval to spindle cells with an oval or elongated nucleus, fine stippled chromatin and a moderate amount of eosinophilic cytoplasm.

Age compared to histopathological diagnosis:
Dogs diagnosed with either leiomyoma or leiomyosarcoma had an median age of 10 years and 6 months. Dogs presented with leiomyoma ranged from 8 years 0 months 3 days up to 12 years 9 months 6 days old. The median age of dogs presented with a leiomyoma was 10 years 4 months 24 days. Dogs presented with leiomyosarcoma had an age range between 6 years 7 months 10 days and 13 years 11 months 18 days. The median age of dogs presented with leiomyosarcoma was 10 years 6 months 24 days. No substantial age difference could be found between patients presenting with the malignant leiomyosarcoma versus the benign leiomyoma.

Neuter status compared to histopathological diagnosis:
At the time of surgery, 17 (53%) dogs were already spayed. Out of 14 dogs presented with a vaginal leiomyosarcoma, 8 out of 14 were previously neutered (57%). Out of 8 dogs presented with either leiomyoma or fibroleiomyoma, 4 out of 8 were previously neutered (50%).
Three dogs presented for a vaginal mass relapse excision. Out of these 3 dogs, 2 were previously neutered (67%).

Mitotic count (MC) and mitotic index (MI):
Mitotic activity is a common component of most tumor grading systems and was measured as a criterion for assessing the vaginal masses. Aggressive neoplasms have a high proliferation rate, which is reflected in a high number of mitotic figures on histology.
The mitotic count (MC) was obtained by counting the number of mitotic figures in 10 consecutive high-power fields (hpf).
The mitotic index (MI) reflects the ratio of the number of cells undergoing mitosis to the number of cells not undergoing mitosis in a population of cells.
Following this method, a mitotic count (MC) was achieved for 11 vaginal masses: mitoses were rare (MC<4) in 7 cases, present (MC:4) in 2 cases and frequent (MC:34) in 1 case.
A mitotic index (MI) was obtained instead of a mitotic count (MC) in 8 cases; mitoses were rare (MI<1) in 6 cases, infrequent (MI:4) in 1 case and moderate (MI:5) in 1 case.

Leiomyosarcoma:
When looking closer at the leiomyosarcomas in specific, a mitotic count (MC) was obtained for 9 masses, a mitotic index (MI) was obtained for 3 masses and 1 mass had no record of mitotic count or index. Mitoses were graded from rare (MI<2 or MC<4) to moderately present (MI<6 or MC<13) and frequent (MI>6 or MC>13). Mitoses were rare in 8 cases, moderately present in 4 cases and frequent in 1 case.

Leiomyomas:
A mitotic count (MC) was obtained for 1 vaginal mass and a mitotic index (MI) was obtained for 5 cases. A mitotic count (MC) or index (MI) couldn't be obtained for 2 cases. Mitoses were graded from rare (MI<2 or MC<4) to moderately present (MI<6 or MC<13) and frequent (MI>6 or MC>13). Mitoses were rare in all 6 available cases.

Follow-up:
The patients were discharged, depending on if they could urinate independently, after one to three days of hospitalisation and sent home with NSAIDs (e.g., carprofen (Rimadyl®)) and antibiotics (e.g., amoxicillin clavulanic acid (Clavubactin®)). A control visit was planned 14 days after surgery to check the wound and discuss the histopathology results. None of the presented dogs for vaginectomy (n=32) were followed-up long-term after discharge.

IV. Discussion:

A combined laparotomy and episiotomy approach for vaginectomy consistently resulted in adequate management of benign and malignant vaginal neoplasia. Clinical data of patients presented at the Ghent University Small Animal Clinic from January 2009 to December 2018, in which partial or total vaginectomy was performed, showed that the procedure was associated with few intra-operative complications which were easily dealt with during surgery. None of the presented patients for vaginectomy (n=32) had major complications intra- or postoperatively.

Although vaginectomy only rarely results in intra- and postoperative complications, it stands to reason how this is possible. During the cadaver study of this dissertation we found out the uterine branch of the vaginal artery and the caudal vesical artery get ruptured during the episiotomy approach of the vaginectomy procedure. Rupture of these decently sized vessels should result in extensive intra-abdominal and -pelvic bleeding. These vessels cannot be ligated during the laparotomy approach due to their localisation deeply within the pelvis. A possible explanation could be the usage of an electrocautery device, which is often used to stop intra-operative bleeding during the episiotomy part of the surgery. It however seems unlikely these vessels could be reached with the electrocautery device during the vaginectomy via episiotomy. Rupture of the caudal vesical artery would also lead to insufficient perfusion of the bladder since the cranial vesical artery is often rudimentary or not present in dogs. Although no long-term follow-up period was available, none of the presented dogs (n=32) had complaints concerning bladder dysfunction due to hypoperfusion, intra-abdominal or intra-pelvic bleeding at the 14 days post-op control visit.

In literature, vaginectomy is described as an invasive surgery mainly used for benign smooth muscle tumors, such as leiomyomas and in lesser frequency for malignant leiomyosarcomas (Mathews, 2001). Over 80% of the reported vaginal tumors are of benign biological nature according to literature (Brodey and Roszel, 1967; Thacher and Bradley, 1983). Leiomyosarcomas are the most frequently observed malignant vaginal neoplasia in our regions, but are much less frequent than benign leiomyomas (Hill et al., 2000; Nelissen and White, 2012). Our retrospective case study of patients presented at the Ghent University Faculty of Veterinary Medicine from January 2009 to December 2018 however, showed a different result. Out of 25 patients presented, the following histopathological diagnosis was obtained: Leiomyosarcoma (14), Leiomyoma (7), Fibro-leiomyoma (1), Inflammatory non-neoplastic granulation tissue (1), Transitional cell carcinoma (1), Lymphangiosarcoma (1). When calculating the frequency of leiomyosarcomas versus (fibro-)leiomyomas we found a much higher frequency of the malignant leiomyosarcomas (63,64%) then what is found in literature (< 20%). If this higher rate of malignant presentation reflects our population of dogs, a more aggressive approach towards vaginal neoplasia should be taken. The more aggressive approach would consist of leaning more towards performing a total vaginectomy instead of partial vaginectomy. A possible explanation for the higher malignant rate could be due to a selection bias at the Small Animal Clinic of Ghent University. Patients presented at the Faculty had been referred by their own veterinarian due to the complexity or delicacy of the surgery. It is possible the actual percentage of malignant vaginal neoplasia is much lower than what we found when not every veterinarian refers their patient or if the owner elects not to have surgery. Perhaps a larger group of patients with benign vaginal neoplasia gets operated at their own veterinarian due to the easier resection of benign processes. Another possibility consists of the owner not noticing the benign vaginal mass. Benign vaginal masses, such as the leiomyoma and fibroleiomyoma, tend to grow slowly and thus resulting in fewer and a later onset of complaints compared to the aggressive growth of malignant vaginal processes, more specifically the leiomyosarcoma.

V. Conclusion:

Although our cadaver study consistently showed rupture of the uterine branch of the vaginal artery and the caudal vesical artery, the surgical technique for vaginectomy, consisting of a combined laparotomy and episiotomy approach, seems to result in few clinically relevant complications. It remains to be elucidated why these ruptured vessels do not result in major post-operative complications.

As our retrospective case study demonstrated that malignant vaginal neoplasia are more prevalent than previously anticipated, a more aggressive surgical approach consisting of total vaginectomy instead of partial vaginectomy is advised if no pre-operative biopsy has been taken. Since literature describes a more frequent presentation of benign vaginal processes, multi-center research is needed to determine if our findings of a more frequent malignant presentation are representative for the whole population or a result of a possible selection bias at the Small Animal Clinic of Ghent University.

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