Título: Vulvovaginal squamous cell carcinoma on female dog. Literature review, comparative aspects and case reports.

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I. Abstract

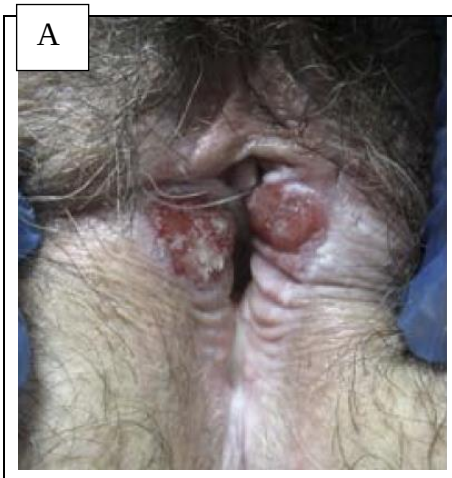
Vulvovaginal squamous cell carcinoma is a rare malignant tumor in female dogs, not published in the literature. However, between 2011 and 2017 the GICOREC-IUSA oncology service received three patients with this type of tumor. Currently, vulvovaginal SCC is the object of study in women, with whom we have found similarities with respect to clinical presentation and tumor behavior, which allows us to study the diagnostic and staging protocols, and treatment, being able to establish a more accurate prognosis. The main treatment of our patients consisted of surgical exeresis of the tumor trying to leave free-tumor cells margins, without knowing its stage or if the patient needed adjuvant therapy; so, special importance was given to the diagnosis protocol and stage of the disease in order to improve the OS of our patients by administering the appropriate treatment. In women surgical treatment is performed together with adjuvant therapy in patients who present VaIN, VIN, or stage I of vulvovaginal SCC; In veterinary medicine, a reliable staging for this type of tumor on female dogs has not been developed, so we do not know if it had an influence on the fact that the treatment used did not cure any of the patients despite there were significant differences between our patients on their FDI and OS.

II. Introduction

II.1. Epidemiology and distribution

Squamous cell carcinoma (SCC) is one of the most malignant skin tumors that arise from the epithelial cells (epidermis and mucosal epithelium), in which the cells demonstrate differentiation to squamous cells (keratinocytes). The incidence of SCC in dogs is 6% of all skin tumors (Hauk and Oblak, 2020). Is relatively common in dogs. These tumors especially occur at a median age of 6 to 13 years in dogs. Blood-hounds, Basset hounds, Standard poodles, Giant schnauzer, Keeshond, Kerry blue terrier may be predisposed breeds to develop cutaneous SCC (Goldschmidt and Goldschmidt, 2017; Hauk and Oblak, 2020). In dogs, the most frequent location occurs on the head, abdomen, limbs, perineum, and digits (nailbed). The individuals that spend a lot of time outdoors have a higher incidence of cutaneous SCC, especially in the head or the ventral abdomen. Occasional cases will arise from the wall of the anal sacs which is lined by stratified squamous epithelium. (Hauk and Oblak, 2020). Vulvovaginal SCC in female dogs is a meager pathology only mentioned in the literature, although vulvar and vaginal SCCs are the most frequent primary vaginal malignancy in women (80-90%). Most of vaginal SCC in people (93%) were preceded by precursor lesions known as high-grade vaginal intraepithelial neoplasia (VaIN) (Wu *et al*, 2008; Kuhlan *et al*,2016; Fedus *et al*, 2017; Lima *et al*, 2019; Bertoli *et al*, 2020; Zhou *et al*, 2022). “SCC lesions may be erythematous, ulcerated or crusted, but they can appear plaque-like to papillary and from crateriform to fungiform; they are usually slow growing, but sometimes it depends on the location of the primary tumor; they can be locally aggressive, but most neoplasms do not show metastatic spread to regional lymph nodes and metastasis to distant sites was not reported in dogs” (Hauk and Oblak, 2020). Local invasion rather than disease spread is the most common cause of death in patients with SCC (Blackwood, 2016).

A



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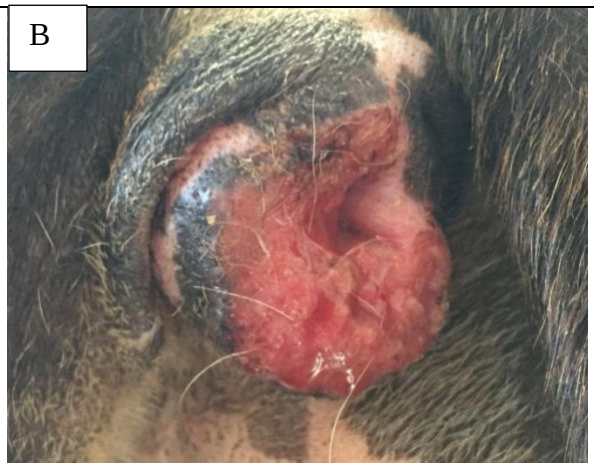


Figure 1. Comparative aspects between the physical appearance of vulvovaginal SCC in women and in female dogs. A) Vulvovaginal SCC in women due to LS (Day *et al*, 2018). B) Vulvovaginal SCC in female dogs.

There are several **predisposing factors** associated with the development of SCC. They can be physical factors, viral factors, immune status, and genetic abnormalities.

Physical factors:

- Ionizing radiation and thermal injury.
- The role of Ultraviolet (UV) radiation in the development of skin tumors in dogs and cats is primarily epidemiologic and supported by case reports on dogs diagnosed with a spectrum of sunlight-induced lesions.
- Solar exposure of skin in light-colored animals, lack of pigment within the epidermis at the sites of tumor development, and lack of hair or a very sparse hair coats at the affected sites. (Individuals that spend a lot of time outdoors)
- Geographic location and climate (UV radiation exposure)
- Chronic inflammation

(Morris and Dobson, 2001; Blackwood, 2016; Henry and Flesner, 2020)

Viral factors: Several studies have linked papillomaviruses to SCC in dogs. Well-documented ability to induce neoplastic transformation in human papillomavirus (PPV) mucosal infection. Viral effects on cell proliferation, integration into the genome, and interactions of papillomavirus proteins with cellular proteins lead to neoplastic transformation. The association of viral infection with the development of squamous cell carcinoma stems from multiple lines of evidence, including evidence of PPV in oral and cutaneous SCC. Oral PPV in dogs has also been demonstrated in several cases of cutaneous SCC. Several novel PPVs were detected at different sites in SCC, including four dogs with skin tumors (Withrow, 2020). Case reports support the correlation between PPV infection and the development of invasive SCC, including lesions of mixed histology. Susceptibility to infection may also be breed-dependent. In people, it has been suggested that UV exposure and PPV infection may act as cofactors in the development of SCC **(**Morris and Dobson, 2001; Macy, 2020).

In women it has been reported that 69% of VUC and 71% of VAC in human patients have an HPV infection indicating that VUC and VAC are probably highly associated with HPV (Zhou *et al*, 2022).

Tumor-promoting inflammation: The importance of inflammation was inferred from the earliest microscopic studies of cancer, when tumors were described as "wounds that never heal" (Dvorak, 1986), which provided synthesis for the recurrent observation that tumors often were infiltrated by inflammatory cells of the innate immune system and the adaptative immune system. Nowadays, mechanistic distinctions between inflammation that retards growth or eliminates the tumor remain to be defined; however, it can be concluded confidently that inflammation contributes to tumor growth and survival by supplying factors that sustain proliferation; factors that limit cell death; proangiogenic factors; extracellular matrix-modifying enzymes that facilitate angiogenesis; invasion and metastasis; and other signals that lead to activation of EMT and other hallmarks- facilitating programs. Inflammatory cells also release reactive oxygen species that are actively mutagenic for nearby cancer cells, accelerating their genetic evolution toward states of heightened malignancy (Modiano and Kim, 2020).

Immune status: immunosuppressed individuals have a greatly increased risk of skin cancer, although this may reflect susceptibility to persistent PPV infection in some instances. A case report of the development of multiple cutaneous hamartomas and SCCs in situ in a dog receiving long-term immunosuppressive therapy demonstrated positive staining for PPV antigens (Hauk and Oblak, 2020).

Genetic abnormalities: Cancer is a genetic disease. The accumulation of multiple alterations in critical genes is usually necessary for full neoplastic transformation. So as was mentioned before, exists some breed predisposition (Hauk and Oblak, 2020).

Hormonal factors: Malignant tumors like vaginal SCC occur more often in spayed animals (Bilbrey *et al*, 1989). The incidence of vaginal SCC was studied, and some authors found an association between previous hysterectomies and cervical dysplasia with the development of primary vaginal SCC in women at 56% and 73% respectively (Lima *et al*, 2019). Previous hysterectomy, endometriosis, chronic irritation, and cervical or vaginal radiotherapy are also among the risk factors. 33% of patients with SCC underwent hysterectomy for benign reasons (Kulhan *et al*, 2016).

Comparative aspects of predisposing factors

In human beings there are two types of vulvar SCC: 30% of cases are associated with HPV, and 70% are usually associated with lichen sclerous (LS). Several studies have shown that vulvar SCC occurs in 5% of women with LS, and some recent studies have shown that effective treatment can reduce this risk. Vulvovaginal lichen planus (LP) has also been associated with vulvar neoplasms in some case reports and series, and SCC is occasionally found in women with erosive LP during long-term follow-up (Day *et al*, 2018).

II.2. Current status

There is no information published about primary vaginal SCC in dogs, and it is a subject of study in human medicine.

II.3. Diagnosis and staging

The evaluation of vulvovaginal SCC is similar to the evaluation of any solid tumor: diagnosis and staging. An entire anamnesis may be informative. Accurate clarification of the duration, growth rate and clinical signs associated with the tumor (Hauk and oblak, 2020). The pelvic examination remains the main method of assessing the extent of disease in women. However, it has several limitations, including the inability to detect metastatic lymph nodes and difficulty imaging tumor infiltration. (Lima *et al,* 2019). For a complete diagnosis, histologic grade of the tumor, and information about vascular and lymphatic invasion, histology (biopsy) is recommended more than cytology (FNA); other characteristics such as degree of differentiation, nuclear morphology and percentage of necrosis may be helpful (Hauk and Oblak, 2020).

Moreover, diagnostic imaging has become an essential tool for diagnosing and establishing the real spread of the tumor (regional or distant metastasis), the sentinel LN can be superficial inguinal nodes or iliac nodes because of its proximity. Therefore, ultrasonographic evaluation of its size and samples obtention via FNA or by biopsy is indicated. CT or MRI are also recommended. One study demonstrated that the use of advanced local invasive techniques increased the primary tumor stage in 69% of patients, so sometimes is preferred to make an abdominal ultrasound, CT or MRI (image techniques) first and then take a biopsy or surgical excision (Hauk and Oblak, 2020). Three-view thoracic radiographs are the next choice (Blackwood, 2016; Hauk and Oblak, 2020). - Assessment of the primary tumor's size by the longest diameter is the first step in the staging process. For large, infiltrative, or fixed masses, local assessment may require advanced imaging (CT or MRI), to accurately determine the tumor's size and extent. (Hauk and Oblak, 2020).

Some techniques require a sufficiently large piece of tissue to accurately diagnose and grade the tumor (ie IHC or PCR).

Because of the unreliable nature of LN cytology and the potential effect of LN metastasis on the prognosis and the adjunctive therapy, histopathology should be considered in cases with a high risk of malignancy.

Metastasis in dogs with cutaneous SCC appears rare, with only four dogs described in the literature. (Hauk and Oblak, 2020). The non-metastasis rate of women with primary vaginal SCC was 77.8%, but the inguinal lymph node metastases rate was found in 2%, and no distant metastases were observed (Kulhan *et al,* 2016).

II.4. Clinical symptoms

Primary vaginal SCC in human beings usually presents with painless vaginal bleeding/discharge, urinary symptoms, pelvic pain, and foreign body sensation (Lima *et al,* 2019). Regarding dogs, no information has been published in this subject.

II.5. Treatment

The treatment of choice for primary vulvovaginal SCC and other carcinoma is wide surgical excision. In women, this approach combined with effective neoadjuvant therapy is adequate for long-term control, but a standardized treatment protocol for primary vulvovaginal SCC has not been established (Diao *et al,* 2017). Again, we do not have information about long-term results after complete surgery on female dogs.

The pathologist will determine if excision is complete studying all the margins which need to be identified. The surgeon must properly prepare the sample and size of the tumor before sending it to the pathologist (Hauk and Oblak, 2020).

Treatment options for SCC (*in situ*, infiltrative or in multiple sites) are surgical excision, radiotherapy, and photodynamic therapy (PDT). Radiation therapy and PDT are most appropriate for early disease, and invasive or extensive disease usually requires surgical management (Blackwood, 2016).
In one article, two dogs with metastatic SCC were treated with cisplatin. One dog with cutaneous SCC metastatic to the axillary LN and lungs had marked reduction in the number and size of lung nodules, in addition to a partial response (PR) of the axillary LN after cisplatin chemotherapy; however, the response duration was only 4,5 months. The other dog had complete and durable response of multiple lesions (>22 months). Bleomycin has demonstrated short-lived clinical activity in the treatment with a total of six doses. Mitoxantrone resulted in a response in 4/9 dogs with SCC in one study (Hauk and Oblak, 2020). There is no proven role for systemic chemotherapy. Intra-lesional chemotherapy requires special formulation to avoid systemic toxicity and is rarely used (Blackwood, 2016)

In human patients with primary vaginal or vulvar SCC, the first choice is complete surgical excision and a complete evaluation of the patient, and a large-period follow-up. The treatment always had a feasible response in patients with early-stage or stage I primary vaginal SCC on women. There is no consensus in the literature regarding the treatment of primary vaginal cancer; Treatment should address individual characteristics of the patient, i.e. age, general medical condition, stage, and tumor location. Although radiotherapy is recommended as the standard treatment for all the stages in many centers, surgical treatment is a better solution for early-stage disease terms of survival. Radical hysterectomy, vaginectomy and pelvic LN dissection are the current approaches to treating vaginal cancer. Vaginal cancer management has not been standardized because prospective studies on the possible treatment on vaginal cancer have not been conducted. There are no uniform treatment options, and highly individualized treatment is necessary. All patients in our study underwent radical surgery, and 44.4% of patients with radiotherapy, 11.1% with only chemotherapy and 33.3% with chemoradiation as adjuvant therapy (Kulhan *et al*, 2016).
Traditionally, the conventional treatment for primary vaginal SCC in women have been radical surgery combined with radiotherapy, but in nowadays highly individualized treatment is necessary and recommended (neoadjuvant therapy with irinotecan and cisplatin is one of them, it showed a complete response in one study with three patients, none of them having recurrence in more than 45 months of follow up) and “a clinical research was enrolled by Benedetti *et al* of 11 patients enrolled, 27% achieved a complete remission after receiving 3 courses of chemotherapy with paclitaxel an cisplatin every 21 days. All the patients were treated with radical hysterectomy and total vaginal resection following chemotherapy” (Diao *et al*, 2017) the use and efficacy of this combined chemotherapy is not studied in dogs.

II.5.1. Surgical procedure

The surgical technique performed - Vulvovaginectomy and perineal urethrostomy- was described by Bilbrey *et al.* (1989) in figures 2 to 4 with modifications attending to each patient's needs. Due to the particular anatomical location of the vagina, the range of resection without injury to the surrounding structures is limited (Diao *et al,* 2017).

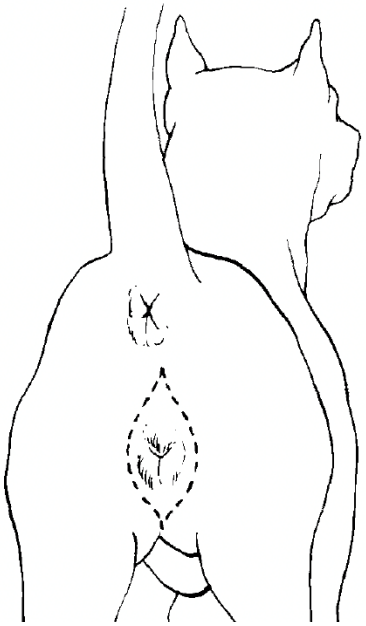


Figure 2. Incision site for vulvovaginectomy (Bilbrey *et al.*

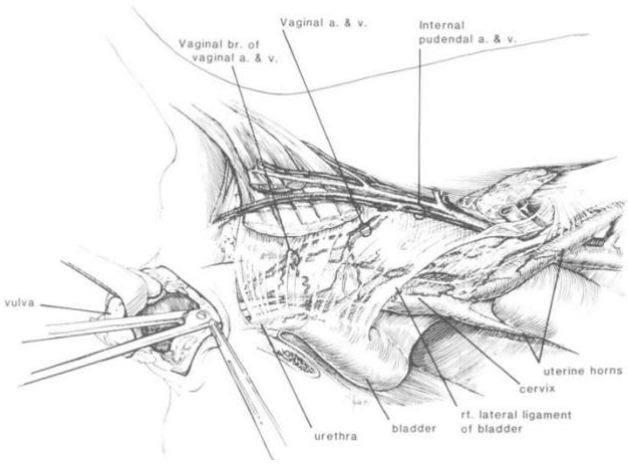


Figure 3. Surgical access. Perivaginal dissection and ligation of vessels (Bilbrey *et al,* 1989).

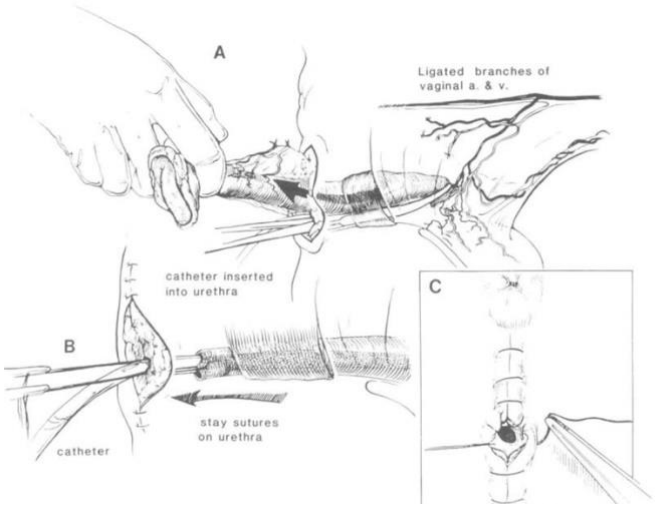


Figure 4. Vulvovaginectomy. A) Vaginal remove. B) The urethra is pulled throught the incision with stay sutures. C) The urethra is sutured to the skin (Bilbrey *et al,* 1989).

Prepubic urethrostomy is a modification technique used in one of the cases, which is performed with the patient in the supine position and consists of approximating the cranial portion of the urethra to the skin in the cranial region of the pubis, for which reason it is necessary to perform a pubic osteotomy to have access to intrapelvic urethra.

In people, the aim of the treatment is not only prolongation of survival, but also perform a personalized and individualized treatment (Diao *et al*, 2017). It has been reported that the treatment result is satisfactory when using neoadjuvant chemotherapy for early-stage vaginal cancer. Three human patients discussed in Diao *et al* were treated with either chemotherapy or chemotherapy combined with surgery and the curative effect was satisfactory.

II.6. Prognosis

The stage is the most important prognostic factor in vaginal SCC. Advanced age, LN involvement, and tumor size are considered poor prognosis factors at diagnosis. Lymphovascular invasion is a powerful indicator of tumor recurrence and short survival (Kulhan *et al*, 2016).

Extended SCC in dogs with an aggressive behavior, like in this location, have a bad prognosis, it needs a complete and exhaustive follow-up, because this type of tumor can fatal to patients because of locally aggressive invasion (Blackwood, 2016).

Moreover, the extension (LN or distant metastasis) is less frequent than local spread of the tumor, but they imply very bad prognosis.

II.6.2. Histopathology

Fully developed SCC is easy to diagnose, but early or precancerous changes can be subtle. Early neoplastic lesions, called actinic keratoses, show epidermal hyperplasia, hyperkeratosis, acanthosis, epidermal reticularis, and keratinocyte dysplasia. Affected keratinocytes, mainly found in the basal and spinous layers, exhibit loss of polarity, nuclear enlargement, hyperchromatin, enlarged and prominent nucleoli, and mitotic figures of basal and suprabasal keratinocytes. Because this damage is caused by prolonged exposure to UV light, some cases may show solar elastosis with degeneration and fragmentation of elastic and collagen fibers in the superficial dermis and thickened basophilic fibers Deposits of like material, van Gieson elastin stain positive, can be stained. At this stage, dysplastic keratinocytes have not invaded the basement membrane, as occurs in SCC described below. SCCs have an association with the overlying epidermis although this may not always be found on microscopic examination. Islands, cords, and trabeculae of neoplastic squamous epithelial cells invade the dermis and subcutis. The amount of keratin, seen as intracytoplasmic, eosinophilic fibrillar material, produced by the neoplastic cells is quite variable.

Invasion of the dermis and subcutaneous tissue may evoke a desmoplastic response. There is often an infiltrate of neutrophils into the islands of neoplastic squamous epithelium; the invasive margins of the neoplasm may show neurotropism as well as invasion of dermal and subcutaneous lymphatics.

Several uncommon variants of SCC have been described (Goldschmidt, Goldschmidt, 2017).

In human beings, the histopathological study is very useful to distinguish between VAIN and SCC, which is an important prognosis factor (Day *et al*, 2018; Zhou *et al*, 2022).

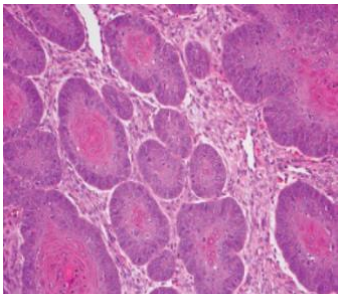
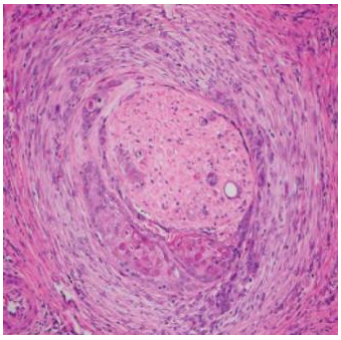


Figure 4. SCC histology (Goldschmidt, Goldschmidt, 2017).



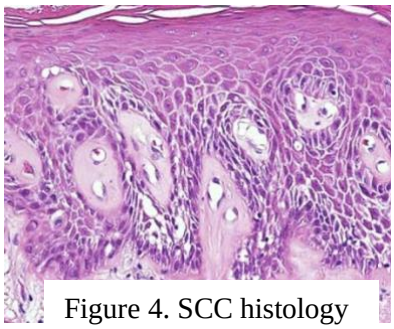


Figure 4. SCC histology (Goldschmidt, Goldschmidt, 2017).

Figure 5. Inva
desmoplasia
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II.7. Aims and objectives

1. Significant aspects of the literature review of clinical application in canine patients:
    a. Vulvovaginal SCC behavior.
    b. Clinical approach and diagnosis of the tumor.
    c. Treatment protocols and efficacy of them.
2. Clinical study of three patients with vulvovaginal SCC.
3. Based on literature review and an small group of patients, consider prognostic factors and clinical behavior.

III. Material and methods

III.1. Patients

Our three patients were remitted, two of them were diagnosed with SCC and one with undifferentiated carcinoma. They were selected for this study because of their similitude between the clinical signs and the macroscopic appearance of the tumor.

III.2. Diagnosis and staging

The first diagnostic method consisted in FNA, and after each surgery, all the tissue resected was sent to anatomopathological services.

Their common anamnesis included anuria and/or dysuria and they presented hematuria or metrorrhagia. No pain caused for the lesions was registered.

Their macroscopic appearance was prominent and ulcerated vulvar hyperplasia and inflammatory signs before the neoplastic lesions. And all of them presented lymphadenomegaly after surgery evaluated by abdominal ultrasound by the same veterinarian.

III.3. Treatment

The main treatment consisted by radical vulvovaginectomy based on the literature (Bilbrey *et al,* 1989) with some variations depending on the patient's needs:

- Patients 1 and 2: complete vaginectomy and prepubic urethrostomy was performed. The prepubic urethrostomy is a modification of the surgical technique described.
- Patient 3: complete vaginectomy, urethrostomy and urinary bladder biopsy was performed, joining the distal portion of the urethra with the perineal skin.

Two of them received adjuvant medical treatment (chemotherapy -patient 1- or Toceranib -Patient 2-). The clinical treatment was based on the oncologist experience.

III.4. Follow-up

The disease-free interval (DFI) and Overall survival (OS) are terms that determine the efficacy of the treatments applied.

III.5. Search sources

The information has been obtained from reliable sources: Pubmed, veterinary scientific literature, and VIN, a forum used by veterinarians to discuss clinical cases.

IV. Case reports

Case 1. Mixed female dog, 12 years old. She came after partial vulvar excision on another veterinary center. This patient has been one week with anuria and metrorrhagia. An exploratory laparotomy was realized, and ovarian cysts were found, so the patient was ovariohysterectomized. In the consulting room a sounding was applied and thanks to endoscopy, a urethral obstruction was diagnosed due to primary vaginal SCC. One month later she was submitted to the surgical procedure described by Bilbrey *et al.* in 1989 with prepubic urethrostomy due to the infiltration of the tumor. The surgical resection was sent to anatomopathology, the result revealed that the neoplasia consisted in SCC. The urethra portion sent presented SCC, but the urinary bladder portion was

neoplasia-free. The margins resected were free-SCC. However, the patient had a bad prognosis. One month later, the patient showed hematuria and a cyst on the vulvar region. The vet prescribed topic Betamethasone/Gentamicin (Diprogenta™: corticosteroid/antibiotic) for 4 days on the vulvar lesion, Firocoxib (Previcox™: NSAID) 0.1mg/kg/24h for one week, monometric dose of cyclophosphamide (chemotherapy) combined with Toceranib (Palladia ™) 2.5mg/kg Monday-Wednesday-Friday, and amoxicillin/clavulanic acid (augmentin™) 12.5mg/kg/12h during one week. Two months later, the patient returned to the consulting room because of anuria since the day before and edema in the left posterior extremity. The patient was sounding, and the veterinarian evacuated 1400ml of urine the next time in the hospital is described for abdominal US and Folley sounding application. OS was more than 4 months and DFI was 1 month. A lot of data about this patient has been lost.

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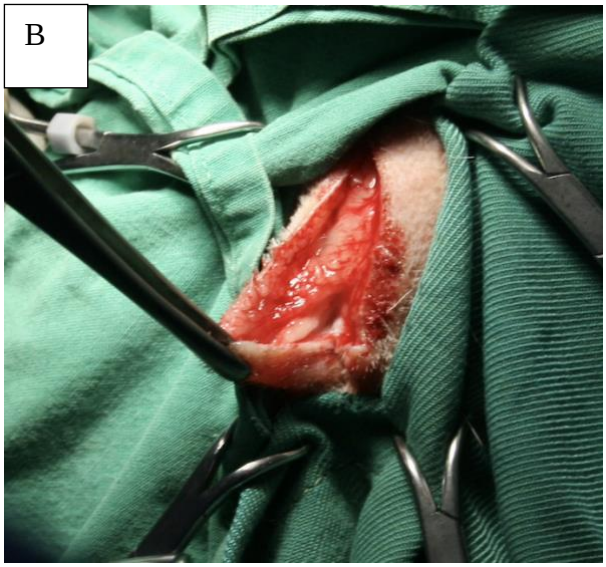

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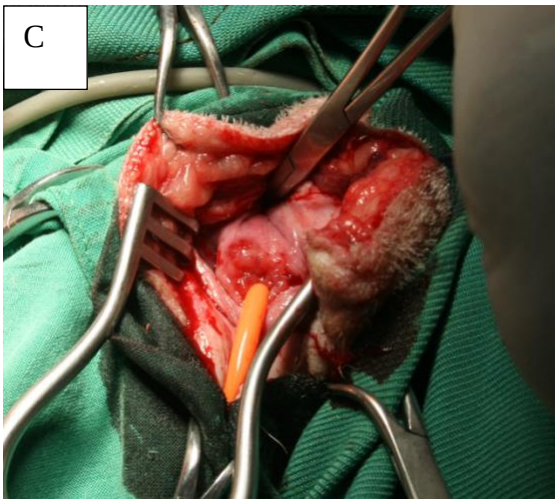

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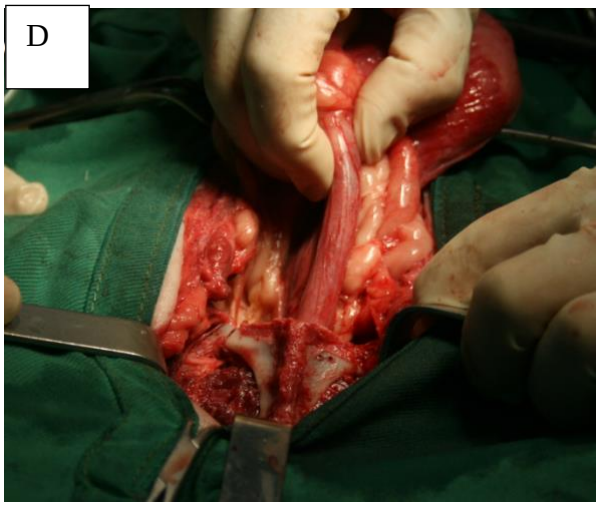


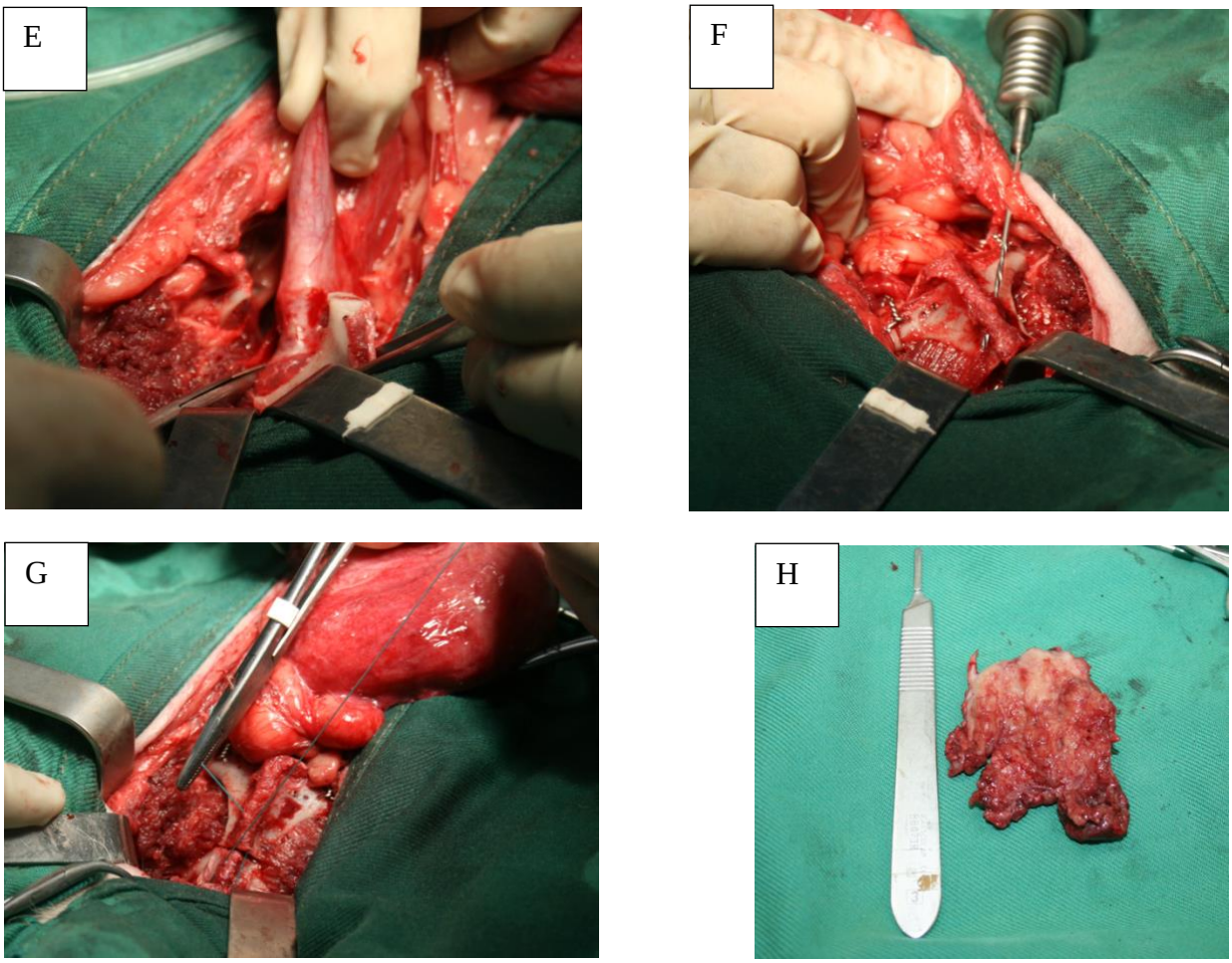
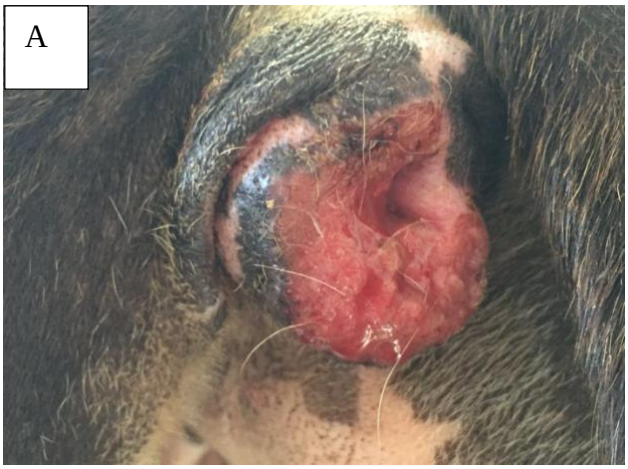
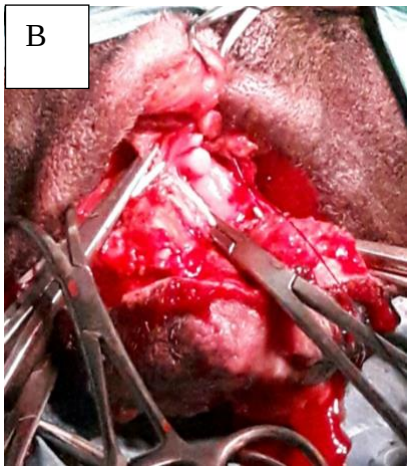


Figure 7. A) Initial image of dog after referal, catheterized and with partial vulvectomy. B-G) Images of the surgical procedure, where we can observe the mass infiltrated in internal structures of the reproductive system. The pubis is cut, and a vaginectomy is performed (D and E) trying to leave healthy margin tissue. The pubis is attached with metal suture (G). H) Picture of the removed tumor tissue.

Case 2. A 6 years and 6 months, female American pit-bull terrier, non-castrated, presented in June 2016 with an ulcerated vulvar lesion and 3-weeks of vulvar bleeding. Punch biopsies result revealed vulvitis and panniculitis, one of the samples consisted of inflammatory process with PMN-neutrophils and neovascularization; the other sample showed an infiltrative inflammatory process from the dermis to the skeletal musculature with neovascularization, and the pathologist commented the absence of neoplastic transformation. 45 days after remission, surgical excision of the lesion (7x6.5x2.5 cm) was performed and the anatomopathological report revealed ulcerated infiltrative neoplastic growth arised in the epidermis with intense desmoplastic reaction and inflammatory infiltration, diagnosing vulvovaginal SCC. Margins were free of neoplastic cells. 33 days after surgery another surgery excision was performed because of the presence of ventroinguinal cutaneous lesions, the anatomopathological report revealed that the lesions consisted of median-grade SCC lesions with infiltrative behavior and inflammatory consequences. Four months later, the patient presented a 3cm cutaneous perineal mass, after one month, vulvar enlargement and ulcerated lesion were described, the veterinarian prescribed ciprofloxacin (fluoroquinolone antibiotic) 10mg/kg/12h for one week and carprofen (Canidryl™) 4mg/kg/24h for 5 days. After four days, abdominal US was performed, and it showed bilateral iliac lymphadenopathy with moderate heterogenicity and slight surrounding reaction, so the veterinarian decided to perform a LN FNA, and the cytology result revealed polymorphic cellular population with a high level of lymphoid cells, the pathologist observed plasmatic cells and PMN-neutrophils too. Epithelial cells were not observed. Thirteen days after, the patient started with apathy and hyperthermia (39.4ºC), so she was medicated with antibiotics and when this clinic presentation ended the veterinarian prescribed Toceranib (Palladia™) 2.5mg/kg (50mg) Sunday-Tuesday-Thursday and Prednisone 2mg/kg/24h. After 3 weeks the patient presented an enlarged and ulcerated lesion, and the patient started to limp, Robenacoxib (Onsior™) 1mg/kg/24h was prescribed until the next review in one week. Four days after the patient was euthanized.





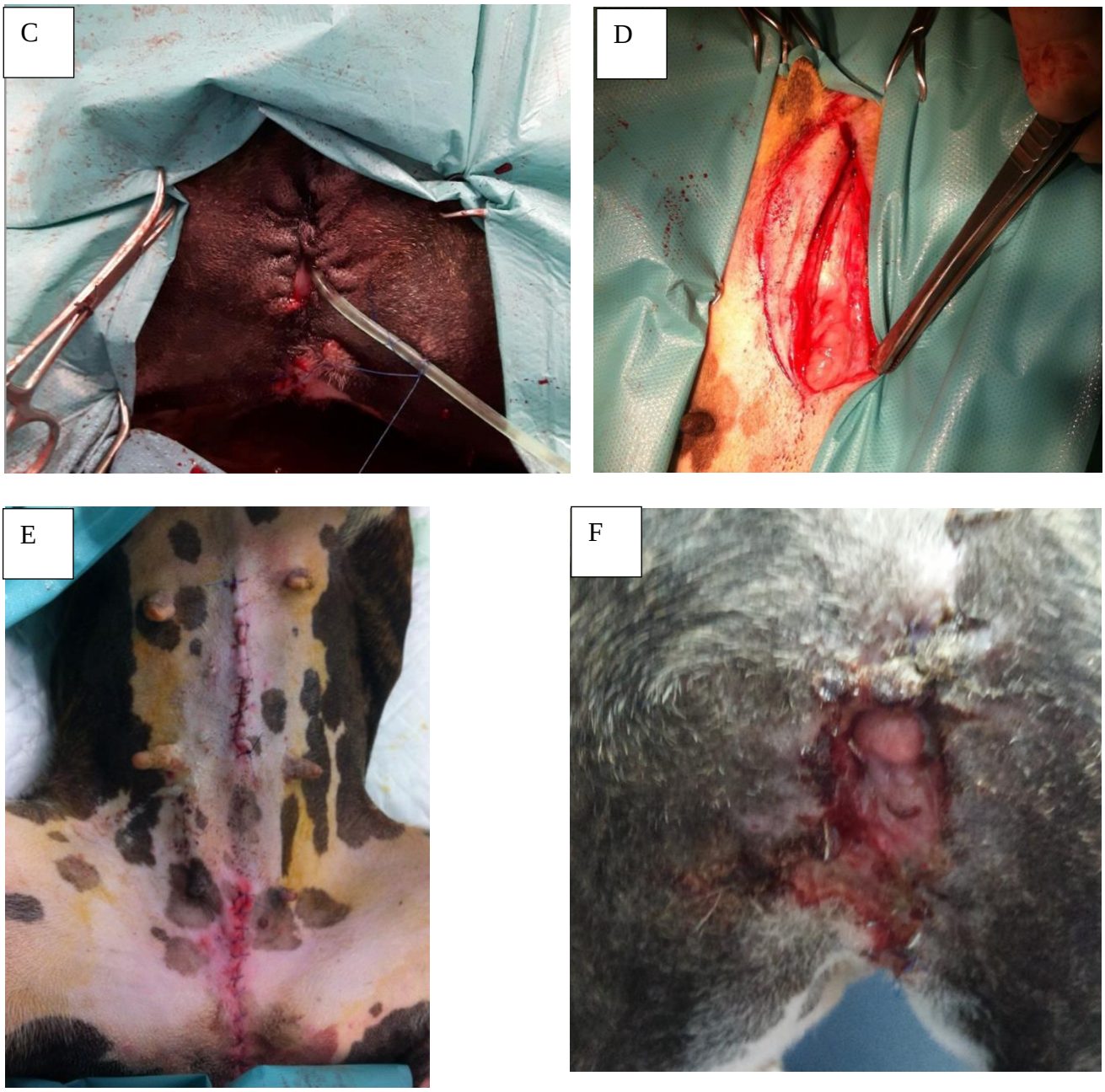
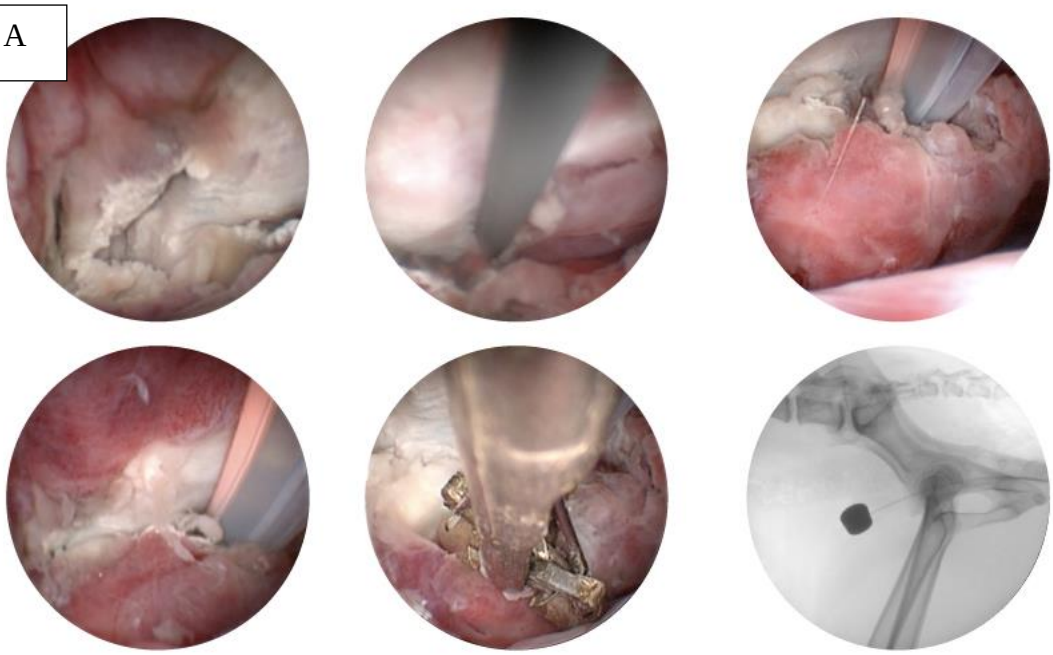


Figure 8. A) External vulvovaginal SCC lesion. B) Picture of the surgical technique; prepubic urethrostomy was performed (C). D-E) Images after the first recurrence (ventroinguinal lesions and stump were removed). F) Local recurrence of the tumor.

Case 3. An 11 years and 6 months old, female Labrador retriever was spayed at 8 years old because of pyometra. She has been treated with corticosteroids and antibiotics because of atopic dermatitis. The patient presents to the consultation because she does not eat, drink, or urinate. three weeks ago, she started urinating large amounts of pee at home. Hemogram without abnormalities, urine sediment and she started with Amoxicillin/clavulanic acid (Augmentin™); it caused vomiting and diarrhea, so she stopped. After two weeks, the patient started with anuria, she tries to pee, but she cannot; two days later, she started again with vomits, and after two days (day before consultation), she started once again with diarrhea. The patient has normal behavior and no changes in her normal activity. On physical exploration, the veterinarian palped lymphadenomegaly on prescapular and popliteal lymph-nodes, an enlarged urinary bladder, and a vaginal mass surrounding the urethra that prevent sounding. One day after the consultation, an abdominal US was performed because of the urinary tract obstruction, and it showed an enlarged urinary bladder with fine, smooth, and homogeneous walls, with no visible alterations. The urethra was distended (8.2mm) and iliac lymphadenomegaly. The following day, a urinary endoscopy (Fig. 9, A) was realized with Hopkins II 30º 2.7mm. During the urinary endoscopy, it was impossible to evaluate the vesical mucosa and urethral proximal portion because of the presence of a 5 cm deep mass at the urethral meatus level. A biopsy was taken and it urinary sounding (Foley Ch. 8). The anatomopathological report revealed undifferentiated carcinoma. One week after the first consultation, the surgical resection was performed, and the patient stayed at UCI until she could urinate, with some medicaments: Robenaxib (Onsior™) (NSAID) 1mg/kg/24h for 5 days and some amoxicillin/clavulanic acid (Augmentin™) 12.5mg/kg/12h for 7 days. This patient died a few days later of unknown causes.

A



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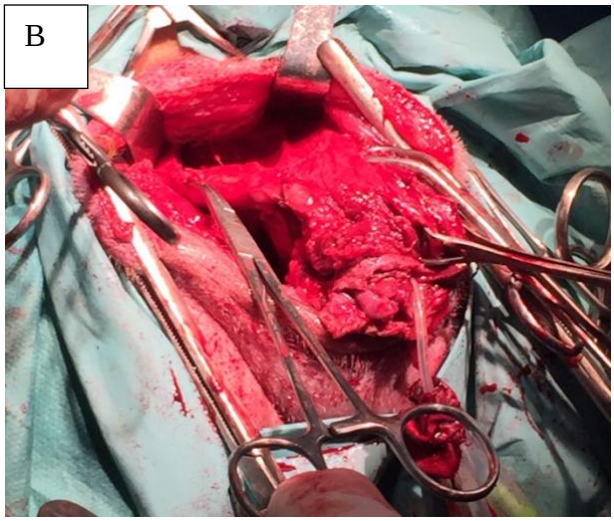

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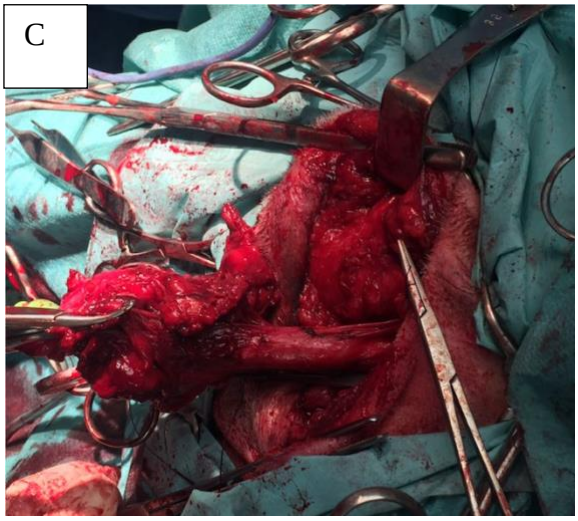

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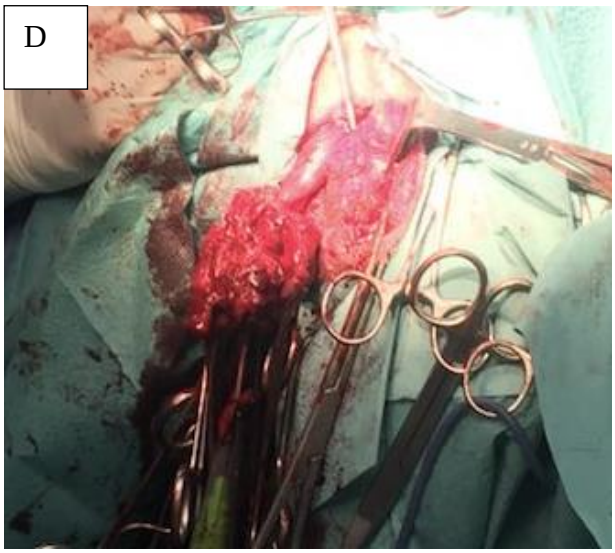

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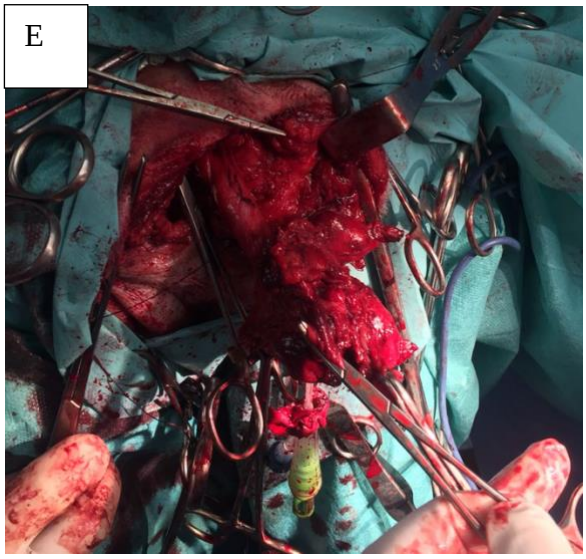

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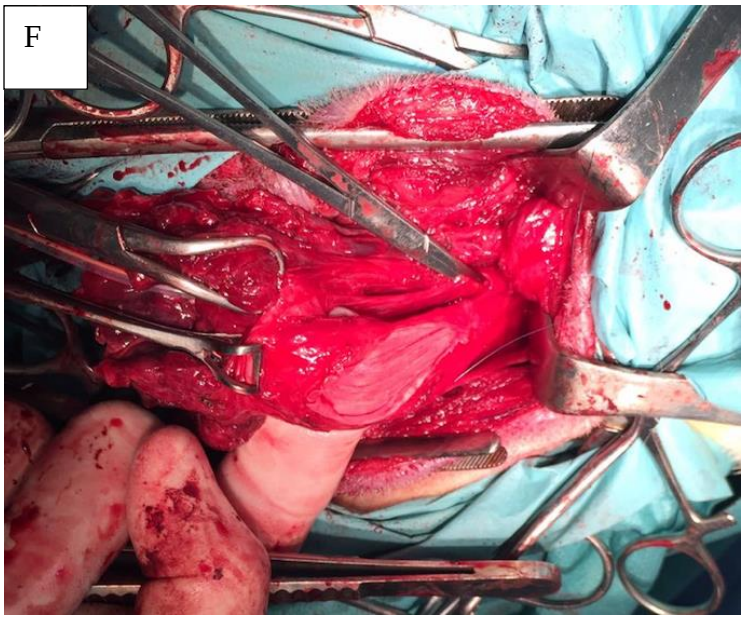

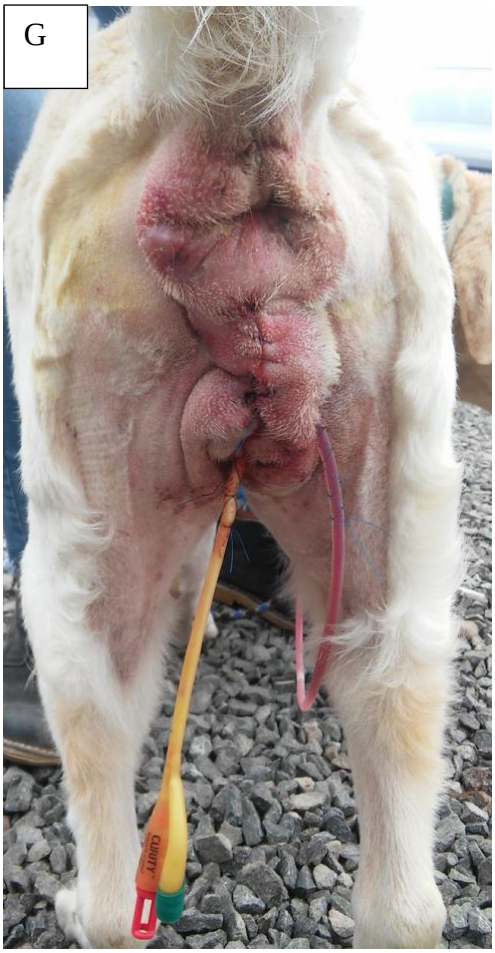

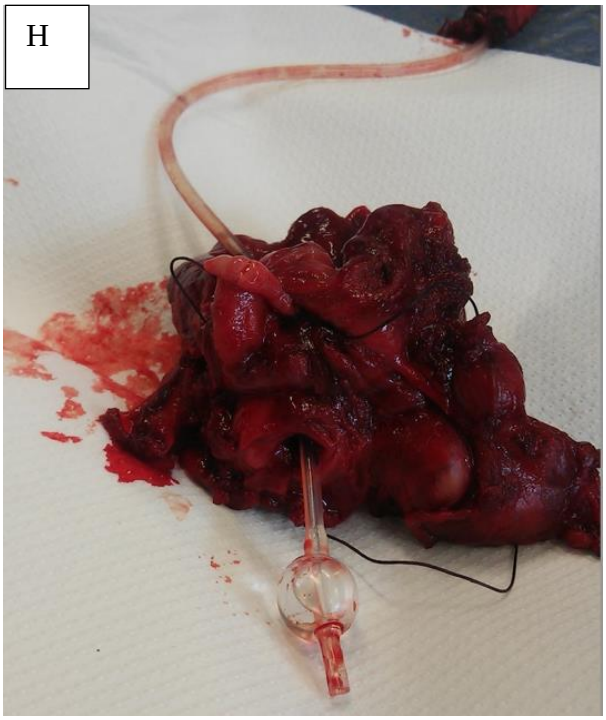

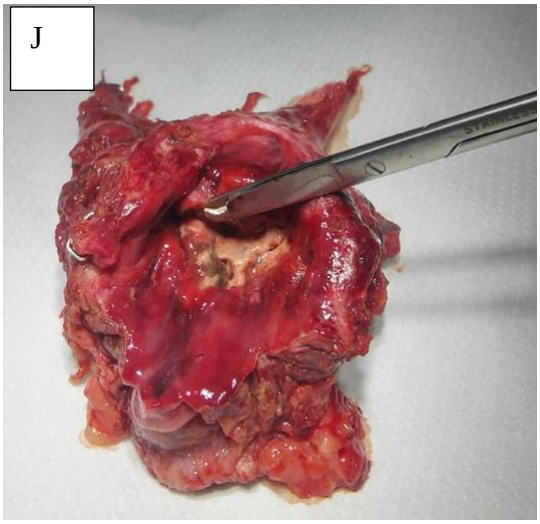

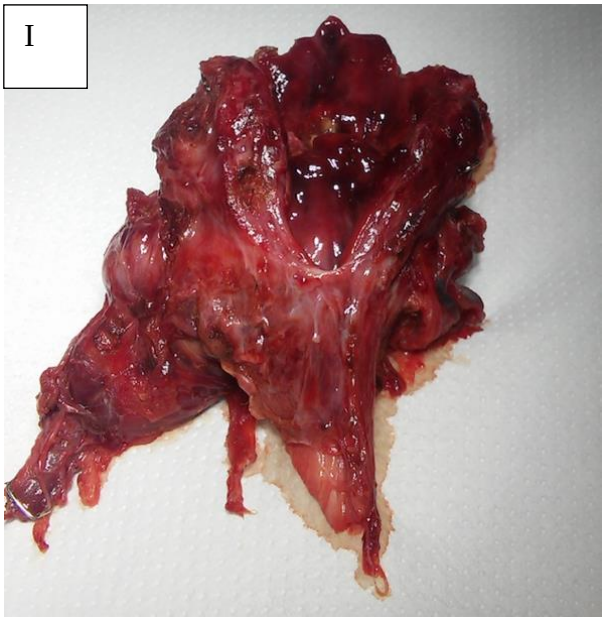

Figure 9. A) Urinary endoscopy and biopsy taking. B-E) Vulvovaginectomy and (F)biosy of the bladder. G) Prepubic utrethrostomy. The proximal urethra was sutured to the skin and the multifenestrated catheter was placed into the surgical site with continuous suction device. H-J) Picture of the removed tumoral tissue, in which a necrotic area can be observed (J).

V. Results.

Patients one and three had advanced disease at the time of surgical intervention, and disease spread was not evaluated since surgical extension included all removable tissues preservation urinary and digestive functions. Pelvic exenteration, practiced sometimes in women (Lewandowska *et al*, 2020) cannot be considered on dogs since creation of multiples stomas was not tolerated by owners nor recommended.

Patient two, that had an earlier diagnosis and lees aggressive surgery, progressed despite of having obtained clean margins.

Patient three died of unknown causes shortly after surgical removal of affected tissues, so local and distant behavior could no evaluated. Following tables illustrate clinical aspects of all three cases.

Patient	**Review**	**Diagnosis**	**Clinical signs**	**Surgical procedure**	**Medical treatment**	**OS and DFI**
Case 1	12 y/o Mixed Spayed	Vaginal SCC	Metrorrhagia and anuria	Vulvovaginectomy and prepubic urethrostomy was performed.	Ciclophosphamide NSAID Topic corticosteroid Antibiotics	OS >4months DFI: 1 month
Case 2	6 y/o American pil-bull terrier Spayed	Vulvovaginal SCC Inguinal cutaneous SCC	Vulvar bleeding	Vulvovaginectomy and prepubic urethrostomy was performed.	Toceranib NSAID Corticosteroid Antibiotics	OS: 8 months DFI: 33 days
Case 3	11 y/o Labrador retriever Spayed	Urethral undifferentiated carcinoma	Anorexia, anuria	Complete vaginectomy, urethrostomy and urinary bladder biopsy was performed, joining the distal portion of the urethra with the perineal skin.	NSAID Antibiotics	NA (Not applicable)

Table 1. Complete medical history of the patients.

Patient	**Tumor behavior**	**Metastasis**	**Recurrence**
Case 1	Local infiltration Invasion to urinary tract	Iliac lymphadenomegaly	Yes in 1 month
Case 2	Local infiltration Cutaneous ventroinguinal lesions	Iliac lymphadenomegaly	Yes in 8.5 months
Case 3	Local infiltration, invasion of urinary tract	Iliac, prescapular and popliteal lymphadenomegaly	Unknown

Table 2. Histological description, metastasis and recurrence of the disease of each patient.

VI. Discussion

In human literature, there is a lot of information about preneoplastic lesions and their transformation to SCC several possible influencing factors that need to be studied on their importance in the transformation from in situ to invasive vaginal SCC (Wu, *et al* 2008).

The UV exposure influence has been discarded in human beings for causing vulvovaginal SCC because of its low exposure in this location but in dogs further investigation is needed. In women the main factors associated to vulvovaginal SCC are multiple sexual partners, low socioeconomic status, family history of anogenital cancer, and cigarette smoking; and apparently, most of these factors increase the risk of vaginal cancer through high exposure to oncogenic HPV-infection. Previous studies suggest that nicotine may block apoptosis and suppress the immune system, possibly promoting the development of vaginal cancer. In women has been studied the association between previous hysterectomy and an increased risk of vaginal cancer, it is believed that the risk may be related to previous HPV-associated anogenital diseases that led to the surgery which reported are more frequent among women with vaginal cancer (Wu, *et al*, 2008).

There is evidence that if the patient has received radiotherapy for other anogenital malignancies is a risk factor for suffering vaginal SCC (Wu, *et al* 2008).

In women, LS is associated with vulvar SCC, but in dogs has not been associated. Moreover, in relation to high-grade VaIN (Vaginal Intralesional Neoplasia) and high-grade VIN (Vulvar Intralesional Neoplasia) could be associated in female dogs like in women. This association is shown in case 2, because on her first anatomopathological report the diagnosis was vulvitis and panniculitis with neovascularization and infiltrative inflammation and she had surgical resection of a vaginal SCC mass one month after the first biopsy. Another association is found between taking a biopsy of a preneoplastic lesion before a CT diagnostic and rapid growth and neoplastic transformation of the tissue, having a more aggressive behavior (Hauk and Oblak, 2020).

Local invasion of the SCC is associated with a later diagnostic time, which in women depends on their socioeconomic context, and in dogs depends mainly on their owners. Local invasion is also associated with a worse prognosis of the disease because it indicates a greater spread, and the possibility of lymphatic and vascular invasion, invasion of other structures, and lymph node metastasis. Furthermore, local invasion makes complete surgical resection of the neoplasm difficult with margins free of tumor cells.

One of the patients (case 3) was diagnosed with undifferentiated carcinoma and is included on this study because of its similar clinical presentation, tumor behavior, and surgical procedure. In addition, because the anatomopathological report corresponding to the resection of the anatomical piece has not been found, which would give more information than the biopsy report. Due to the similarity of behavior and symptoms with a case described in the human literature, it is worth mentioning that cases have been described in which SCC has been found in samples of the urinary bladder and vaginal

wall. This patient was evaluated with endoscopy and This patient was evaluated by endoscopy and a mass was observed 5 cm deep in the vaginal meatus that produced stenosis and even blockage of the urinary tract; adding that the mucosa was hyperemic, friable, slightly ulcerated, and edematous, having behavior similar to SCC; In addition, its location can be compared with that described in the human medicine literature: women vaginal's middle third.

In human beings when a primary vaginal or vulvar mass is diagnosed with FNA or by biopsy, the woman undergoes a diagnostic imaging study, which normally consists of a CT study to be able to stage the disease and visualize the actual invasion of the neoplasia. There is a study that specifies the utility of performing MRI in patients with vaginal SCC (Lima *et al*, 2019). However, in our patients, no imaging study was performed prior to surgery that included the region where the tumor was located.

In human medicine, there are strict action protocols, since metastasis to adjacent or distant lymph nodes changes the patient's prognosis significantly, as does the size of the tumor. This protocol is so important, since it will also change the way of approaching the treatment, because in the literature it is described that in cases involving vulvovaginal SCC - grade II or higher, surgical treatment is not indicated, since the local recurrence of the tumor is fast, and it can have a more aggressive behavior that significantly worsens the quality of life of the patient as well as its survival period. Medical treatments for vaginal SCC - grade II are still under study, with one study using pembrolizumab (immunotherapy) without objective responses observed (Jeffrey *et al*, 2021), in such a way that the standard treatment in these cases would be to administer palliative treatment to the patient. Nowadays, we do not know the tumor grade of our patients because no diagnostic protocol for vulvovaginal SCC in dogs has been standardized. Even though none of our patients underwent a CT study, in patient 3, who was evaluated with abdominal ultrasound, bilateral iliac lymphadenomegaly with a slight reaction around the lymph nodes was found. However, in the rest of the patients, abdominal ultrasound was performed only after remission of the tumor once they had already undergone surgery.

In human patients, despite needing radical surgery, in some cases, an attempt has been made to preserve the vulva due to the moral damage it can cause in patients. In one of the studies, one patient refused to undergo surgery, so it was performed. only medical treatment consisting of a combination of radiotherapy and chemotherapy, having a positive response from the tumor. Due to the complete study of the disease, in humans, it has been proposed to standardize a treatment according to the location of the tumor within the vagina, in this study the authors classified uniform treatment options:

- “Partial vaginectomy with adequate surgical margins + pelvic lymphadenectomy ± hysterectomy for mass located in the superior part of the vagina.
- Radical vaginectomy with vulvectomy and inguinal lymphadenectomy for vaginal lesions located in the inferior part of the vagina.
- Radical vaginectomy + pelvic lymphadenectomy + elective inguinal lymphadenectomy are recommended for lesions located in the middle part of the vagina.”

But in this article, 55.6% of the patients with vaginal SCC had a recurrence. (Kulhan *et al,* 2016). There have not been such extensive studies in female dogs, so less aggressive surgical techniques are not proposed, in addition to the fact that only one surgical technique has been described for tumors similar to the one presented by our patients, described by Bilbrey *et al.;* despite being a radical surgery (with margins free of neoplastic cells) and making modifications taking into account the needs of each patient, all showed tumor recurrence.
Patient 2 had local recurrence of the vaginal SCC after 8.5 months, so surgery excision can be a long-term control of the tumor when it is realized on early-stage, but there is no information available for this long-term control because it can depend on the age of the patient, the stage of the tumor and/or the surgical procedure applied.

In human patients, surgical resection of the mass combined with radiotherapy is the typical treatment of primary vaginal SCC, because there is evidence that women with positive groin LN who received adjuvant radiotherapy directed at the groins had improved survival (Olawaiye *et al,* 2021). However, it has been demonstrated that radiotherapy can be a risk factor for the appearance of other anogenital tumors because vulvovaginal adjacent structures are not protected from radiation (Wu *et al,* 2008). This therapeutic option could not even be considered for our patients because at that time there was no radiotherapy equipment in Spain.

Currently, in humans, the individualized treatment of patients is being investigated and favorable results have been obtained with respect to the disease-free period and survival time. In veterinary medicine, this type of treatment is not yet taken into account and there are no articles that describe individualized treatments of vaginal SCC.

In human patients, total disease remission has been achieved after complete surgical excision with margins free of tumor cells; however, our patients had a rapid recurrence. This may be because in humans the surgical action protocol not only includes excision of the lesion but also of the iliac LN, in addition to the fact that patients are generally subjected to neoadjuvant therapies. Our patients received treatment (patient 1: cyclophosphamide; and patient 2: Toceranib) once it was evident that the disease had recurred; both without evident therapeutic effect at the time of administration.

An important factor to consider is the grade of the tumor, since depending on said grade the treatment will be more or less effective. In human patients, surgery is an effective treatment when the patient presents VaIN, vulvar SCC or vaginal SCC grade I. In female dogs, a scale that defines the grade of this type of tumors has not been carried out, so we do not know the grade of the tumor at the time of surgery. In addition, due to the great advances in human medicine compared to veterinary medicine, it is much easier to detect low-grade neoplastic lesions or even pre-neoplastic lesions in human patients rather than in dogs.

However, it seems that in patient 2 at the time of the biopsy the vulvovaginal lesion was a preneoplastic lesion, since as the pathologist specified in the report: the samples consisted of an inflammatory process with PMN-neutrophils and neovascularization,

and showed an infiltrative inflammatory process from the dermis to the skeletal musculature with neovascularization; so, the pathologist commented the absence of neoplastic transformation. Therefore, in this case, the similarity that the vulvovaginal SCC has in bitches and women is evident.

Radiotherapy is the concomitant therapy to surgery traditionally used in people with vulvovaginal SCC, however, this treatment is being replaced because radiotherapy can emit radiation to adjacent structures, in addition to carrying out individualized therapies according to the needs and efficacy that the patient may have. On personal communication, recognized veterinarians recommend radiotherapy after surgical excision of the lesion - in addition to monitoring the patient using diagnostic imaging techniques: evaluating iliac nodes and possible spread of the disease, either locally, regional metastases or distant metastases - however, no studies have been published on this type of tumors to date, so we are completely unaware of the efficacy and possible side effects that this therapy may have on female dogs.

The efficacy with respect to the use of non-steroidal anti-inflammatory drugs or corticosteroids has not been proven in human medicine, however, in some studies non-steroidal anti-inflammatory drugs have been used in people who presented preneoplastic lesions, although they always consider the origin of the lesion, since which are mostly caused by HPV or by evolution of LS. In the study Day *et al* suggests that it is recommended that women who are part of the high-risk group be given maintenance treatment with topical steroids or calcineurin inhibitors to prevent severe relapses. In our patients, both drugs were used at different times without showing significant efficacy that improved the clinical presentation of the disease or slowed or decreased the growth of the lesions (Day *et al*, 2018).

In humans, as well as in our patients, antibiotics were used due to the local infection that the patients presented.

In women, a study was carried out with three patients treated with surgery and a combination of irinotecan with cisplatin, achieving a complete response confirmed by pathological examination and biopsies performed in all patients. (Diao *et al*, 2017) In one study (Kulhan *et al*, 2016), a group of patients was treated with radiation alone, another with chemoradiation, and another with chemoradiation as adjuvant therapy, and 55.6% of the patients relapsed. On the other hand, immunotherapy is being developed in human oncology, due to the fact that it is having good results, for which a study was carried out in which Pembrolizumab was used in vaginal and vulvar grade II SCC, caused by HPV, without obtaining responses favorable in none of the patients, in whom up to 10 cycles of Pembrolizumab were used. (How *et al*, 2021) Imiquimod - topical treatment - has been studied to be useful in preneoplastic SCC lesions even in veterinary medicine (Hauck and Oblak, 2020), however, due to the location of the tumor and the price of this drug in Spain, its use was completely ruled out, even in the patient who apparently had pre-neoplastic lesions because she could lick the product and we do not know its secondary effects. In veterinary medicine, no studies have been published regarding medical therapies applied to female dogs with vulvovaginal SCC. Therefore, in patient 1, cyclophosphamide was used as chemotherapy combined with surgery,

which did not show improvement in symptoms or clinical presentation, and in patient 2, Toceranib was used, without showing favorable responses in the patient -both treatments were used based on the experience of the veterinarian responsible for the oncology service-.

In human epidemiological studies of vaginal SCC, the importance of postoperative care has been highlighted with respect to the survival period of the disease, finding differences between black and white women, because the latter usually have a better quality of life, in addition that the disease was detected when they were younger, which improved their prognosis factor. (Wu *et al*, 2008) This can be related to the fact that the patient who attended the consultation the most pre and postoperatively, due to the fact that she received more veterinary check-ups, tests and therapies for the disease could be anticipated, preventing it from spreading more quickly, despite the aggressiveness of the tumor. Moreover, it is important to highlight the speed of recovery that our patients had after surgery, since on the same day two of the patients and the next day they were able to go home with hospital discharge, since they could walk, ate, drank, and urinated normally.

In women, the mortality of vaginal SCC increases significantly with the age of diagnosis, it has been shown in our patients, because patient 2 (the 6 years old one) had a higher OS than the other two.
Compared to women with a localized vaginal SCC, women with regional spread have an increased hazard of shorter survival time (Zhou, *et al* 2022). It can be predicted that in female dogs the behavior will be the same, since a greater expansion of the tumor is associated with a higher grade, which in turn will be indicative of a worse prognosis and therefore will cause a shorter OS and free-disease period too. Which it can be de reason that when the lymph nodes are observed to be enlarged in the abdominal ultrasound follow-up after surgery, it is when it is predictable that the patient will begin to worsen and present clinical symptoms again.

VII. Conclusions

1.- The behavior of the vulvovaginal SCC is similar in women and female dogs.
2.- Human morphological criteria in could be used in veterinary medicine to classify the stages prior to the neoplastic transformation of epithelial tissue into SCC, improving the quality of life of patients, their OS, reducing the invasiveness of treatments and their side effects.
3.- Human medicine diagnostic protocols can also be used as a reference in the case of suspected vulvar or vaginal SCC in female dogs.
4.- Lack of information on vulvovaginal SCC in female dogs is notorious, and our low number of cases may not be representative.

5.- In veterinary medicine, a reliable staging for this type of tumor in female dogs has not been developed. Therefore, the significance of stage on prognosis is not reliable at present but may be similar to SCC in other locations. Differences in DFI and OS observed in case 2 can be attributed to an early diagnosis, and it may favor a radical surgery from the first stage diagnosis.

6. Advanced imaging techniques should be recommended from the first diagnosis in order to plan the extension of surgery.
7.- Adjuvant combined therapies need to be evaluated
8.- Radical surgery in advanced cases did not prove to have a clear influence on DFI or OS. In our patients, surgery did not prove to be the cure of the disease in any of the patients, delaying the growth and aggressiveness of the SCC in at least one of our patients (case 2), who presented recurrence 8.5 months later.

ABBREVIATIONS:

CT: Computerized Tomography
DFI: Disease-free interval
FNA: Fine needle aspiration
HPV: Human Papillomavirus
LN: Lymph node
LS: Lichen Sclerous
MRI: Magnetic resonance image
NA: Not applicable
OS: Overall survival
PMN-Neutrophils: Polymorphonuclear neutrophils
PPV: Papillomavirus
SCC: Squamous cell carcinoma
US: Ultrasound
UV: Ultraviolet
VaIN: Vaginal intraepithelial neoplasia
VIN: Vulvar intraepithelial neoplasia

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