SHORT COMMUNICATION
HER-2/neu (c-erbB-2) oncoprotein in hyperplastic endometrial polyps detected in two cats

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The presence of HER-2/neu (c-erbB-2) oncoprotein, oestrogen-α receptor (ER), and progesterone receptor (PR) in hyperplastic endometrial polyps (EPs) of two cats with cystic endometrial hyperplasia-pyometra (CEH-P) complex was investigated. Immunohistochemistry assay for ER, PR and c-erbB-2 oncoprotein in the glandular and stromal tissue of the EPs was performed. ER and c-erbB-2 immunoreactivity was observed in the glandular epithelium of the EPs whereas PR immunoreactivity was detected only in the stromal fibroblasts. The c-erbB-2 oncoprotein may play a role with the ER in the pathogenesis of the hyperplastic EPs, although the role of this oncoprotein in the pathogenesis of EPs has yet to be determined.

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Endometrial polyps (EPs) are generally single or multiple benign growths consisting of a focal proliferation of both glandular and stromal elements of the endometrium. The EPs usually project into the lumen of the affected uterus as pedunculated to broad-based masses emanating from the endometrium. They can become large and may readily be confused with tumours of the uterus.

Studies on the pathogenesis of human EPs have pointed to the role of oestrogen and progesterone receptors (ER and PR). More recently the malignant potential of the hyperplastic EPs in women has been reported. Although well documented in humans, there are only two reports concerning to the role of exogenously administered oestrogen in the occurrence of EPs in zoo felids and in a rhesus monkey.

It is a well known fact that epidermal growth factors (EGFs) are mitogenic for endometrial cells via mediating local effects of steroid hormones. HER-2/neu (c-erbB-2) oncoprotein is a transmembrane glycoprotein with intrinsic tyrosine kinase activity which shares functional and morphological homologies with the EGF receptor (EGFR). Activation of c-erbB-2 receptors in the endometrium leads to epithelial cell proliferation. Studies have shown that the expression of c-erbB-2 in the hyperplastic uterus is higher than that in normal uterus and the highest quantities can be reached in malignant uterine samples in humans. There is only one report which observed the presence of c-erbB-2 oncoprotein in the hyperplastic cat endometrium. The aim of this study was to investigate the presence of ER, PR, and c-erbB-2 oncoprotein in EPs of two cats.

Uterine tissue and EPs collected from two privately owned cats undergoing ovariohysterectomy because of clinical signs of cystic endometrial hyperplasia-pyometra (CEH-P) were used in this study. A 7-year-old female mixed breed cat was noted to have vaginal bleeding for 3 weeks. A second female mixed breed cat (10 years of age) was referred with a 4-week history of vaginal bleeding. Both of the cats were nulliparous and none of them had ever been exposed to hormonal treatment (contraceptives) for oestrus suppression. General and vaginal examinations (vaginoscopy and vaginal smear) were performed. The clinical signs in both cats were anorexia, lethargy, abdominal distention, vomiting, dehydration, weight loss and bloody vaginal discharge.

The haematological parameters, analysed by using an Abbott Cell-Dyn 3500 haematological analyser (Table 1), revealed a leukocytosis and thrombocytopenia in both cats. Intermediate and parabasal cells with a high number of neutrophils and red blood cells have been determined in vaginal smears stained with May Grunwald and Giemsa (Merck, Darmstadt, Germany). Transabdominal ultrasonography, performed by using a B-mode scanner...
real-time ultrasound scanner 5 MHz linear array transducer (Pie Medical Scanner 450, Maastricht, The Netherlands), revealed an enlarged uterus with convoluted, tubular horns filled with anechoic fluid in both cats. Endometrial polyps were detected as determinate hypoechogenic areas (Fig 1A).

At macroscopic examination, the presence of corpus luteum (CL) was detected in both cases. In both cats the cornu uterine were dilated and a single, pale coloured, hard spheroid tissue mass (approximately 2.5 × 3.5 cm in size) attached to the endometrium by a thin stalk were present in the lumen of the uterus. Hyperplastic areas of the adjacent uterine mucosa and the presence of blood clots and purulent exudate on the endometrium and the surface of the endometrial masses were detected (Fig 1B).

After macroscopic examination, the tissue samples taken from the uterus and the EPs were fixed in 10% buffered formalin, processed routinely, embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin. Immunohistochemistry was performed using a streptavidin–biotin–peroxidase method described previously18 to investigate the presence of ER, PR, and c-erbB-2 oncoprotein in the EPs. The primary antibodies used were monoclonal mouse-anti-human ER receptor antibody (clone 1D5 + 6F11), monoclonal mouse-anti-human PR antibody (clone hPRa 2 + hPRa 3) and monoclonal mouse-anti-human c-erbB-2/HER-2/neu oncoprotein antibody (clone e2-4001 + 3B5) (NeoMarkers, Fremont, CA, USA).

Histologically, the masses were lined by endometrial epithelium and contained endometrial glands surrounded by a well vascularised connective tissue stroma. In some areas of the EPs there was clustering of endometrial glands composed of densely packed, columnar epithelial cells with increased basophilia interpreted as adenomatous hyperplasia; several glands were dilated or cystic. Based on the gross and histological findings, a diagnosis of single hyperplastic EPs was made. Histological examination of the uterine tissue confirmed the clinical diagnosis of CEH-P in both cases. Based on immunohistochemical staining of the hyperplastic EPs; weak positive cytoplasmic c-erbB-2 immunostaining was observed in all endometrial glands (Fig 2A and B) whereas positive nuclear ER immunoreactivity was detected in approximately 40% of the endometrial glands (Fig 2C). No immunolabelling for c-erbB-2 or ER could be seen in the stromal cells. Nuclear PR immunoreactivity was detected in stromal cells (Fig 2D), but no PR staining was observed in the glandular epithelium.

Reports of spontaneously occurring EPs in animals are rare and have only involved a few species.8,19–28 There are just three reports of EPs in cats.24,27,28 Table 1. Haematological parameters of the cats

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Cat 1 (7 years old)</th>
<th>Cat 2 (10 years old)</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte</td>
<td>3.98 ×10^{12}/l</td>
<td>4.61 ×10^{12}/l</td>
<td>4.95–12.53</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>8.2 g/dl</td>
<td>9.2 g/dl</td>
<td>8.5–14.4</td>
</tr>
<tr>
<td>PCV (haematocrit)</td>
<td>28.0%</td>
<td>24.2%</td>
<td>24–45</td>
</tr>
<tr>
<td>Mean corpuscular volume</td>
<td>70.3 fl</td>
<td>52.6 fl</td>
<td>30–50</td>
</tr>
<tr>
<td>Mean corpuscular haemoglobin concentration</td>
<td>20.6 pg</td>
<td>20.0 pg</td>
<td>12.2–16.8</td>
</tr>
<tr>
<td>WBC (total leukocyte count)</td>
<td>26.8 ×10^{9}/l</td>
<td>46.0 ×10^{9}/l</td>
<td>3.8–19</td>
</tr>
<tr>
<td>Platelet</td>
<td>130 ×10^{9}/l</td>
<td>122 ×10^{9}/l</td>
<td>160–660</td>
</tr>
</tbody>
</table>

Fig 1. (A) Ultrasound picture of cat 2. The anechoic area indicates the purulent exudate filled the uterus (black arrow) and the hypoechoic scene noted the polyp (white arrow).
morphological evidence suggests that EPs arise from focal areas of endometrial hyperplasia (EH). Authors reported that most of the animals with EP had EH in adjacent areas of the uterus. In the present study hyperplastic EPs and CEH-P complex was observed in two aged cats (7 and 10 years old). This finding supports the suggestion that EH predisposes to EP formation in animals and confirms the association of EPs with advanced age, which has been reported previously in different species of animals.

In the present study CL and presence of pyometra in addition to EH was detected in both cats. These findings are not fully in accordance with Potter et al who suggested that most cases of pyometra or endometritis, but not EH, are associated with CL in cats.

The role of ER and PR on the pathogenesis of EPs has been well documented in humans. It has been suggested that especially the ERs, play a crucial role in the pathophysiology of EPs and that EPs could arise from localised overexpression of ER in women. In a previous report we noted elevated ER scores in glandular endometrium of cats with severe hyperplasia in comparison to mild hyperplasia, and concluded that the increased ER expression in cats with severe hyperplasia emphasises the importance of ER activation in the development of EH in cats. Connection between the EPs and EH has been reported previously.

In the present study positive ER immunoreactivity was detected in approximately 40% of the glandular epithelium of the EPs whereas PR immunoreactivity was detected only in the stromal cells. These findings are in accordance with our previous report.

The EPs in women have been classified as atrophic, atrophic cystic and hyperplasic types. From the various human studies, it is clear that the hyperplastic EPs have a malignant potential and that pre-malignant and malignant transformations can occur in EPs of women.

There are no data on the malignant potential of the EPs in animals. In the two EPs examined in this study, some areas (including clustering of endometrial glands composed of densely packed, columnar epithelial cells with increased basophilia) have been interpreted as adenomatous hyperplasia and diffuse weak c-erbB-2 immunostaining was observed in all endometrial glands. No malignant transformation was observed.

Some authors suggested a relationship between oestrogen and c-erbB-2 activity in the endometrial cell proliferation. Markogiannakis et al observed that oestradiol increases the expression of c-erbB-2 gene in the Ishikawa human endometrial adenocarcinoma cells, and Maia et al suggested that the activation of c-erbB-2 receptor probably potentiates the proliferative effects of oestrogens on the endometrium by some still unknown mechanisms. In our previous report we suggested that c-erbB-2 oncoprotein may play a role in the pathogenesis of EH together with ER in cats because we have observed elevated c-erbB-2 and ER scores in glandular endometrium of cats with severe EH in comparison to the cats with mild EH.

The data in this study suggests that c-erbB-2 oncoprotein may play a role in the pathogenesis of the hyperplastic EPs together with ER in cats. To the best of our knowledge this is the first report of the ER, PR and

![Fig 2](image-url)
c-erbB-2 status in EPs of cats. The role of this oncoprotein in the pathogenesis of EPs and the malignant potential of the EPs in cats has to be further investigated.

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References