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Sex Steroid Hormones and Tumors in Domestic Animals

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1. Introduction

Sex steroid hormones play a role in the development and control of animal tumours, particularly in those arising in their target organs. Due to their incidence and prevalence, mammary tumours of female dogs and cats are among the most frequently studied with focus on the role of ovarian oestrogen and progesterone. In these tumours, sex steroid hormones have been shown to act during the three steps of the carcinogenesis cascade: initiation, promotion and progression. Experimental data have shown the mutagenic effect of oestrogens [1] while epidemiologic and clinical studies highlighted the role of ovarian hormones as promoters on mammary tumours in both the dog and the cat [2-9]. Finally, oestrogens and progesterone further act during tumour progression. Their role in the last two steps of carcinogenesis makes it possible to control the evolution of the disease.

Studies on the role of ovarian hormones during tumour progression depend on the capability of demonstrating the presence of oestrogen receptors (ER) and progesterone receptors (PR) in tumours. Earlier studies on the field were published in the seventies of the 20th century, all related to mammary gland tumours. Some of these studies revealed the presence of unoccupied hormone receptors in tissue homogenates from mammary tumours of dogs and cats [10-13]. However, actual knowledge comes from the late nineties, when different groups of researchers standardized immunohistochemical (IHC) methods of analysis for ER and PR in feline and canine tissues [14-19]. Once the IHC analysis revealed the presence of sex steroid hormones receptors in mammary tumours, several studies analyzed their value as favourable prognostic indicators [18, 20-25] adding new data to the well-known similari-

ties between and human and animal mammary tumours [26]. However, the important role of ER and PR as predictive factors of response to endocrine treatment of breast cancer has been rarely analyzed in animal tumours although recent studies based on the blockade of PR in canine mammary carcinomas and reproductive tract tumours of female and male dogs and cats support their value in the control of these diseases [27-30].

Mammary gland tumours are the most frequent tumours in female dogs and the third in the cat. However, they are not the only tumours known to be sex steroid-dependent. Tumours of the reproductive tract of female and male dogs and cats and some skin appendages can be defined as hormone-dependent on the basis of the IHC expression of ER α and PR [31, 32]. In addition, epidemiological and clinical studies support the role of steroid hormones as tumour promoters [28]. The most common tumours in the genital tract of the bitch are benign smooth muscle tumours of the vagina, vulva and perineal skin. Their hormone-dependence is similar to that of mammary gland tumours as the majority of canine genital tract leiomyomas express PR and also respond to neoadjuvant treatments with the PR antagonist aglepristone with a reduction in size [28].

In the dog, and to a lesser extent in the cat, benign and malignant sebaceous gland neoplasias are among the most common skin tumours. Studies concerning the role of sex steroid hormones during tumour promotion and progression are scarce but do show there is ER and PR expression in sebaceous gland tumours of dogs. Therefore, not only androgens but also progesterone and estrogens may regulate hormone-related physiology. In fact, preclinical studies indicate partial clinical remission after chemical castration in old dogs with hepatoid gland tumours and heart failure [30].

2. Methods for studying steroid hormone receptors

Immunohistochemical (IHC) methods based on antigen-antibody reactions are widely used today to detect steroid hormone receptors in tumours. New specific antibodies have been developed against a range of steroid hormone receptors, enabling reliable detection by ordinary light microscopy. Traditionally, however, steroid hormone receptors were detected by biochemical assay based on the binding of radiolabelled ligands to unoccupied receptors. Among the most commonly used biochemical techniques was the dextran-coated charcoal (DCC) method [33], which – until the late nineties – provided all the data available on steroid hormone receptor expression in mammary carcinomas in female domestic animals. Biochemical techniques, however, were not without drawbacks: they had to be applied to frozen tissue samples, were very expensive, needed specialised equipment and were not widely accessible. For this reason, published research on the use of these techniques was scanty. The development of monoclonal antibodies highly specific to oestrogen receptor (ER α) and progesterone receptor (PR) proteins enabled the development of immunohistochemical techniques based on the binding of receptors to specific antibodies [34-37]. These techniques offered several major advantages over biochemical methods [38-40]:

1. They could be applied to tissue samples routinely processed for histopathology and could be performed in the pathology laboratory, rendering them both more accessible and less costly.
2. They provided information regarding the specific location of ER and PR, enabling analysis of the relationship between receptor expression and tissue structures in normal and/or tumoural mammary glands, thus avoiding the false-positive findings common in biochemical examinations.
3. They furnished information on all the receptors present in tissue samples, irrespective of the occupation status.

The process of standardizing IHC methods of ER and PR detection in animal tissues involved the use of commercially available antibodies raised against human antigens first as antibodies raised against canine and feline antigens were not produced. Then, these IHC methods for detecting ER and PR in formalin-fixed, paraffin wax-embedded tissue samples needed to be validated before they could be routinely implemented, as stipulated by the “National Institutes of Health Consensus Conference on Estrogen Receptors in Breast Cancer” (NIHCC), held in 1979. The first step in the validation procedure was the comparison of ER and PR detection using both biochemical and IHC techniques and the correlation of IHC results with those of the gold-standard radioligand-binding assays in order to evaluate their specificity and sensitivity. In 1999, Graham et al. [15] analysed ER α expression in formalin-fixed, paraffin-embedded tissue samples from canine mammary tumours using biochemical and IHC methods, reporting good correlation between the two. In 2000 and 2002, Martín de las Mulas et al. [17, 19] validated the IHC method for the detection of ER α and PR detection, respectively, in formalin-fixed, paraffin-embedded tissue samples from feline mammary tumours using the avidin-biotin-peroxidase complex (ABC) method, and compared their findings with those obtained using the DCC biochemical method; reported agreement between the two methods was 72.7% for ER α and 95.6% for PR. The IHC technique used demonstrated good specificity (true-negatives) and sensibility (true-positives) (Table 1).

DCC versus IHC	ER α	PR
SPECIFICITY	95.6%	89.4%
SENSIBILITY	47.6%	87.5%

Table 1. Specificity and sensibility of an IHC method for detecting ER and PR, versus a biochemical method (DCC) in feline mammary carcinomas [17, 19].

Second, the NIHCC (1979) also recommended the clinical validation of technically validated IHC methods since the presence of ER and/or PR in human breast cancer was known to be a favourable prognostic indicator as well as a predictive marker of the response to adjuvant hormone therapy [41, 42]. The prognostic value of the IHC expression of ER α and PR expression in canine mammary carcinoma has been demonstrated in the dog and the cat [18,

20-25]. Finally, preclinical and clinical studies of our group have shown that the PR antagonist Aglepristone produces partial clinical remission of canine mammary carcinoma [29].

At present, IHC methods are routinely used in a number of veterinary laboratories for the detection of ER and PR. However, no consensus has yet been reached regarding tissue preparation, the antibodies and techniques to be used, and the evaluation of results. New image-analysis systems may help to objectively evaluate receptor expression and to standardise the results obtained by different pathologists [43]. The guidelines issued by the American Society of Clinical Oncology/College of American Pathologists to improve the accuracy of immunohistochemical ER α and PR testing in breast cancer and their utility as predictive markers of the response to hormone therapy may serve as a model for veterinary pathologists [44].

3. Mammary gland tumours in dogs and cats

Mammary gland tumours are the most common neoplasms in female dogs and the third most common in female cats, but are rare in other domestic animals. Between 41% and 53% of mammary tumours in dogs and between 85% and 93% in cats are malignant. Most authors agree that these tumours account for around 50% and 17% of all neoplasms in dogs and cats, respectively, with an incidence of 205 and 25.4 per 100,000 dogs and cats at risk. Median age at tumour presentation is 10-12 years in both species [45]. Tumours develop almost exclusively in females, appearing only rarely in males [46-48]. Breed is also a risk factor: mammary tumours are more common in pure-bred than in mixed-breed animals, and some breeds – including Poodles, Boxers, Beagles, a number of Spaniel breeds, and Siamese cats – display a higher incidence than others [45, 49-51]. Other risk factors are more controversial, or have been the subject of less research: diet, exposure to radiation, family background and individual history of benign and malignant mammary lesions. The action of ovarian hormones – oestrogens and progesterone – on mammary gland tissue during different stages of development is also a risk factor associated with the development of mammary tumours, while breeding-related factors such as parity, age at first gestation, number of pregnancies, pseudopregnancy and changes in the oestrous cycle, do not appear to influence the risk of developing mammary tumours, although not all authors agree on this [45].

The development of mammary tumours in female dogs and cats is clearly hormone-dependent, and they offer a good spontaneous model of human breast cancer. The preventive effects of castration on the development of mammary tumours in both species have been reported [52-54]. Early ovariectomy in dogs and cats has a protective effect against both benign and malignant mammary tumours, the intensity of that effect depending on the number of cycles before spaying. In dogs spayed before the first oestrus, the risk of developing a mammary tumour is 0.05 %, compared to 8% after the first oestrus and 26% after the second oestrus; the protective effect of spaying disappears after the age of 2.5 years. In cats, spaying before 6 months of age reduces the risk of mammary carcinoma by 91% with respect to unspayed cats, while spaying before 1 year of age cuts the risk by 86%.

While prolonged administration of oestrogens has not been shown to increase the incidence of mammary tumours [55,56], administration of medroxyprogesterone acetate and progestins over a prolonged period to young female dogs increases the risk of mainly benign mammary tumours; the intensity of that risk depends on the dosage received and on the regularity or irregularity of treatment [6]. A number of authors, however, also report an increased incidence of malignant tumours [7]. The risk of developing a malignant tumour rises following long-term experimental administration of oestrogens combined with high-dose progestins as shown by experimental studies to analyze the effects of the pill [55, 57], and following the administration of drugs with combined progestagenic-oestrogenic activity to control the signs of oestrus [6-9]. Thus, prolonged administration of high-dose (125 x human dose) of a progestational compound (lynestrenol) prompted the development of breast cancer in 40% of intact treated dogs [6]. By contrast, a combined regime of low-dose progestagen+oestrogen appears to afford some protection [6]. Cats treated with synthetic progestins or oestrogen-progestin combinations displayed a threefold higher risk of developing benign and malignant mammary tumours than untreated cats [58].

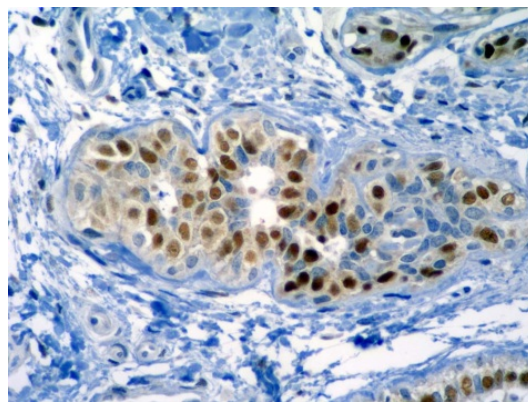


Figure 1. Details of a canine simple carcinoma showing expression of ER α in the nucleus of luminal epithelial cells (brown colour). ABC. 40X.

The role played by ovarian steroid hormones during tumour progression (i.e., once the tumours are detected clinically) has been studied through their expression of ER α and PR. Early studies using biochemical methods of ER α and PR analysis showed that between 65% and 92% of mammary tumours contain hormone receptors [17, 19]. With IHC methods, differential expression between benign and malignant tumours has been observed in dogs and cats as the expression was higher in the former. However, data concerning ER α expression in mammary carcinomas are very different among different laboratories. Thus, in the dog, 7% [59], 10% [16], 11% [14], 22% [20], 24% [15], 59% [60, 61], 87.5% [18] and 92.6% [62] of canine mammary carcinomas have been shown to express ER α (Figure 1). Concerning PR expression, reported data are the following: 14% [63], 33% [14], 42%-52% [62, 64], 60% [16] and 66% [20](Figure 2). In the cat, 7% and 17% of in situ carcinomas expressed ER α and PR, respectively [59] while invasive carcinomas expression figures range from 10% [65] to 25% [17] for ER α and from 38.5% and 43% for PR [19, 65]. Many of these studies point to proges-

terone, and not oestrogens, as the sex steroid hormone driving proliferation in mammary gland tumours as all benign tumours of the canine mammary gland and 2/3 of malignant tumours express PR [14, 16, 20, 22].

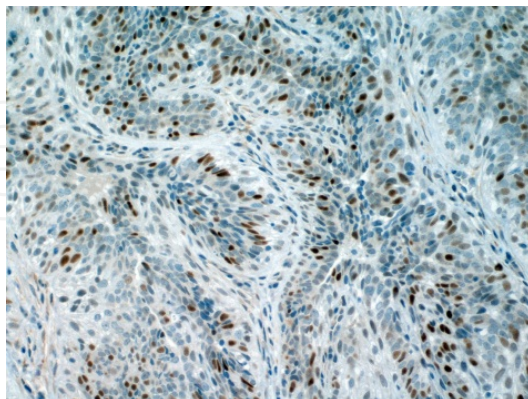


Figure 2. Canine simple carcinoma showing PR expression in the nucleus of neoplastic epithelial cells. (brown colour). ABC. 20X.

After the cloning of a second ER in rats and humans, designated ER β [66, 67], it became known that currently reported data correlated with the isoform α of ER (ER α). Our group was the first to publish data on the expression of the second isoform of the ER (ER β) in mammary tissues of dogs [68], and one more study has been performed up to date [69]. ER β expression was observed in the ductal and acinar epithelium of normal mammary glands and in one third of mammary tumours. Expression was greater in benign than in malignant tumours [68]. Among malignant tumours, ER β expression was greater in complex and mixed tumours than in simple carcinomas, indicating that ER β may also be a prognostic factor of these tumours (Figure 3).

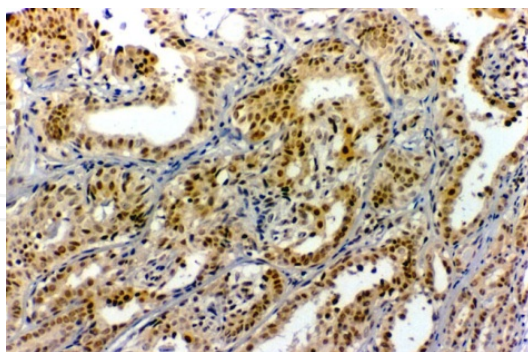


Figure 3. ER β immunoreaction in the nucleus of neoplastic epithelial cells of canine mammary carcinoma. ABC. 20X.

The prognostic value of steroid hormone receptor activity in malignant tumours (lower risk of relapse and metastasis, together with greater survival time) is now widely accepted after several univariate and multivariate prognostic studies using IHC methods for detection of ER and PR [18, 20-25]. In a study using IHC techniques to detect ER and PR expression in

228 tumours (155 malignant and 73 benign) from 100 bitches, Martín de las Mulas et al. [20] found that a total of 76% of tumours (96% benign and 66% malignant) expressed ER α and/or PR. In seven cases with lymph node metastasis, both the primary tumours and their metastases were ER α and PR negative, indicating a loss of hormone influence and thus greater aggressiveness. Expression of PR alone in 66% of malignant tumours suggested that PR played a more important role than ER α in tumour proliferation.

Surgery is the treatment of choice for feline and canine mammary tumours. Treatment with drugs such as tamoxifen has been tested in dogs with mammary carcinoma, but is not recommended due to their considerable side effects; these drugs are therefore not currently in use [70]. Ovariohysterectomy (OHE) performed at the time of surgery does not appear to produce any clear benefit in dogs with mammary carcinoma [71, 72]. The only adjuvant treatment administered at present is chemotherapy, and results are not wholly satisfactory [45].

In 1998, Cappelletti et al. [73] reported different ER α and PR counts in tumours analysed before and after treatment with a range of drugs including tamoxifen, concluding that malignant mammary neoplasms were sensitive to steroid hormone treatment. A feline dysplasia of the mammary gland, the feline fibroadenomatous change, is highly sensitive to Alizin®, a PR antagonist [74, 75]. The fact that all benign tumours and two thirds of malignant tumours in dogs express mainly PR [16, 17, 22] points to the potential use of progesterone receptor antagonists as a neoadjuvant and/or adjuvant treatment for these tumours. A recent study performed by our group [29] has shown that administration of the PR receptor antagonist Aglepristone (Alizin®, Virbac, France) as neoadjuvant treatment in female dogs inhibits the proliferation of PR-expressing mammary carcinomas. Twenty-seven non-spayed bitches with mammary tumours were treated with Alizin® before surgical tumour removal. Tumour tissue samples were analysed before and after treatment, and PR expression was reduced following treatment (36.4% versus 59.1% prior to treatment). The proliferative index (PI) was also analysed before and after treatment, using the avidin-biotin-peroxidase complex technique and a proliferative marker (Ki67). A significant decrease in the PI was recorded in tumours expressing PR, while no change was observed in those not expressing PR, suggesting that the PR antagonist Aglepristone inhibited tumour proliferation in PR-positive tumours by blocking the PR.

4. Female and male reproductive-tract tumours in dogs and cats

4.1. Female reproductive tract

The actual incidence of female reproductive-tract tumours is difficult to ascertain, presumably because a significant percentage of dogs and cats are neutered [76]. These tumours may arise in the vagina, vulva, uterus or ovaries; vulvar and vaginal tumours are the most common (after mammary gland tumours), accounting for 2.4% to 3% of all canine neoplasms [77, 78]. No incidence rates are available for vulvar and vaginal tumours in cats. Uterine tumours are rare in both dogs and cats, accounting for 0.3%-0.4% and 0.2%-1.5% of all canine

and feline tumours, respectively [77, 79-84]. Ovarian tumours are also uncommon in dogs and cats; although the true incidence is unknown, the reported incidence in intact bitches is 6.25%, thus representing 0.5% to 1.2% of all canine tumours [85, 86], while reported incidence in cats ranges from 0.7% to 3.6% [87]. The most common neoplasms in the canine female reproductive tract are benign tumours of mesenchymal origin, classified as leiomyomas, fibroleiomyomas, fibromas and polyps depending on the amount of connective tissue present [88,89]. The use of markers for smooth-muscle differentiation (e.g. desmin, calponin, smooth muscle actin) is valuable for the accurate identification of smooth muscle involvement in tumour growth [31, 90, 91] (Figure 4).

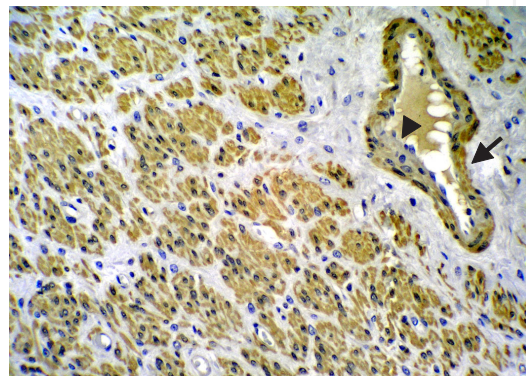


Figure 4. Immunohistochemical detection of the smooth muscle protein calponin in a leiomyoma. Most of the tumour cells show immunoreactive products to calponin antibody in their cytoplasms (brown colour). The vascular smooth muscle cells are also reactive (arrow) while endothelial cells are unreactive (arrowhead). ABC. 20X.

Leiomyoma is among the most common tumours of the female reproductive tract in many domestic species. It is located primarily in the vulva and vagina, followed by the uterus and its incidence is greater in older non-neutered bitches [77, 78]. In two different studies, all dogs diagnosed with leiomyoma were non-neutered bitches [78, 93] and the recurrence rate was 15% in bitches left unneutered after local excision. On the contrary, there was no recurrence in any animal when ovariohysterectomy was performed at the same time as excision. In another study [77], no leiomyomas were diagnosed in ovariectomised bitches under two years old. These epidemiological and clinical findings support the hormone-dependent nature of leiomyomas [88]. In addition, the role of ovarian steroid hormone receptors in the progression of female reproductive tract leiomyomas in the dog has also been demonstrated. Thus, Millán et al. [31] performed the first study on canine leiomyomas from the reproductive tract (uterus, vagina and vulva) demonstrating the IHC expression of ER α and PR in tumour tissue samples. Half of the leiomyomas (50%) expressed ER α and more than three quarters (82.1%) expressed PR (Figure 5).

Finally, a pioneer study of our group reported that the expression of PR in a canine vaginal fibroma was a predictive factor of favourable response to hormone therapy. Aglepritone at a dose of 10 mg/kg injected at days 1, 2, 8, 15, 28 and 35 prompted a progressive reduction in the size of the mass, which measured 9.1 x 5.4 cm on day 1 and 6.4 x 4.7 cm on day 45 [28] (Figure 6). The authors evaluated the proliferation index in the same study using a prolifera-

tion marker (Ki67), recording similar low values at days 15 and 25 of treatment, suggesting that Aglepristone did not reduce tumour size by reducing the tumour cell proliferation rate but rather through increasing apoptosis. It was therefore concluded that the size of PR-expressing benign tumours of the canine vagina could be reduced by palliative or neoadjuvant therapy with the PR antagonist Aglepristone. These findings regarding steroid hormone receptor expression in canine tumours of the female reproductive tract highlight the potential of hormone therapy in selected cases. In cats, leiomyomas located in the mammary gland and in the perineal region are reported to express ER α and PR [27]; however, no research has yet focussed on the expression of steroid hormone receptors in feline leiomyomas located in the vagina, vulva or uterus.

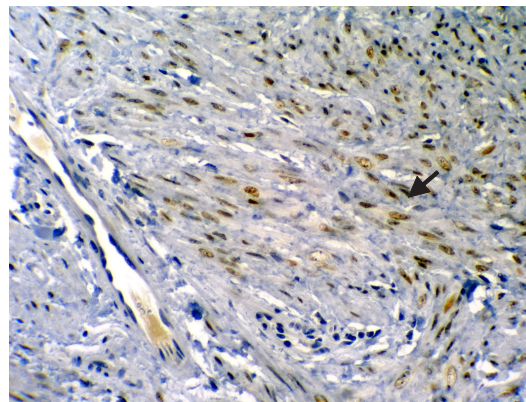


Figure 5. Immunohistochemical detection of ER α in a leiomyoma. Most of the tumour cells show immunoreactive products to ER antibody in their nucleus (brown colour)(arrow). ABC. 40X.

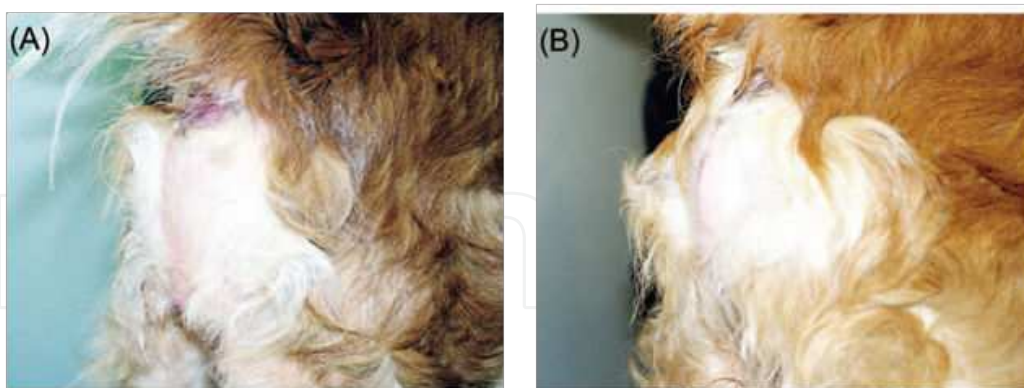


Figure 6. A) Female dog with a perineal mass measuring 9.1 x 5.4 cm. (B) The appearance of the tumour mass 28 days after treatment with aglepristone. The size is reduced to 6.4 x 4.7 cm [28].

4.2. Male reproductive tract

Male reproductive-tract tumours in dogs and cats may arise in the testes, prostate, penis or foreskin. Testicular tumours are the second most common cancer in intact dogs, accounting

for roughly 90% of all reproductive-tract tumours [96]. In cats, however, testicular tumours are rare [97]. Prostate tumours are uncommon in dogs, with a reported incidence of between 0.2% to 0.6% [98, 99], and equally rare in cats [97, 100-102]. Many penis and foreskin tumours affect the epithelial surface of these structures, the most common being the transmissible venereal tumour of the penis in dogs [103].

Currently, the only male reproductive-tract tumour in domestic species for which published data is available regarding the possible influence of steroid hormones on tumour development is canine prostate carcinoma. The dog is the only non-primate species that develops spontaneous prostate cancer [104]. Most tumours in this location are of epithelial origin [98, 105] and mainly affect older dogs (average age 10 years), prostate carcinoma being common [98]. The prostate is an androgen-dependent organ [106], and androgens achieve their effect through activation of androgen receptors (AR). However, there is a good deal of controversy concerning the influence of androgens on the development and biological behaviour of malignant prostate tumours. While some studies have found no evidence that castration has a protective effect against prostate carcinoma [99], others argue that castration before sexual maturity reduces the risk of this malignancy [105]; still others suggest that castration may actually increase the incidence and aggressive behaviour of canine prostate carcinomas [98]. The expression of steroid hormone receptors in tumour tissue samples of canine prostate carcinoma has been rarely studied. The more complete study up to date showed that expression of AR, ER α and ER β was lower in malignant tumour epithelial cells than in normal prostate tissue and benign lesions, suggesting that oestrogen actions in the prostate are complex and may play a dual role in the aetiology of prostate cancer [32, 107]. This study also demonstrated for the first time that PR expression in canine prostate tumours is greater than in normal prostate tissue [32], although the effect of progesterone at this location still remains to be demonstrated, as does the potential for hormone therapy.

5. Cutaneous tumours in dogs and cats

Tumours of the skin and subcutaneous tissue are the most common neoplasms affecting dogs and the second most common in cats [108]. Incidence rates have been estimated at around 450 per 100,000 dogs and 120 per 100,000 cats [108]. In dogs, most skin tumours are benign, the most frequent being histiocytomas and sebaceous gland adenomas. In cats, however, approximately 50% to 65% of skin tumours are histologically malignant, the most frequently-reported being squamous cell carcinomas [109].

Epidemiological research has identified breed and age as major risk factors for these tumours. A number of authors note a linear increase in risk by a factor of 1.1 per year of increasing age; additionally, pure-bred dogs appear to be twice as likely to develop a malignancy as cross-breeds. When all types of tumours are considered together, no significant sex predilection is apparent [110]. A number of etiological factors – physical, viral, genetic and molecular – have been reported for some skin tumours [111]. Over the last few years, it has become increasingly evident that steroid sex hormones may play an

important role in the pathogenesis of these tumours, as they do in mammary tumours. It is well known that oestrogens, progesterone and androgens not only help regulate skin development and function, such as the development and/or physiology of sebaceous glands and hair follicles, but are also involved in the development and biological behaviour of certain skin neoplasms [112].

To date, a number of studies have demonstrated a possible relationship between sex steroid hormones and sebaceous, perianal and hepatoid gland tumours and mast-cell tumours in domestic animals. To better understand this relationship, recent research has focussed on pinpointing the site of action of these hormones and on locating their receptors in both normal and tumour tissues. As a result, biochemical and immunohistochemical studies carried out over the last few years have detected androgen receptors (AR), oestrogen receptor α (ER α) and progesterone receptors (PR) in epidermis, hair follicles and fundamentally in the sebaceous glands of canine skin [113-117]. These results suggest that not only androgens and oestrogens, but also progesterone, may play a major role in the regulation of normal skin appendage function and in the pathogenesis and development of neoplasms.

Sebaceous gland tumours are among the most common skin tumours in the dog. They can be divided into four groups based on histological appearance: sebaceous hyperplasia, sebaceous epitheliomas, sebaceous adenomas and sebaceous adenocarcinomas. They account for between 6.8% to 7.9% of all skin tumours in dogs, and between 2.3% to 4.4% in cats [118]. Canine benign and malignant sebaceous gland neoplasias may provide a suitable experimental model for the study of hormone influences on the development of glandular tumours [114]. As in human skin, specific staining for ER α and PR is seen mainly in the basal cells of normal sebaceous glands [115-117]. However, unlike in human medicine, no data are available regarding the possible involvement of these steroid sex hormones in the pathology of canine and feline skin and the development of cutaneous neoplasms. A single study by the present authors analysed ER α and PR expression in canine sebaceous gland hyperplasias, adenomas/epitheliomas and carcinomas [117], revealing that canine sebaceous glands express both ER α and PR (Figure 7 y 8). Moreover, differences were recorded between types of lesion in the number of cells expressing ER α and PR. Compared with normal sebaceous glands, ER α expression was significantly lower in sebaceous gland epitheliomas and adenocarcinomas, suggesting that ER α plays a key physiological role in the maintenance of normal sebaceous glands, and that a decrease in levels influences the development of both benign and malignant neoplasms. A number of studies indicate that this drop in ER α could be secondary to changes in androgen or oestrogen production, but further research is required to confirm this hypothesis in canine sebaceous glands. The cited study also found that PR expression in adenocarcinomas was significantly lower than in normal and hyperplastic sebaceous glands, suggesting that tumour growth may become less hormone-dependent during the progression phase of carcinogenesis, as reported in certain human mammary tumours. Unlike ER α , the proportion of PR-positive cells did not differ significantly from that found in normal sebaceous glands, a finding also reported in humans [112]. This may indicate that progesterone does not necessarily influence the growth of this type of tumours. Conversely, sebaceous gland carcinomas display a significant loss in PR staining

intensity; PR loss may be one factor involved in the pathogenesis of canine sebaceous gland neoplasms.

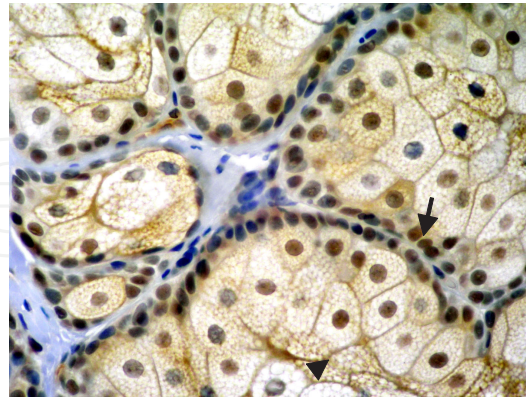


Figure 7. Immunohistochemical localization of PR in sebaceous gland hyperplasia. Nuclear staining is observed in basal cells (arrow) and differentiated sebocytes (arrowhead) [117]. ABC. 40X.

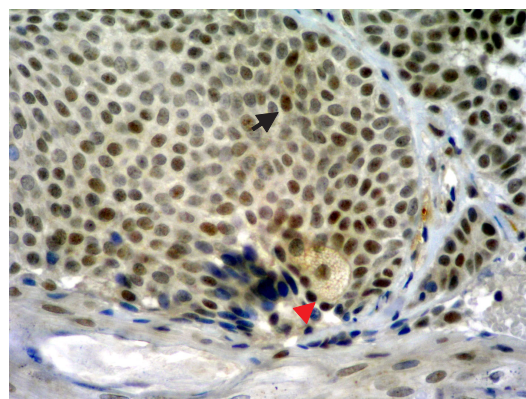


Figure 8. Immunohistochemical localization of PR in a sebaceous gland epithelioma. Nuclear staining is observed in basal cells (arrow) and differentiated sebocytes (arrowhead) [117]. ABC. 40X.

The influence of female sex steroids on their receptors in skin has been highlighted by several studies. When an ovariectomised bitch received a local estradiol (E2) implant, ER levels in the affected skin were found to be six times higher than in control skin [119]. However, despite the considerable amount of work done to date in this field, further research is still required to demonstrate the presence of AR and examine the effects of these hormones and their pharmacological antagonists on tumour development.

Other tumours considered clearly hormone-dependent include those arising in the perianal or hepatoid glands, modified sebaceous glands located in the perianal dermis. The most frequently observed tumours of this region in dogs are perianal adenoma, perianal adenocarcinoma and apocrine gland adenocarcinoma of the anal sac. Perianal adenomas account for between 58% and 96% of all canine perianal tumours [120]. Unlike adenomas, perianal gland carcinomas are rare, accounting for between 3% and 17% of all per-

ianal neoplasms [121]. Although found in both male and female dogs, the highest incidence is reported in intact males of mean age 10 or more [122]. This evidence has for years been interpreted as proof of a clear stimulation of tumour development by sex steroid hormones, particularly androgens [123]. Adenomas have been found to be hormone-responsive; a full or partial regression has been observed following castration or oestrogen treatment [113, 120, 124]. In females, perianal adenomas occur almost exclusively in ovariectomised animals whose low oestrogen levels fail to suppress tumour growth. Similarly, testicular interstitial cell tumours – which clinical observation suggests are associated with an increase in systemic androgen levels – occur more frequently in association with perianal tumours [125]. While hormone dependence has been clearly demonstrated in the case of perianal adenomas, there appears to be no link between perianal adenocarcinomas and steroid sex hormones. Perianal gland carcinomas do not regress following castration and are not responsive to hormone therapy with oestrogens [121, 124]. However, receptors for these hormones have been found in normal, hyperplastic and neoplastic perianal glands in dogs (Figure 9). An early report identified androgen-binding sites in perianal adenomas [124]. Research by Pisani *et al.* [126] detected AR expression in all normal and abnormal glands, although in hyperplastic tissues the proportion of positive nuclei was significantly greater than in normal tissue. A similar increase in the percentage of positive-staining nuclei was also observed in perianal epitheliomas, while in adenomas the increase with respect to normal tissue was only slight. In adenocarcinomas, the proportion of AR-positive cells was similar to that observed in benign tumours.

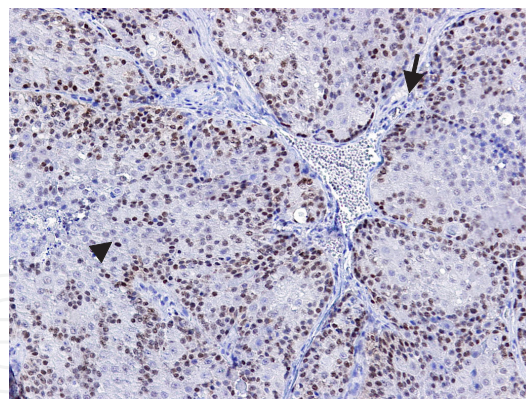


Figure 9. Immunohistochemical localization of ER α in a hepatoid gland adenoma. Nuclear staining is observed in basal cells (arrow) and differentiated hepatoid cells (arrowhead). ABC. 20X.

All these observations support the view that therapy based on antagonists of these hormones could prove beneficial in the treatment of these tumours. The present authors have carried out a preliminary clinical trial in order to evaluate the effect of Deslorelin (SuprelorinTM Virbac), a GnRH antagonist, on the clinical response of perianal gland adenomas [127]. This antagonist suppresses pituitary production of the hormones LH and FSH and of steroid sex hormones; its effect has been compared to that of surgical castration. The trial found that

subcutaneous deslorelin implants induced complete remission in at least 50% of dogs and a partial response to treatment in a further 29%. This antagonist could thus be considered as a new option for the treatment of perianal gland adenomas.

Finally, the association between mast-cell tumours (MCTs) and steroid sex hormones remains controversial. The role of these hormones in tumour pathogenesis is poorly understood, although some evidence is now available: one study has detected cytosolic receptors for oestrogen and progesterone in canine MCTs [127], while another found that 6 out of 9 MCTs contained no ER α and 3 were questionable [128].

6. Conclusion

Sex steroid hormones are involved in the development of animal tumours with high epidemiological and clinical impact. Research in the field has shown the potential benefits of endocrine manipulations to control tumour progression in a neoadjuvant or adjuvant setting. From a comparative point of view, steroid hormone dependent animal tumors represent an accessible and natural model of human disease.

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