This is an author produced version of a paper published in Reproduction in Domestic Animals

This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Citation for the published paper:

Published in final form at:
http://dx.doi.org/10.1111/rda.12642

Copyright: Blackwell Verlag GmbH

Access to the published version may require subscription.
Changes in c-erbB-2 Immunoexpression in Feline Endometrial Adenocarcinomas

AL Saraiva1,2, R Payan-Carreira1, F Gartner3,4, F Faria3, LM Lourenço1 and MA Pires2

1CECAV, Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal; 2Escola Universitária Vasco da Gama (EUVG), Coimbra, Portugal; 3Abel Salazar Institute of Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal; 4Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal

Contents
Human epidermal growth factor receptor type 2 (c-erbB-2), an oncoprotein with potential prognostic marker and therapeutic use, is overexpressed in several human and animal tumours. But information regarding this molecule in feline tumours is scarce. This study aimed to assess the changes in the immunohistochemical expression of c-erbB-2 in feline endometrial adenocarcinomas (FEA) compared to normal endometrium. An immunohistochemistry assay using a specific antibody against c-erbB-2 was performed in FEA samples (n = 34) and in normal endometrium in the follicular (FS; n = 12) and luteal (LS; n = 11) stages. In FEA, the c-erbB-2 immunoreactivity was assessed in neoplastic epithelial cells whilst in normal endometria it was individually evaluated in the surface and the superficial and deep glandular epithelia (SE, SGE and DGE, respectively). In FS and in LS, all the epithelia were positive for c-erbB-2; positivity was higher in the SE and the SGE than in DGE. Twenty of the 34 FEA samples (58.8%) were positive for c-erbB-2 immunolabelling. Nevertheless, its expression was higher in all the epithelia in the FS compared to FEA (p ≤ 0.0001) or the LS (p = 0.016). The results presented herein suggest that c-erbB-2 molecule is differently expressed in the feline endometrium through the oestrous cycle and though it may also be involved in feline endometrial carcinogenesis, a question remains unanswered on the importance of additional pathways of epithelial proliferation in the neoplastic changes in feline endometrium.

Introduction
Human epidermal growth factor receptor type 2 (c-erbB-2) is overexpressed in several human cancers, including endometrial adenocarcinomas (Yarden and Sliwkowski 2001), and its amplification is often associated with tumour invasiveness, particularly in breast cancer (Brix et al. 2014). In women, the reported rates of c-erbB-2 overexpression in endometrial carcinomas vary from 1% to 80% depending on its histological type (reviewed by Buza et al. 2014). This very wide range of results is pointed as related to small sample sizes, different clinicopathological features considered and/or differences in the staining and the scoring systems used in immunohistochemistry evaluation of the tumours. For these reasons, it is possible that in human endometrial carcinomas c-erbB-2 overexpression and tumour prognosis and outcome may not always be correlated (Buza et al. 2014). On the other hand, an increased expression of c-erbB-2 is particularly well-studied in women mammary carcinomas (Yarden and Sliwkowski 2001). In c-erbB-2-overexpressing human breast carcinomas, targeted therapy against c-erbB-2 is proven to be effective. Therefore, standardization of c-erbB-2 assessment is well-established in these tumours (Wolff et al. 2013), unlike in endometrial cancer (Buza et al. 2014).

Scoring guidelines for c-erbB-2 evaluation by immunohistochemistry have been recently established for the canine mammary tumours (Peña et al. 2014), which are similar to those existing in humans. The evaluation of c-erbB-2 in feline mammary tumours has also been studied, but no similar guidelines exist (Millanta et al. 2005; Winston et al. 2005; Ordás et al. 2007; Rasotto et al. 2011). Several studies suggest c-erbB-2 overexpression in feline mammary carcinomas as a marker of malignancy and a prognostic indicator (Millanta et al. 2005; Winston et al. 2005; Ordás et al. 2007), but this is controversial, Rasotto et al. (2011) defending that c-erbB-2 may not play a major role in mammary carcinogenesis and prognosis. Technical factors and subjective interpretation of c-erbB-2 immunolabelling may explain the disagreements in the incidence of c-erbB-2 expression between studies (Rasotto et al. 2011).

Increasing reports in feline endometrial adenocarcinomas (FEA) (Gil da Costa et al. 2009; Anderson and Pratschke 2011; Cho et al. 2011; Payan-Carreira et al. 2013; Sontas et al. 2013; Saraiva et al. 2015a) suggest that the disease may be more common than previously described. The definitive diagnosis is usually accomplished by histopathological exam. Histopathological features of FEA, although not constant, include recognized criteria of malignancy such as nuclear atypia, bizarre mitoses, necrosis, and vascular, myometrium and/or serosa invasion (Papparella and Roperto 1984; Miller et al. 2003; Anderson and Pratschke 2011; Saraiva et al. 2012). Despite the growing number of studies in FEA, little is known about the molecular events related to the carcinogenesis of the endometrium in queens. Moreover, immunohistochemical characterization of FEA is limited and performed in small case series (Espinosa de los Monteros et al. 1999; Miller et al. 2003; Gil da Costa et al. 2009). Nevertheless, the pathogenesis of FEA remains elusive.

Considering that c-erbB-2 could play a role in feline endometrial carcinogenesis, the present study aimed to characterize the c-erbB-2 immunohistochemical pattern of expression in FEA compared to normal endometrium, and to explore putative roles of c-erbB-2 in feline cyclic and neoplastic endometrium.
Materials and Methods

Source of samples
The samples of FEA (n = 34) and of normal post-pubertal feline uterus (n = 23, 12 in the follicular stage – FS – and 11 in the luteal stage – LS) used in the present work were collected from the archives of four different laboratories in Portugal, during a period of 8 years (from 2003 to 2011). The age, breed, and past or present progestin-based contraception of animals enrolled in the study were assessed by the request forms accompanying the surgical specimens for histopathological evaluation. For FEA, the clinical history leading to the submission of specimens to the laboratories was also recorded.

Histopathological examination
Tissues were fixed in 4% buffered formalin and paraffin embedded. Sections with a thickness of 3 μm were cut, one slide routinely stained with haematoxylin and eosin for histopathological diagnosis. The tumours were evaluated according to several criteria of malignancy as described in Saraiva et al. (2015a). Briefly, the histopathological criteria used included potential indicators of tumours dedifferentiation, namely: nuclear atypia, considered as low to moderate or high; mean number of mitoses per high power field (HPF; 400x), valued as lower than 1 (<1), 1–5 and more than 5 (>5); and the myometrium, serosa and/or vascular invasion, evaluated as present or absent. Twenty-three normal uterine samples were staged as FS (n = 12) or LS (n = 11) based on the presence of follicles or corpora lutea in the ovaries and the epithelial cell height and the degree of development and coiling of endometrial glands (Saraiva et al. 2015a).

Immunohistochemistry
Immunohistochemistry was performed using a polymer-based system (Novolink® Polymer Detection System, Product No: RE7280-K Leica Biosystems, Newcastle, UK), according to the manufacturer’s instructions. The sections were deparaffinized with xylene and rehydrated through graded alcohols; antigen retrieval was performed in a pressure cooker (−96°C) in 10 mmol/l sodium citrate buffer (pH 6.0) for 3 min. The slides were cooled for 10 min at room temperature and rinsed twice in triphosphate buffered saline (TBS) for 5 min. Endogenous peroxidase activity was blocked by treating the sections with the Peroxidase Block® provided in the kit. Sections were incubated overnight at 4°C in a humid chamber with 1:40 Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein® (Code A0485 Dako, Denmark) that labels an intracellular domain of c-erbB2 oncprotein. Colour was developed with 3,3'- diaminobenzidine (DAB), incubated at room temperature and sections were then counterstained with Mayer’s haematoxylin, dehydrated and mounted. Sections of human colonic adenocarcinoma were used as positive control. For negative controls, the primary antibody was omitted.

Quantification of immunolabelling
The immunoreactivity of c-erbB-2 was assessed semi-quantitatively, in morphologically representative areas of the tumour at low power fields, based in the Hercep-Test scoring criteria, described in the American Society of Clinical Oncology (ASCO) guidelines (Wolff et al. 2013). Briefly, samples were classified according to the score: IHC3+, when the circumferential membrane staining is complete, intense and within >10% of tumour cells (‘chicken-wire pattern’); IHC2+, if the circumferential membrane staining is incomplete and or weak/moderate but within >10% of tumour cells, or complete and circumferential membrane staining that is intense in only ≤10% of tumour cells; IHC1+, if incomplete membrane staining is faint/barely perceptible and within >10% of tumour cells; IHC0, no staining is observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of tumour cells (Wolff et al. 2013). Scores of IHC0 and 1+ were considered negative, whereas scores of IHC2+ and 3+ were considered positive (Grushko et al. 2008; Rasotto et al. 2011; Togami et al. 2012). In the normal uterus, the analysis of immunoreaction against c-erbB-2 was assessed independently in the surface epithelium (SE), the superficial and deep glandular epithelia (SGE and DGE, respectively) and stromal compartment, using the same scoring system.

Statistical analysis
Statistical analysis was performed using chi-square and Fisher exact tests in the IBM SPSS Statistics Base 20.0 software®. p Values < 0.05 were regarded as statistically significant.

Results

Animals
The 34 FEA used in this study were diagnosed in queens with an age range of 1–15 years (mean 7.8 years); this information relates to 26 queens, since this information was not declared for the remaining eight animals. Breeds included Domestic Shorthaired cats (n = 24; 70.6%), Siamese (n = 2; 5.9%) and Persian (n = 1; 42.9%); in seven cases, breed was omitted. Contraception was confirmed in nine (26.5%) animals, whilst it was denied in 23 (68.6%) cases. In the remaining 22 cases, data concerning contraception was not mentioned in the form. When such information existed, the length of the treatment was not declared. The reasons leading to the request of histopathological analyses included clinical signs of uterine disease (n = 14; 41.2%), concurrent mammary tumour (n = 10; 29.4%) and detection of uterine lesions during routine ovariohysterectomy (n = 4; 11.8%).

© 2015 Blackwell Verlag GmbH
Normal uterine samples (n = 23) used as controls were obtained from post-pubertal animals submitted to routine ovariohysterectomy. Queens’ age was declared for 16 controls and ranged between 7 months and 8 years of age (mean 1.4 years). Breeds included Domestic Shorthaired cats (n = 7; 30.4%) and Persian (n = 1; 4.3%); this information was not specified for the remaining animals. In controls, none of the queens were submitted to contraceptive treatment.

**Histopathological features**

Feline endometrial adenocarcinomas (FEA) morphology was characterized by papillary proliferation, lined by multiple layers of neoplastic cells supported by a fibrovascular stroma. Other architectural patterns scarcely present within the tumours included solid, tubular and glomeruloid patterns. These features supported the classification of the tumours as a papillary serous type (Mutter and Crum 2000; Saraiva et al. 2012). Neoplastic cells were plasmorphic, mostly columnar shaped, with a moderate amount of eosinophilic cytoplasm and round-to-oval, vesicular or hyperchromatic nuclei that lost the normal polarity. Numerous multinucleated cells were present within and at the surface of the lesions. A variable number of clear cells was also observed. Nucleoli were evident and occasionally intranuclear clear inclusions were also found. Randomly distributed areas of necrosis within the tumours were frequently present. Histopathological features presumably related to tumour dedifferentiation are summarized in Table 1. Seventeen tumours (50.0%) had a low to moderate degree of atypia, whereas the other half of the sample (n = 17; 50.0%) was characterized by high atypia. The majority of tumours (n = 24; 70.6%) showed a mean number of mitoses per HPF between 1 and 5, while in 7 tumours (20.6%) it was <1 and in a minority of cases (n = 3; 8.8%) it was >5 mitoses. In normal samples, the surface epithelium in either FS or LS consistently presented <1 mitosis per HPF (n = 12 and n = 11, respectively; 100.0%). The mean number of mitoses per HPF in tumours was higher, compared to the normal epithelia (p ≤ 0.0001) (Table 1).

In the FS, the glandular epithelia (both the SGE and DGE) showed <1 mitosis per HPF. In the LS, the mean number of mitoses differed between the SGE and the DGE, though without statistical meaning; the SGE regularly presented a mean number of mitoses <1 (90.9%; n = 10) with only 1 sample showing a mean number of mitoses between 1 and 5 (9.1%), whilst the DGE showed a mean number of mitoses either <1 (n = 7; 63.6%) or between 1 and 5 mitoses (n = 4; 36.4%). Myometrial invasion was observed in 22 tumours (64.7%). Vascular and serosa impairment were present in 3 (8.8%) and 1 (2.9%) tumours, respectively. These cases occurred independently but they always co-existing with myometrial invasion.

**c-erbB-2 immunohistochemistry**

The immunoreaction against c-erbB-2 oncprotein in FEA is summarized in Table 2. Of the 34 FEA cases, 14 (41.2%) were considered negative to c-erbB-2: four cases were classified as IHC0 and 10 cases as IHC1+ (11.8% and 29.4%, respectively). The remainder 20 FEA samples (58.8%) were positive for c-erbB-2.

**Table 1.** Major histopathological features assessed in FEA cases

<table>
<thead>
<tr>
<th>Histopathological features</th>
<th>Number of FEA in 34 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear atypia</td>
<td></td>
</tr>
<tr>
<td>Low to moderate</td>
<td>17 (50.0)</td>
</tr>
<tr>
<td>High</td>
<td>17 (50.0)</td>
</tr>
<tr>
<td>Mean number of mitoses per HPF</td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>7 (20.6)</td>
</tr>
<tr>
<td>1–5</td>
<td>24 (70.6)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>3 (8.8)</td>
</tr>
<tr>
<td>Myometrium invasion</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22 (54.7)</td>
</tr>
<tr>
<td>No</td>
<td>12 (35.3)</td>
</tr>
<tr>
<td>Serosa invasion</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>No</td>
<td>33 (97.1)</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (8.8)</td>
</tr>
<tr>
<td>No</td>
<td>31 (91.2)</td>
</tr>
</tbody>
</table>

FEA, feline endometrial adenocarcinomas; HPF, high power field.

<table>
<thead>
<tr>
<th>c-erbB-2 IHC score n (%)</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IHC0</td>
<td>IHC1+</td>
</tr>
<tr>
<td></td>
<td>IHC2+</td>
<td>IHC3+</td>
</tr>
<tr>
<td>Follicular Stage (n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>1 (8.3)</td>
<td>0</td>
</tr>
<tr>
<td>SGE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DGE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Luteal Stage (n = 11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>0</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>SGE*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DGE*</td>
<td>2 (18.2)</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td>FEA</td>
<td>4 (11.8)</td>
<td>10 (29.4)</td>
</tr>
</tbody>
</table>

IHC, immunohistochemical; FEA, feline endometrial adenocarcinomas; SE, surface epithelium; SGE, superficial glandular epithelium; DGE, deep glandular epithelium.

Different superscripts indicate a statistical significance: *P = 0.016; bP < 0.0001; cP = 0.004; dP = 0.024.
being 18 (52.9%) scored as IHC2+ and 2 (5.9%) as IHC3+. The immunoreaction of c-erbB-2 in both normal and FEA stroma was consistently negative.

The stage of the cycle influenced the immunoreaction against c-erbB-2 in the normal uterus. Concerning the overall epithelial expression of c-erbB-2 in follicular and luteal stages, samples in FS were most commonly positive for c-erbB-2 (p = 0.016). In FS, the endometrial epithelia were generally positive for c-erbB-2 (Table 2; Fig. 2), with only 1 case (8.3%) being negative for this molecule in the SE. Scores varied between IHC2+ and IHC3+, accounting 75.0% (n = 9) and 16.7% (n = 2) in the SE, 33.3% (n = 4) and 66.7% (n = 8) in the SGE, and 58.3% (n = 7) and 41.7% (n = 5) in the DGE, respectively. In LS, the SE was negative in only 1 sample (9.1%), being positive in 10 specimens (90.9%), represented by six samples (54.5%) with a score IHC2+ and 4 (36.4%) with IHC3+. While the SGE in LS was considered positive in all the cases, being 9 (81.8%) scored as IHC2+ and 2 (18.2%) scored as IHC3+, the DGE was mainly negative (n = 6; 54.5%); 2 cases (18.2%) were scored as IHC0 and 4 (36.4%) samples had a score of IHC1+. Only five samples (45.4%) were found positive for c-erbB-2 with a score of IHC2+. In LS, the SGE was more frequently positive for c-erbB-2 than the DGE (p = 0.024). Positivity for c-erbB-2 was more frequent in DGE of the FS than in the homologous counterpart of the LS (p = 0.004). FEA were more likely negative for c-erbB-2 expression than controls of FS (p ≤ 0.0001). No significant differences were detected between FEA and LS.

In tumours, c-erbB-2 expression was compared with nuclear atypia, number of mitoses per HPF and myometrium, serosa and vascular invasion, all features presumably indicative of tumour dedifferentiation. However, no statistical differences were recorded.

**Discussion**

The present study proposed to establish the pattern of immunohistochemical expression for c-erbB-2 oncoprotein in FEA, compared to the normal feline endometrium and to explore putative associations between c-erbB-2 and the histopathological features of FEA that might be related to poor prognosis and worse outcome. To the best of our knowledge this is the first description of c-erbB-2 immunohistochemical expression in feline neoplastic endometrium.

Despite the rarity of endometrial adenocarcinomas in domestic species except cows and the rabbits (Cotchin 1964; McEntee and Nielsen 1976; Kennedy et al. 1998), the increasing number of reports on FEA questions the possibility that these tumours may be more
The c-erbB-2 molecule was expressed in 58.8% of the cases under study. In women, c-erbB-2 positive phenotype has been associated with high-grade tumours (Ferrandina et al. 2005; Mariani et al. 2005) and to early stages of the disease (Sugimoto et al. 2007). Data gathered in the present study failed to evidence an association between c-erbB-2 scoring and histopathological malignant features mentioned above, as it has been also referred regarding the women histopathological features of malignancy (Miller et al. 2003; Horn et al. 2007; Goldschmidt et al. 2011), namely nuclear atypia, mitotic activity and myometrial, vascular and/or serosa invasion.

The c-erbB-2 molecule was expressed in 58.8% of the cases under study. In women, c-erbB-2 positive phenotype has been associated with high-grade tumours (Ferrandina et al. 2005; Mariani et al. 2005) and to early stages of the disease (Sugimoto et al. 2007). Data gathered in the present study failed to evidence an association between c-erbB-2 scoring and histopathological malignant features mentioned above, as it has been also referred regarding the women histopathological features of malignancy (Miller et al. 2003; Horn et al. 2007; Goldschmidt et al. 2011), namely nuclear atypia, mitotic activity and myometrial, vascular and/or serosa invasion.

The present study also localized the c-erbB-2 protein in the normal feline uterus in the FS and LS of the cat oestrous cycle. Differences in the expression of c-erbB-2 between the normal feline endometrium and in endometrial hyperplasia had been determined (Misirlioglu et al. 2006). In that study, it was referred that the c-erbB-2 content in SE of normal feline uterus was lower than that of the glandular epithelium, contrasting with the findings in the present study. The differences may be explained by the fact that a different antibody or scoring systems were used, as well as by the fact that the oestrous cycle was not staged, therefore impairing any inference on eventual cyclic changes in this molecule expression. Still, the results reported herein showed that c-erbB-2 expression was confined to the epithelial compartment, as it has also been reported for human endometrium (Wang et al. 1992). Interestingly, in the present study, the expression of c-erbB-2 was significantly higher in follicular stage of feline endometrium compared to the luteal stage. Van Dam et al. (1991) detected non-significantly higher levels of c-erbB-2 oncoprotein in the proliferative phase of pre-menopausal endometrium (grossly equivalent to the FS described herein), compared with the secretory phase (which would correspond to the LS in our work), alike to the results presented herein.

Oestrogens increase the levels of epidermal growth receptor (EGFR) in the endometrium (Mukku and Stancel 1985), and the cyclic interchange between oestrogens and progesterone has been proved to have a greater effect on EGF synthesis (Imai et al. 1995). Steroids also determine the interplay between the epithelial and stromal elements of the endometrium, which seems to have a major role in the endometrial proliferation and differentiation (Pierro et al. 2001).

Once c-erbB-2 shares functional and morphological homologies with EGFRs, it could mediate oestrogen-induced proliferation (Maia et al. 2002). However, in a previous report it was showed that ER-α is underexpressed in FEA compared to normal endometrium, suggesting that the proliferation of FEA might be controlled by a different pathway involving local growth factors, rather than by ER-α expression (Saraiva et al. 2015b). It was also evidenced that the proliferative indexes, estimated on the basis of Ki-67 (MIB-1) immunolabelling, were higher in FEA than in the normal endometrium, irrespectively of the oestrous stage considered (Saraiva et al. 2015b). However, a positive correlation between c-erbB-2 and MIB-1 was described in human endometrial carcinomas (Ioachim et al. 2002), which seems to contrast with results reported herein. In fact, c-erbB-2 expression in LS was higher in the SGE compared to DGE. However, the persistency of proliferation of the basal glands during LS, which is reflected by the increased coiling reported in this stage (Chatdarong et al. 2005), should accompany an increase in c-erbB-2 expression if this pathway was involved in epithelial proliferation of feline endometrium. Watson et al. (1996) proposed that differentiation and decidualization in the LS is a progesterone-mediated event, regulated by EGF. Payan-Carreira et al. (2014) also suggested that progesterone is an important driver for differentiation events occurring in the canine endometrium during the LS. All the above agreed with the involvement of c-erbB-2 in differentiation phenomena occurring during trophoblast invasion, as suggested by Mühlhauser et al. (1993).

These could explain the absence of association between the c-erbB-2 protein expression and the
histopathological malignant features expressed in FEA, such as the mitotic index or the invasiveness. Therefore, data gathered in the current study regarding c-erbB-2 molecule sustain the hypothesis raised in previous reports that other pathways controlling epithelial proliferation in the feline endometrium may be involved in FEA carcinogenesis (Saraiva et al. 2015b). Therefore, further studies ought to address the importance of the loop between oncogenes, growth factors and steroid hormones in the determination of proliferative and differentiation phenomena in feline cyclic and diseased endometrium.

Concluding, the c-erbB-2 immunohistochemical location and pattern of distribution in FEA and the normal endometrium in FS and LS is described herein for the first time. The majority of the FEA cases under study were positive to c-erbB-2 immunolabelling; however, a decrease in the expression of this protein in FEA was observed when compared to the FS of normal endometrium. No associations were established between c-erbB-2 expression and the histopathological features potentially related to FEA dedifferentiation and poor outcome. The expression of c-erbB-2 was significantly higher in the epithelia of the FS compared to LS, which suggests that this protein is influenced by functional changes occurring during the feline oestrous cycle. Additional studies are required to further investigate the role of c-erbB-2 in feline endometrial physiology and carcinogenesis.

Acknowledgements

The authors thank Prof. Dr. Conceição Peleteiro and Prof. Dr. Pedro Faisca for archived material provision, Prof. Dr. Adelina Gama for the scientific discussions and Prof. Dr. Margarida Saraiva for language assistance. This work was sponsored/financed/founded by the Portuguese Science and Technology Foundation (FCT) under the Project PEst-OE/AGR/UI0772/2011 and 2014. Preliminary results were presented as an Abstract at the XX Meeting of the Portuguese Society of Animal Pathology, Lisbon, 2015.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

ALS, RP-C, FG and MAP were involved in the acquisition of clinical and histopathological data, data analysis and interpretation, as well as the manuscript writing and the reviewing of the literature. MAP and ALS were responsible for the immunohistochemical analysis. FF and LML were responsible for technical assistance. ALS, RP-C, FG and MAP contributed to the manuscript writing and the reviewing of the literature. All authors read and approved the final manuscript.

References


© 2015 Blackwell Verlag GmbH
c-erbB-2 Expression in Feline Endometrium

filament proteins in 50 feline neoplasms.
Vet Pathol 32, 692–701.
Vet Pathol 42, 30–34.

Submitted: 6 Aug 2015; Accepted: 23 Oct 2015

Author’s address (for correspondence): Maria dos Anjos Pires, CECAV, Laboratório de Histologia e Anatomia Patológica, Edifício de Blocos Laboratoriais Sala C0.11, Universidade de Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal. E-mail: apires@utad.pt

© 2015 Blackwell Verlag GmbH