in vivo *32*: 1175-1181 (2018)
doi:10.21873/invivo.11361

EGF Level in Hepatoid Gland Adenomas and Hepatoid Gland Epitheliomas in Dogs After Administering Tamoxifen

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Abstract. *Background/Aim: Neoplastic lesions of perianal glands account for approximately 10% of all skin cancer cases in dogs. They occur in many dog breeds, usually in male animals aged over 6 years. Due to their hormone-dependency, tamoxifen can be used in antineoplastic treatment. The aim of the study was to measure epidermal growth factor (EGF) levels in the serum of dogs with perianal tumours after tamoxifen treatment and to use it as a prognostic factor for further treatment. Materials and Methods: The study was performed on 19 male dogs aged between 6 and 14 years, diagnosed with neoplastic hyperplasia in the perianal region. The control group comprised 10 healthy dogs brought in for routine castration. The research material comprised blood drawn from the animals and tumour specimens for histopathology. The study group received 1-month treatment with tamoxifen. Blood serum was then tested for 17-β oestradiol level, and for EGF level on the first day of the therapy and 6 months after treatment completion. Results: Hepatoid gland adenomas were diagnosed in 10 cases, and hepatoid gland epitheliomas in nine cases. Elevated 17-β oestradiol levels were observed in all dogs. On the first day of treatment with tamoxifen, the serum EGF levels in all study groups were higher than in the control group. At the 6-month follow-up, the EGF levels were significantly reduced in hepatoid gland adenoma cases compared to those taken on the first day of treatment of tamoxifen, while in animals with hepatoid gland epithelioma, it was greatly increased and was correlated with relapse. Conclusion: Perianal gland tumours are characterised by EGF overexpression, which can be helpful in early-stage prognosis and treatment. An increase in EGF levels 6 months after tamoxifen therapy correlates with disease progression and may be a useful prognostic factor.*

This article is freely accessible online.

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Key Words: EGF, epidermal growth factor, perianal tumours, dog, tamoxifen.

Perianal gland tumours can occur in many dog breeds and are usually diagnosed in non-neutered male animals aged over 6 years. Perianal tumours in dogs may originate from three distinct structures. These include the anal glands: modified apocrine, alveolar-tubular sweat glands, and anal sac glands: a skin diverticulum located on both sides of the anus between the internal and external rectal sphincter muscle, originating from tubular apocrine glands. The third group comprises hepatoid glands, which are modified sebaceous glands of skin located in the perianal area as well as the prepuce, base of the tail, groin, inner thigh and the dorsal back (1). Histologically, they comprise groups of hepatocyte-like cells which in females regress to single islets, whereas in males they form glandular masses (2). Hypertrophic lesions of hepatoid glands account for approximately 10% of all skin cancer cases diagnosed in dogs. Three main types of neoplastic lesions that can be encountered in the perianal area include adenomas, adenocarcinomas and epitheliomas.

On the surface of both healthy and neoplastic hepatoid cells, the presence of androgen receptors (ARs) and oestrogen receptors (ERs) has been observed (3). They are influenced by gonadal steroids, which means that sometimes it is difficult to distinguish between glandular hypertrophy and a developing tumour. Benign tumours such as hepatoid gland adenomas (HGA) typically grow slowly and reach only limited sizes, whereas adenocarcinomas are characterised by rapid growth and can metastasise to local lymph nodes, abdominal organs or lungs (1, 4-6).

Hepatoid gland epithelioma (HGE) is characterised by low-grade malignancy and is clinically similar to adenomas but shows local invasiveness, *i.e.* infiltrates its surrounding tissues (7, 8). Due to the specific location, the tumours are often susceptible to ulceration, which can lead to infections in the surrounding tissues which hinder defecation (5).

The Ki-67 nuclear antigen can be used to obtain objective data on the character of a tumour and the process of carcinogenesis. Its assay provides important information about the tumour's mitotic activity.

The process of perianal tumour oncogenesis is affected not only by hormones but also by growth factors responsible for tumour angiogenesis. Studies conducted in both humans and animals revealed that the main factor stimulating tumour vascularisation is vascular endothelial growth factor (VEGF) (9). However, another important factor influencing tumour growth is epidermal growth factor (EGF), which acts as a potent stimulator for epidermal and epithelial cell growth, both *in vivo* and *in vitro* (10). It was first described in 1962 by Cohen (11) while the detailed biochemical structure of EGF was determined 10 years later by Savage *et al.* (12). The factor was isolated from salivary glands of male mice. EGF consists of a single peptide chain comprising 53 amino acids, six of which constitute cysteine residues determining the biological activity of EGF. It is synthesised in all tissues of the organism and secreted into the bloodstream. The highest EGF concentrations have been observed in the pancreas, thyroid and kidneys, as well as in systemic fluids such as urine, saliva, milk, and blood serum (13).

EGF plays a particular role in the mammary glands (14, 15), where it is responsible for the growth of both healthy and tumorous epithelium. It also stimulates the synthesis and secretion of hormones: luteotropin (LH), growth hormone (GH) and prolactin.

EGF has been demonstrated to stimulate epithelial tissue growth *via* receptors whose presence has been confirmed in healthy epithelial tissue, macrophages, platelets, and many mesenchymal cells (16). The family of epidermal growth factor receptors (EGFR) includes a group of transmembrane proteins with tyrosine kinase enzyme activity. These comprise: EGFR1 (HER1 or c-erbB1), a receptor for EGF and transforming growth factor alpha (TGFα); EGFR2 (HER2 or c-erbB2), whose ligand remains unknown; and EGFR3 (HER3 or c-erbB3) and EGFR4 (HER4 or c-erbB4), serving as receptors for neuregulins (6, 10, 17-19). EGFR was among the first proteins responsible for intracellular signal transmission to be identified and described. The ligands of the EGFR family are growth factors, which means that the activated signal paths are responsible for the correct process related to proliferation, differentiation, maturation, and survival of epidermal cells, growth and implantation of the embryo, and repair of damaged organs (10, 16). It has been shown that EGF and its receptors, in cooperation with TGF, have the ability to induce VEGF synthesis in the cells of malignant tumours and affect the process of neoangiogenesis (20). In human medicine, elevated levels of EGF and its receptors are observed in a number of malignant tumours, including of the head and neck, lung, colorectum, breast, bladder, pancreas, prostate, ovaries, and stomach, where it is believed to be a negative prognostic factor (16, 21).

The therapy of perianal gland tumours depends on the particular type of the tumour, extent of the lesion and its invasiveness. It typically involves surgical removal of the tumour together with a margin of healthy tissue, coupled with castration, pharmacological treatment (cytostatics), cryotherapy or radiotherapy (22).

Due to the hormone dependency of these particular tumours, one viable treatment method involves antihormonal therapy using selective oestrogen receptor modulators such as tamoxifen (23). Tamoxifen belongs to a group of synthetic non-steroidal agents widely used in human medicine for the treatment and prevention of oestrogen receptor-positive breast cancer. Tamoxifen competitively blocks with oestrogen receptors, effectively preventing their ability to bond with 17-β oestradiol, which limits the biological impact of oestrogens on tumour cells (24). On the other hand, tamoxifen has agonistic properties and is capable of inducing some oestrogenic responses. The manifestation of these two different actions is not completely understood and depends on each species, organ, tissue, and cell type (24, 25).

The goal of this study was to determine EGF levels in the serum of dogs diagnosed with perianal tumours after pharmacological treatment with tamoxifen and to use it as a prognostic factor in further treatment.

Materials and Methods

The study was performed on 19 male dogs aged between 6 and 14 years. The animals were diagnosed with perianal tumours at the Department and Clinic of Animal Surgery of the University of Life Sciences in Lublin. The routine protocol before every surgical procedure in our Department involves blood test including, blood smear and biochemical serum analysis of alanine aminotransferase, aspartate aminotransferase, kidney profile (urea, creatinine and total protein level), as well as electrocardiogram (ECG).

The control group comprised 10 healthy dogs aged between 2 and 7 years which were brought in for routine castration. Physical examination of dogs from the control group showed no signs of any disease and their blood tests and ECG remained within the reference range.

In all the studied animals, elevated (above 7 pg/ml) levels of 17-β oestradiol were observed, which was an indication for hormonal therapy with selective oestrogen receptor modulators (tamoxifen) dosed at 2 mg/kg body weight administered orally. The therapy lasted 1 month.

The research material consisted of blood drawn from the animals and tumour specimens collected during trepanobiopsy which was performed twice: on the day of admission and after 6 months from the completion of tamoxifen treatment in cases of visible tumour

Table I. *Results of biochemical and immunohistochemical tests in the group of dogs with hepatoid gland adenoma.*

Patient description, age (years)	Ki-67 index (%)	17-β Oestradiol (pg/ml)	EGF (pg/ml)	
			At diagnosis	6 Months after therapy
Dachshund, ♂, 7	>50	35.00	15.93	3.81
German Shepherd, ♂, 10	>50	31.00	20.82	4.64
Dachshund. ♂. 8	<50	12.29	12.93	5.52
Mixed breed, ♂, 13	>50	21.00	27.28	3.33
Mixed breed, ♂, 8	>50	12.4	13.64	6.47
Mixed breed, ♂, 10	<50	9.20	9.14	3.15
Mixed breed, ♂, 9	>50	8.24	16.77	8.46
Cocer spaniel, ♂, 9	<50	9.46	8.62	0.01
Schnauzer, ♂, 8	>50	7.68	9.73	5.52
Mixed breed, ♂, 7	>50	12.40	3.83	0.62

EGF: Epidermal growth factor; Ki-67 index (%): percentage of immunopositive cells per 500 neoplastic cells. No recurrence was observed in the above group of dogs.

progression. Blood was drawn from the cephalic vein into vials with Cakutest clotting activator (Vacutest Kima, Arzegrande, Włochy, Poland). Blood was collected twice: on the day of diagnosis and 6 months after treatment completion. The serum obtain from centrifuged blood was stored at –75˚C until the assay. The EGF levels were determined in the serum of animals from both groups. The measurements were performed using an immunoenzymatic ELISA essay (Canine Total Epidermal Growth Factor ELISA Kit; MyBioSource, Inc., San Diego, CA, USA), in accordance with the test protocol provided by the manufacturer.

Tumour specimens were collected using a 6 mm trepan. Histopathological analysis was performed at the Department of Pathological Anatomy at the University of Life Sciences in Lublin. The material was solidified in 10% buffered formalin (pH 7.2) for 24 (48 h) and then in a tissue processor (Leica TP-20), processed using alcohol solutions of increasing concentration, acetone and xylene into paraffin blocks. Tissue specimens of 4 μm in thickness were stained with haematoxylin and eosin (HE) for analysis under a Nikon Eclipse E-600 light microscope (Nikon Instruments Europe BV, Amsterdam, the Netherlands). Diagnoses were based on the World Health Organization (WHO) Histological Classification of Tumours (7). Specimens intended for immunohistochemical analyses were placed on Super Frost slides coated with silane (Menzel-Glaser, Braunschweig Germany, DAKO) and stored for 12 h in a thermostat in 56˚C. Next, the specimens were deparaffinised with xylene and processed using alcohol solutions of decreasing concentration to distilled water.

Dako-ARK™ set (Animal Research Kit; Dako, Glostrup, Denmark) was utilised for immunohistochemical staining of tissues samples in order to assess Ki-67 antigen expression. Mouse monoclonal antibody (clone MIB-1) against Ki-67 protein was used (Dako) which was intended for processing of paraffin-embedded tissues at 1:300 dilutions. The preparations were incubated with 3,3'-diaminobenzidine tetrahydrochloride solution used as a chromogen to obtain reaction in colour. PC unit equipped with image analysis software (NIS-Elements BR-2.20; Laboratory Imaging) and computer microscopic image analysis system were used for the assessment of Ki-67 antigen expression. Ki-67 expression was determined at a lens magnification of ×40. The index value was counted on the basis of the percentage of immunopositive cells per 500 neoplastic cells. A result of Ki-67+ represents more than 50% immunopositive cells in the Ki67 immunohistochemical test.

All procedures conducted in the study group (blood drawing and tissue sampling, collection of tumour sections) and in the control group (blood drawing) were approved by the Local Ethics Committee II in Lublin (permit no. 26/2015, 28.04.2015 to conduct experiments on animals; experiments on tissues and animal organs). Furthermore, the owners of the animals were informed about the goal of the study and gave their consent to the experimental procedures.

A statistical analysis was performed to determine whether significant correlations existed between serum EGF levels in the experimental and control groups, as well as whether statistically significant differences were present in terms of EGF levels 6 months after the administration of pharmacological treatment. Statistical analysis was evaluated using STATISTICA 10 (StatSoft Polska Sp. z o.o., Cracow, Poland). To verify the normality of the distribution of the obtained results, the Shapiro–Wilk test was used. In comparison of results of two groups, in the absence of a normal distribution whose test function was designated as 'Z', nonparametric the Mann–Whitney *U*-test was used. The correlation between Ki-67 and EGF level, and between EGF level and tumour recurrences was calculated using Spearman test.

Results

Of the selected cases of pathological perianal hyperplasia, HGA was diagnosed in 10 (Table I) and HGE in nine on the basis of trepanobiopsy (Table II).

In all dogs with HGA and in five animals diagnosed with HGE, the tumours regressed for a period of at least 6 months from the end of treatment. Continued progression of the neoplastic disease in the form of individual nodules was observed in the remaining four animals with HGE (Table II).

Expression of the Ki-67 proliferative antigen was observed in most of the studied tumours but statistical

Table II. *Results of biochemical and immunohistochemical tests in the group of dogs with hepatoid gland epithelioma*

Patient description, age (years)	Ki-67 index (%)	17-β Oestradiol (pg/ml)	EGF (pg/ml)		Recurrence
			At diagnosis	6 Months after therapy	
Mixed breed, ♂, 10	>50	36.20	40.83	82.09	+
Mixed breed, ♂, 14	>50	16.80	114.64	38.01	–
German Shepherd, ♂, 12	>50	48.02	64.59	17.92	–
Dachshund, ♂, 7	>50	50.00	184.15	71.22	–
German Shepherd, ♂, 6	<50	23.69	38.46	18.24	–
Terrier, ♂, 10	>50	24.00	82.09	112.82	+
Cocer spaniel, ♂, 9	<50	16.32	32.85	7.92	–
Mixed breed, ♂, 13	>50	23.79	40.68	86.32	+
Mixed breed, ♂, 11	>50	18.49	22.95	42.74	+

EGF: Epidermal growth factor; Ki-67 index (%): percentage of immunopositive cells per 500 neoplastic cells.

Table III. *Comparison of epidermal growth factor concentrations (pg/ml) in hepatoid gland adenomas (HGA) and hepatoid gland epitheliomas (HGE) with control groups at the beginning of treatment.*

Group	N	Median	Min	Max	*p*-Value*	Mean	SD
Control	10	3.82	2.15	5.85		3.88	1.17
HGA	10	13.28	3.83	27.28	<0.01	13.86	6.75
HGE	9	40.83	22.95	184.15	<0.01	69.03	51.83

**Versus* control.

Table IV. *A breakdown of epidermal growth factor levels in hepatoid gland adenomas for consecutive samplings.*

Group	N	Median	Min	Max	*p*-Value	Mean	SD
At diagnosis	10	13.28	3.83	27.28	<0.001	13.87	6.75
6 Months after therapy	10	4.64	0.01	8.46		4.19	2.72

analysis did not confirm its correlation with EGF level (p=0.593 for HGA and p=0.153 for HGE).

The EGF level in the control group ranged between 2.15 and 5.85 pg/ml (median=3.82 pg/ml). The median EGF concentration in the serum of dogs with HGA on the first day of treatment was 13.28 pg/ml. In HGE, EGF levels were higher and ranged between 22.95 and 184.15 pg/ml (median=40.83 pg/ml). For both tumour types, statistically significantly higher EGF levels were observed on the first day of treatment when compared to the control group (Table III). Upon analysing EGF levels after 1 month of tamoxifen administration, values for HGA after pharmacological treatment decreased considerably (p<0.001; Table IV).

The statistical analysis of HGA revealed a statistically significant and higher level of EGF 6 months after therapy, which was correlated with relapse (R_s=0.732, p=0.039).

Discussion

Data available in literature indicate the presence of ERs and ARs in perianal tumour tissue, which suggests hormone dependency of these tumours, whereby hormonal fluctuations can influence tumour growth (26). Hormones bind with specific receptors present in the hepatoid gland tissue and stimulate cell division, thus facilitating carcinogenesis. This observation has been confirmed in a number of studies where the percentage of ERs or ARs was significantly higher in proliferative cells compared to healthy gland tissue (3, 27, 28).

It has been demonstrated that administration of oestrogen-based formulations or performing castration to dogs diagnosed with perianal hepatoid adenoma may result in partial or even complete remission of the tumour (22). Our

study is consistent with this, where at 6 months follow-up after treatment with tamoxifen, no relapse was observed in any dogs suffering from HGA.

To date, many various treatments of perianal gland tumour have been tested, starting from relatively non-invasive hormonal therapies and ending with surgery and radiotherapy. The choice of the correct method depends on the type, size and malignancy of the tumour, as well as the presence of metastasis. Tamoxifen used in the treatment of perianal gland tumours, apart from serving as a selective modulator for ERs, is also an angiogenesis inhibitor and causes apoptosis. It acts as competence inhibitor by occupying the place which binds oestrogen to the ER, thus inhibiting the influence of oestradiol on the growth and development of cancer cells. Tamoxifen is used in targeted treatment of hormone-dependent breast cancer in women (16, 18). Research conducted by Cuzick *et al*. revealed that it reduces the incidence of breast cancer by 26-38%, although its effectiveness has only been confirmed with regard to oestrogen-dependent cancer (ER+). However, although tamoxifen therapy has proven effective, certain side-effects have been reported: osteoporosis, thrombus, embolism, increased risk of endometrial cancer, vision disorders, and elevated liver enzyme levels (29). It was observed that prolonged use of tamoxifen can result in cancer cell immunity to the drug and even proliferation of tumour cells (30).

In one study, 11/20 female dogs treated with 1 mg/kg of tamoxifen developed complications such as pyometra, oedema of the external genitalia, vaginal discharge and pseudogestational behaviour (31). Due to its agonistic stimulation of uterine ER, endometrial cell proliferation is likely to occur in bitches. Thus, the authors suggested using tamoxifen at a lower dose of 0.5 mg/kg (31). In male dogs, tamoxifen negatively influences testis size and libido as well as reducing blood testosterone concentrations (24). However, in our own study, no adverse effects were observed throughout entire observation period in the dogs exposed to 1 mg/kg tamoxifen. Thus, our research showed that hormonal tamoxifen therapy is safe and well tolerated by dogs. It is an effective method applicable in the treatment of HGA, while in the case of tumours characterised by local invasiveness, *i.e*. HGE, it was shown to be insufficient, in most cases leading only to temporary suppression of the disease process.

In such cases, repeat pharmacological therapy or combining antihormonal treatment with surgery is required. Similar conclusions were reached by Tozon *et al*. in a study on treatment effects in cases of perianal gland tumours (28). Their research demonstrated that the most effective therapeutic method, under which 2-year remission was observed in 70% of the cases, is radical tumour resection together with adequately large margin of healthy tissue. It was also demonstrated that good therapeutic effects, particularly in adenoma and epithelioma cases, were obtained by employing the multiple electrochemotherapy strategy (22). In our own research, in four of the dogs with epitheliomas, despite treatment with selective oestrogen receptor modulators, relapse occurred. The obtained results indicate a poor sensitivity of HGE and their receptors to tamoxifen.

The Ki-67 nuclear antigen is a good marker of neoplastic process, useful when monitoring the effectiveness of chemotherapy. High expression of the antigen, which correlated with the presence of metastasis, was observed in many types of canine tumour (32,33). Furthermore, in a study on canine perianal gland neoplasms, Periera *et al*. demonstrated the usefulness of Ki-67 measurements in the assessment of the risk of relapse (33). High Ki-67 expression was also observed in cases of canine mammary gland tumours. It correlated with higher invasiveness or tumour relapse and shorter animal survival, and was indicative of a poor prognosis (32, 34). In our own research on dogs with HGE, the presence of relatively high Ki-67 antigen level was confirmed in 14 of the cases but the statistical analysis revealed that it did not correlate with relapse incidence.

The autocrine hypothesis with regard to neoplasm development was first proposed by Sporn and Todaro in 1980 (35). It states that tumour cells are capable of synthesising growth factors which stimulate the receptors on the cell surface, thus causing uncontrolled neoplastic growth. A number of factors participating in oncogenesis have been identified, but it would seem that VEGF and EGF play the most significant roles in this context. VEGF is responsible for neoplastic angiogenesis. Although under physiological conditions EGF influences growth, embryo implantation and repair of damaged mature organs, its overexpression can lead to uncontrolled cell division (16). It is the key factor stimulating growth of epidermal and epithelial cells and is responsible for maintaining their integrity. Overexpression of EGF intensifies the processes of proliferation, and apoptosis inhibition, increasing cell survival and angiogenesis, which influence the incidence of distant metastasis and progression of neoplastic disease (16). Sabattini *et al*. demonstrated that EGFR overexpression correlates with a negative prognosis in cases of feline skin squamous cell carcinoma. Furthermore, they hypothesised that the use of EGFR inhibitors in such animals, alongside surgery, can significantly improve the chances for successful treatment (36). It was demonstrated that EGF inhibits apoptosis of cells of solid tumours. Overexpression of EGF was detected in these cells (37). It is also a neoplastic marker in canine transitional cell carcinoma – TCC (38). In a study on the EGF level in the serum of patients suffering from unresectable hepatocellular carcinoma, a significantly elevated EGF level (784.49 pg/ml) was observed when compared with healthy patients (297.15

pg/ml) or other patients suffering from non-cancerous liver diseases (338.64 pg/ml). It was concluded that EGF overexpression correlated with negative prognosis and short patient survival (39). Similar results were obtained in our own research. The serum EGF level on the day of the diagnosis was statistically higher in all study group animals when compared to the control group. A statistically significant increase in the EGF level was also observed after the treatment in four dogs with HGE. Our results are similar to other studies (36, 38, 39) and seem to confirm that EGF plays an important role in tumour development, and that its overexpression correlates with the incidence of tumour relapse in dogs. Thus, EGF can be treated as another prognostic factor.

Conflicts of Interest

The Authors declare that they have no conflict of interest in regard to this study.

References

1 Simeonova G and Simeonov R: Correlation between tumour diameter and presence of metastases to the regional lymph nodes in spontaneous canine hepatoid adenicarcimomas. Trakia J Sci *6*: 54-56, 2008.

2 Shabadash SA and Zelikina TI: Once more about hepatoid circumanal glands of dogs. History of their discovery and reasons for revision the structural and functional data. Izv Akad Nauk Ser Bio *2*: 176-185, 2002.

3 Pisani G, Millanta F, Lorenzi D, Vannozzi I and Poli A: Androgen receptor expression in normal, hyperplastic and neoplastic hepatoid glands in the dog. Res Vet Sci *8*: 231-236, 2006.

4 Bray J: Tumours of the perianal region. BSAVA Manual of Canine and Feline Oncology, 3r dedition Dobson J.M and B.D.X. Lascelles (eds.), 2011.

5 Javanbakht J, Tavassoli A, F Sasani F, Sabbagh A, Hassan MA, Samakkhah AS, Shafiee R, Jani M, Alimohammadi S, Samani R, Barati F and Ghalee VR: An overall assessment of circumanal gland adenoma in a terrier mix breed dog. Asian Pac J Trop Biomed *3*: 580-583, 2013.

6 Park K, Han S, Shin E, Kim HJ and Kim JY: *EGFR* gene and protein expression in breast cancers. Eur J Surg Oncol *33*: 956-960, 2007.

7 Goldschmidt MH, Dunstan RW, Stannard AA, von Tscharner C, Walder EJ and Yager JA: Histological classification of epithelial and melanocytic tumors of the skin of domestic animals. WHO International Histological Classification of Tumors of Domestic Animals, 2nd series, vol. III, Armed Forces Institute of Pathology, Washington D.C., 1998.

8 Jakab C, Rusvai M, Glif P and Kulka J: Expression of claudin-4 molecule in canine perianal gland tumours. ActaVet BRNO *79*: 127-133, 2010.

9 Sobczyńska-Rak A: The Role of VEGF in the process of neovasculogenesis. *In*: Ran S. (eds): Tumor Angiogenesis. Intech, Rijeka, Croatia. pp. 181-196, 2012.

10 Sadłecki P, Walentowicz-Sadłecka M and Grabiec M: Serum epidermal growth factor levels in epithelial ovarian tumours. Przeg. Menopauzalny *3*: 187-190, 2011.

11 Cohen S: Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. J Biol Chem *237*: 1555-62, 1962.

12 Savage C R Jr, Hash J H and Cohen S: Epidermal growth factor: location of disulfide bonds. J Biol Chem *248*: 7669-7672, 1973.

13 Sieja K, Stanosz S, Grobelny W, Stanosz M and Puchalski A: Concentrations of growth factors in serum women with primary ductal breast cancer. Wspol Oncol *13*: 201-205, 2009.

14 Bergkvist GT and Yool DA: Epidermal growth factor receptor as a therapeutic target in veterinary oncology. Vet Comp Oncol *9*: 81-94, 2011.

15 Gama A, Gärtner F, Alves A and Schmitt F: Immunohistochemical expression of epidermal growth factor receptor (EGFR) in canine mammary tissues. Res Vet Sci *87*: 432-437, 2009.

16 Wojtukiewicz MZ, Rybałtowski M and Sierko E: Biologic basis of therapy targeted to EGFR. Nowotwory J Oncol *3*: 260-271, 2008.

17 Hynes NE and Lane HA: ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer *5*: 341-354, 2005.

18 Sassen A, Rochon J, Wild PJ, Hartmann A, Hofstaedter F and Schwarz S, Brockhoff G: Cytogenetic analysis of HER1/EGFR, HER2, HER3, and HER4 in 278 breast cancer patients. Breast Cancer Res *10*: 1-3, 2008.

19 Shiomitsu K, Johnson CL, Malarkey DE, Pruitt AF and Thrall DE: Expression of epidermal growth factor receptor and vascular endothelial growth factor in malignant canine epithelial nasal tumours. Vet Comp Oncol *7*: 106-114, 2009.

20 Kennedy KC, Qurollo BA, Rose BJ and Thamm DH: Epidermal growth factor enhances the malignant phenotype in canine mammary carcinoma cell lines. Vet Comp Oncol *9*: 196-206, 2010.

21 Quaranta M, Divella R, Daniele A, Di Tardo S, Venneri MT, Lolli I and Troccoli G: Epidermal growth factor receptor serum levels and prognostic value in malignant gliomas. Tumori *93*: 275-280, 2007.

22 Tozon N, Kodre V, Juntes P, Sersa G and Cemazar M: Electrochemiotherapy is highly effective for the treatment of canine perianal hepatoid adenoma i epithelioma. Acta Veterinaria *60*: 285-302, 2010.

23 Brodzki A, Sobczyńska-Rak A, Brodzki P, Tatara MR and Silmanowicz P: Occurrence, etiology and antihormonal treatment of perianal gland tumors in male dogs. Med Weter *70*: 638-643, 2014.

24 Corrada Y, Arias D, Rodríguez R, Spaini E, Fava F and Gobello C: Effect of tamoxifen citrate on reproductive parameters of male dogs. Theriogenology *61*: 1327-1341, 2004.

25 Hoffmann B and Schuler G: Receptors blockers – general aspects with respect to their use in domestic animal reproduction. Anim Reprod Sci *61*: 295-312, 2000.

26 Queiroga FL, Pérez-Alenza D, Silvan G, Peña L and Illera JC: Positive correlation of steroid hormones and EGF in canine mammary cancer. J Steroid Biochem mol Biol *115*: 9-13, 2009.

27 Burdzińska A and Idziak M: Perianal gland tumors in dogs. Mag Wet *16*: 1130-1134, 2008.

28 Tozan N, Kodre V, Sersa G and Cemazar M: Effective Treatment of Perianal Tumors in Dogs with Electrochemotherapy. Anticancer Res *25*: 839-846, 2005.

29 Cuzick J: Aromatase inhibitors for breast cancer prevention. J Clin Oncol *23*: 1636-1643, 2005.
30 Frasor J, Chang EC, Komm B, Lin CY, Vega VB, Liu ET, Miller LD, Smeds J, Bergh J and Katzenellenbogen BS: Gene expression preferentially regulated by tamoxifen in breast cancer cells and correlations with clinical outcome. Cancer Res *66*: 7334-7340, 2006.
31 Tavares WLT, Lavalle GE, Figueiredo MS, Souza AG, Bertagnolli AC, Viana FAB, Paes PRO, Carneiro RA, Cavalcanti GAO, Melo MM and Cassali GD: Evaluation of adverse effects in tamoxifen exposed healthy female dogs. Acta Vet Scand *52*: 67-73, 2010.
32 Pena LL, Nieto AI, Perez-Alenza D, Cuesta P and Castano M: Immunohistochemical detection of Ki-67 and PCNA in canine mammary tumors: Relationship to clinical and pathologic variables. J Vet Diagn Invest *10*: 237-246, 1998.
33 Pereira RS, Schweigert A, Dias de Melo G, Fernandes FV, Sueiro FA and Machado GF: Ki-67 labeling in canine perianal glands neoplasms: a novel approach for immunohistological diagnostic and prognostic. BMC Vet Res *9*: 83-93, 2013.
34 Zuccari DA, Santana AE, Cury PM and Cordeiro JA: Immunocytochemical study of Ki-67 as a prognostic marker in canine mammary neoplasia. Vet Clin Pathol *3*: 23-28, 2004.
35 Sporn MB and Todaro GJ: Autocrine secretion and malignant transformation of cells. N Engl J Med *303*: 878-880, 1980.
36 Sabattini S, Marconato L, Zoff A, Morini M, Scarpa F, Capitani O and Bettini G: Epidermal growth factor receptor expression is predictive of poor prognosis in feline cutaneous squamous cell carcinoma. J Feline Med Surg *12*: 760-768, 2010.
37 Snider AI, Zhang Z, Xie Y and Meier KE: Epidermal growth factor increases lysophosphatidic acid production in human ovarian cancer cells: roles for phosphalipase D2 and receptor transactivation. Am J Physiol Cell Physiol *298*: 163-170, 2010.
38 Hanazono K, Fukumoto S, Kawamura Y, Endo Y, Kadosawa T, Hidetomo I and Uchide T: Epidermal growth factor receptor expression in canine transitional cell carcinoma. J Vet Med Sci *77*: 1-6, 2015.
39 Kohla MAS, Al-Haddad OK, Nada A, Al-Warraky M, Obada M, Amer M, Ezzat S and Gabal AA: Association of serum levels of epidermal growth factor with disease severity in patients with unresectable hepatocellular carcinoma. Hepatoma Res *2*: 18-25, 2016.

Received April 27, 2018
Revised May 28, 2018
Accepted May 30, 2018