Expression of Prolactin in Feline Mammary Adenomas and Adenocarcinomas

By

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An Abstract of the Thesis of
Danielle K. Trummel for the degree of Bachelor of Science in Bioresource Research with an option in Animal Reproduction and Development and Bachelor of Science in Animal Science with an option in Pre-Veterinary Medicine. Thesis presented on June 4th, 2007.

Title: Expression of Prolactin in Feline Mammary Adenomas and Adenocarcinomas

Mammary tumors are the third most common tumor in cats after cutaneous neoplasia and lymphosarcoma. More than 80% of feline mammary tumors are adenocarcinomas, which are similar to human mammary adenocarcinomas in their biologic behavior and histologic characteristics. Despite treatment with radical mastectomy, feline mammary adenocarcinoma is associated with a high incidence of metastasis to regional lymph nodes, spleen, liver and lungs. Prolactin (PRL), a protein hormone synthesized by the anterior pituitary gland, stimulates mammary epithelial cell proliferation and differentiation. In many mammalian species, PRL is also produced within the mammary gland. In humans and rodents, PRL is an important mitogen for mammary neoplasia. However, the role of PRL has not been investigated in feline mammary adenomas and adenocarcinomas. The purpose of this study was to determine if feline mammary tumors expressed PRL. Formalin-fixed paraffin-embedded sections of archived mammary tissues (n=6) previously submitted to the Oregon State University Veterinary Diagnostic Laboratory were cut with a width of 6 microns and studied by a modified indirect immunohistochemistry protocol with a polyclonal PRL antibody. Feline anterior pituitary tissue was used to verify the validity of the procedure as PRL
expression by lactotrophs in the tissue had positive and specific staining. Prolactin expression was positive and specific in glandular epithelial cells of the mammary gland of some cats (2 of 4) with adenocarcinoma. In conclusion, these results suggest a contribution of PRL in the development of feline mammary neoplasms. Further exploration into the relationship between PRL and feline mammary adenocarcinomas is needed.
Bachelor of Science in Bioresource Research thesis of Danielle Trummel presented on June 4th, 2007

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Date

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Date

I understand that my thesis will become part of the permanent collection of Oregon State University Libraries. My signature below authorizes release of my thesis to any reader upon request.

________________________________________________________________________
Danielle K. Trummel
Date
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**Introduction:**

The cat has four pairs of mammary glands (cranial thoracic, caudal thoracic, cranial inguinal and caudal inguinal) located in the subcutaneous fat layer of the ventral body wall. Mammary tumors are the third most common tumor in cats after cutaneous neoplasia and lymphosarcoma [21]. More than 80% of feline mammary tumors are adenocarcinomas. Due to similarities in the biologic behavior and histologic characteristics of human and feline mammary cancer [25], feline mammary adenocarcinomas would be an appropriate model for the study of breast cancer in women. One in nine women is diagnosed with breast cancer each year [23]. The feline model would allow developments to be made in improving treatment options for both women and cats.

Mammary neoplasia has been reported to occur in cats from 9 months to 23 years of age [25]. The mean age of diagnosis is 10 to 12 years. Cats spayed at 6 months of age had a 40-60% lower risk of developing mammary cancer compared to intact [25]. In contrast to dogs, information pertaining to the success rate of spaying at the time of diagnosis in cats is not known. However, research pertaining to mammary neoplasia in rodent models and humans allows for a comparison to cats.

A guarded to poor prognosis is typically given with the diagnosis of feline mammary adenocarcinoma due to its aggressiveness, invasiveness, and tendency to metastasize. Tumor size is the most important factor determining prognosis. Larger tumor size significantly decreases the median disease-free interval and survival time [25]. The benefit of surgery for feline mammary adenocarcinoma is limited due to early metastases when tumor volumes are large. In one study, two-thirds of the cats that had
conservative surgery to remove only the affected mammary gland developed local recurrence of the tumor [25]. Radical mastectomy (chain mastectomy or the removal of all four mammary glands) is the treatment of choice [26]. Despite treatment by radical mastectomy, feline mammary adenocarcinoma is associated with a high incidence of metastasis to regional lymph nodes, spleen, liver and lungs [20]. In more than half of cats with metastases, chemotherapy using doxorubicin alone or in combination with cyclophosphamide has been shown to induce partial responses (>50% regression) [25].

All tumors should be histologically classified based on descriptive morphology as stated by the World Health Organization in order to provide similarities and differences between species and uniformity within a species. Mammary tumors are classified as adenocarcinoma, sarcoma, carcinosarcoma and adenomas [10]. For the purposes of the current study, only adenomas and adenocarcinomas will be discussed [10]. Adenomas and adenocarcinomas are subclassified as simple or complex. A simple mammary tumor is one that is composed of either secretory epithelial or myoepithelial cells [10]. A complex mammary tumor is one that is composed of both secretory epithelial and myoepithelial cells [10]. In the cat, the complex type of adenoma is extremely rare.

Prolactin (PRL) is a neuroendocrine protein hormone (23 kD) synthesized by lactotrophs in the anterior pituitary gland (adenohypophysis). Prolactin is a necessary hormone for the development of the mammary gland. Prolactin stimulates mammary epithelial cell proliferation and differentiation and is essential for lactogenic activity at parturition [11, 22]. In addition to the adenohypophysis, PRL is found in extra-pituitary sites including the adrenal gland, bone, corpus luteum, lacrimal gland, lymphocytes, pancreas, placenta, prostate, testes, and uterus [11, 22]. Prolactin enhances the immune
system and supports local angiogenesis [28]. In the testes, PRL induces expression of luteinizing hormone receptors [28]. In dogs, humans and rodents, another extra-pituitary source of PRL is the mammary gland [22]. The predominant source for PRL within the mammary gland appears to be of epithelial origin with appreciably less observed in the stroma and endothelium [23]. The role and regulation of PRL production within the mammary gland may be related to the development of neoplasia.

Of the hormones secreted by the adenohypophysis, PRL is the only hormone that does not stimulate secretion of another hormone, such as growth hormone (GH). The adenohypophysis synthesizes and secretes GH in a similar manner as PRL. Structurally, PRL is similar to GH [29]. Growth hormone is the primary hormone responsible for regulating body growth and in intermediary metabolism [28]. Both normal and neoplastic canine mammary tissues are extra-pituitary sources of GH [30]. However, it is not known if the same is true in cats. There are many similarities between PRL and GH and further study of the relationship between the two hormones is worth developing.

In humans and rodents, PRL is an important mitogen for mammary neoplasia. In 1959, Muhbock and Boot reported that PRL was important in the pathogenesis of mammary tumors [11,32]. In dogs, Saluja and coworkers (1973) found that PRL was higher in adenocarcinoma than in normal mammary tissue [26]. In 1995, Glusburg and colleagues found PRL in normal and neoplastic mammary tissue women [3]. Prolactin has been shown in the last decade to be a promising prognostic factor in human breast cancer [22]. However, the role of PRL has not been investigated in feline mammary neoplasia.
Objective:

To determine the immunoexpression of PRL in feline adenomas and adenocarcinomas and correlate expression with established histopathological grading and survival outcomes.
Chapter I: Immunohistochemistry

**Hypothesis**

Prolactin is expressed in feline mammary adenomas and adenocarcinomas.
**Materials and Methods:**

*Attainment of Tissue Sections*

Formalin-fixed tissue samples of feline mammary adenomas and adenocarcinomas were collected from private veterinary hospitals within the state of Oregon that had been sent to the Oregon State University Veterinary Diagnostic Laboratory (OSUVDL) for histologic diagnosis. Tissue samples were paraffin-embedded and embedded tissues were cut into 6 μm thick sections and prepared onto slides. Slides were stained with hematoxylin and eosin (H&E) stain and interpreted by a pathologist. The database of archived samples within the OSUVDL contained 71 feline mammary tumor cases. The H&E slides from each of these cases were re-evaluated for the presence of necrosis by Dr. Christiane Löhr, by a board-certified Veterinary Medical Anatomic Pathologist within the OSUVDL. Necrosis is a common finding in tumors. During the immunohistochemistry procedure, necrotic tissue will stain positively in a non-specific manner. Therefore, samples with abundant necrotic tissue had to be excluded. Following exclusion of these samples, 26 cases remained. A survey was prepared (see Chapter II) and sent to the hospitals that had submitted samples. Survey responses were only obtained from six cases: two adenomas and four adenocarcinomas. Although rare, one case was represented by a male cat. In addition to the neoplastic mammary tissue samples, tissue samples from two cases of feline benign mammary hyperplasia were included as a non-neoplastic (normal) control. Adenohypophysis tissue samples from two cats were also used as a positive control for the PRL antibody.
Immunohistochemistry
(See Appendix 1 for detailed list of solutions)

Mammary adenoma (n=2), adenocarcinoma (n=4), benign hyperplasia (n=2) and adenohypophysis tissue sections were cut (6 µm thick) from paraffin blocks and mounted on poly-L-lysine coated slides. Slides were deparaffinized in xylene and rehydrated in a graded ethanol series (100%, 100%, 80%, 80%) to tap water and double distilled water (ddH₂O). The action of tissue specific endogenous peroxidases (e.g. red blood cells) was inhibited by incubating slides in a pre-treatment solution of 30% hydrogen peroxide in automation buffer for 10 minutes at room temperature (20°C). When using a secondary antibody containing horseradish peroxidase (HRP), it is essential to block endogenous peroxidase activity to diminish non-specific staining. Slides were then blocked for 10 minutes at room temperature with a protein block. The protein block prevents nonspecific binding of the primary antibody with tissue components other than its target protein.

A cytokeratin primary antibody was applied at a ready-to-use dilution to one slide of each of the mammary neoplasia tissue sections. Cytokeratins are a family of water-soluble proteins that form the cytoskeleton of epithelial cells. This antibody was used to differentiate mammary epithelial cells from other mammary cell types, which allowed for the classification of the tumor as simple or complex.

The optimal dilution for the PRL primary antibody was determined through dilution testing. Dilutions were calculated by:

\[
\frac{\text{Antibody(µl)}}{\text{Antibody(µl)} + \text{Antibody diluent (µl)}}
\]
On separate slides, a PRL primary antibody was applied at three dilutions (1:250, 1:500, 1:1000) to each of the mammary neoplasia and adenohypophysis tissue sections for 1 hour at room temperature. The 1:250 dilution was determined to be ideal as it yielded the least non-specific staining and the highest PRL intensity staining. The PRL primary antibody was also applied to the benign hyperplasia mammary tissue at the 1:250 dilution. Specificity of PRL immunostaining was verified by replacement of the primary antibody with a universal negative at a ready-to-use dilution.

Slides were washed with automation buffer six times and then reacted with a secondary antibody (Envision HRP+ goat anti-rabbit) for 30 minutes at room temperature. Slides were again washed with automation buffer six times. Nova Red® was used as a chromagen and slides were counterstained with hematoxylin, followed by a bluing agent (1% lithium carbonate). Hematoxylin stains the cell nucleus blue unless it has already been stained by the Nova Red® chromagen. Slides were dehydrated and coverslips were mounted. To coverslip the slides, a toluene-based liquid mounting media was placed on the slide followed by a coverslip. A fine pointed needle was used to diminish any air bubbles underneath the coverslip.

**Histologic Analysis**

A board-certified Veterinary Medical Anatomic Pathologist within the OSUVDL re-examined all of the H&E stained slides to identify tumor characteristics and verify histologic diagnoses. The same pathologist examined the immunostained slides. Cytokeratin immunostained slides from the neoplastic tissues were classified as simple or
complex mammary tumors. Prolactin immunostained slides were evaluated for the location and specificity of positive PRL staining.
Results:

1. Pituitary: Prolactin Positive
2. Adenocarcinomas: Prolactin Positive
3. Adenocarcinomas: Prolactin Negative
4. Adenomas: Prolactin Negative
5. Benign Hyperplastic Mammary Tissue: Prolactin Negative
1. *Pituitary: Prolactin Positive*

The lactotrophs within the feline adenohypophysis synthesize and secrete PRL. In both feline pituitary samples, positive and specific PRL expression was observed (Figure 1).

Figure 1. Positive PRL staining (arrows) within the feline adenohypophysis at a 1:250 dilution (A) and 1:1000 dilution (B) (40X magnification).
2. Adenocarcinomas: Prolactin Positive

In two cases of feline mammary adenocarcinoma, there was positive and specific PRL immunostaining (Figure 2A and 2E). Prolactin was expressed in the glandular epithelial cells and not within every tumor cell. Prolactin positive cells were present in clusters, rather than individual cells. There was no immunostaining observed in the negative controls (Figure 2B and 2F). Cytokeratin was localized to epithelial cells (Figure 2C and 2G). Both of the PRL positive adenocarcinomas were classified as simple adenocarcinomas based on the cytokeratin staining. One of these tumors was histologically identified as a very aggressive tumor with lymph invasion (Figure 2D).

Figure 2. (Opposite page) Positive PRL staining (arrows) within the feline mammary gland at a 1:250 dilution in two cats with adenocarcinoma (A, E). The specificity of immunostaining was verified using a Universal negative antibody (B, F). Positive cytokeratin staining within the feline mammary gland of the same two cats (C, G). Serial sections stained with H&E were used to identify tumor type (D, H). All images were digitally captured at 40X magnification.
Figure 2.
3. Adenocarcinomas: Prolactin Negative

In two cases of feline mammary adenocarcinoma, there was no positive PRL immunostaining (Figure 3A and 3E). There was also no immunostaining observed in the negative controls (Figure 3B and 3F). Cytokeratin was localized to epithelial cells (Figure 3C and 3G). Both of the PRL negative adenocarcinomas were classified as simple adenocarcinomas based on the cytokeratin staining.

Figure 3. (Opposite page) Negative PRL staining within the feline mammary gland at a 1:250 dilution in two cats with adenocarcinoma (A, E). The specificity of immunostaining was verified using a Universal negative antibody (B, F). Positive cytokeratin staining within the feline mammary gland of the same two cats (C, G). Serial sections stained with H&E were used to identify tumor type (D, H). All images were digitally captured at 40X magnification.
Figure 3.
4. Adenomas: Prolactin Negative

In two cases of feline mammary adenomas, there was no positive PRL immunostaining (Figure 4A and 4E). There was also no immunostaining observed in the negative controls (Figure 4B and 4F). Cytokeratin was localized to epithelial cells (Figure 4C and 4G). One of the adenomas was simple (Figure 4C), while the other was complex (Figure 4G). A complex mammary adenoma is a rare tumor in cats.

Figure 4. (Opposite page) Negative PRL staining within the feline mammary gland at a 1:250 dilution in two cats with adenoma (A, E). The specificity of immunostaining was verified using a Universal negative antibody (B, F). Positive cytokeratin staining within the feline mammary gland of the same two cats (C, G). One of the adenomas was simple (C), while the other was complex (G). Serial sections stained with H&E were used to identify tumor type (D, H). All images were digitally captured at 40X magnification.
Figure 4.
5. **Benign Hyperplastic Mammary Tissue: Prolactin Negative**

In two cases of benign feline mammary hyperplasia, there was no positive PRL immunostaining (Figure 5A and 5D). Benign hyperplasia is a non-neoplastic tissue, which demonstrates that PRL expression is specific to the neoplastic mammary tissues in cats. There was also no immunostaining observed in the negative controls (Figure 5B and 5E). Cytokeratin was localized to epithelial cells (Figure 5C and 5F). There was no evidence of neoplastic processes using H&E staining (data not shown).

Figure 5. (Opposite page) Negative PRL staining within the feline mammary gland at a 1:250 dilution in two cats with benign hyperplasia (A, D). The specificity of immunostaining was verified using a Universal negative antibody (B, E). Positive cytokeratin staining within the feline mammary gland of the same two cats (C, F). All images were digitally captured at 40X magnification.
Figure 5.
Chapter II: Survivability Rates

**Hypothesis**

Survival duration and tumor recurrence is positively correlated with prolactin expression.
Materials and Methods:

Survey Preparation

A survey was constructed to collect case history from each of the cats represented in this study. Data on the quality of life, tumor recurrence, cause of death, and survival duration post-diagnosis were obtained (Appendix 4). The survey was sent to veterinary hospitals for 26 cases of feline mammary neoplasia. Survey responses were only obtained from six cases: two adenomas and four adenocarcinomas.

Data Interpretation

An Oregon State University Statistical graduate student aided in the analysis portion of this study after collecting case history. The survival duration post-diagnosis of mammary neoplasia was analyzed. The date of tissue submission to OSUVDL was considered the time of diagnosis. Kaplan-Meier (KM) estimates were used to determine survival probabilities. The KM estimator used for this data set was:

\[
\hat{S}_{KM}(t) = \prod_{j:t_j \leq t} \frac{n_j - d_j}{n_j},
\]

where \(n_j\) is the number of lives at risk (alive and uncensored) just before time \(t_j\), and \(d_j\) is the number of deaths at time \(t_j\). For studies with large sample sizes, the Kaplan-Meier estimate will approach the true survivability rate of the population.
Results:

Survivability Survey

The survivability rates in months post-diagnosis (time of biopsy) are graphically represented in Figure 6. In the sample population of six cases, there was a survivability rate of 30%. After eight months post-diagnosis, four of the cats had died. The two cases that were still alive as of the time of the survey (September 2006) were both benign adenomas. The data obtained in this study does not represent a simple random sample from the population. Therefore, the combination of a non-response bias and a small number of cases did not allow for the estimate to approach the true survivability rate.
Figure 6. Kaplan-Meier estimate of six cases of feline mammary neoplasia. Reference lines have been added to the plots so that median survival times and 1-year survival times can be estimated. The blue line indicates the survival rate at 1 year and the green line indicates the median survival duration. The uncensored plus signs indicate two cases that were still alive at the time the survey was collected (September 2006).
Discussion:

Although rare, a complex mammary adenoma was identified in a 12-year-old castrated male cat. This tumor was negative for PRL expression. Both cases of feline mammary adenomas were alive at the time of survey. This was expected as complete excision of the benign tumor is typically not associated with recurrence.

In half (2 of 4) of the feline mammary adenocarcinomas, PRL was expressed in glandular epithelial cells. The location of expression was similar to what has been reported in human breast cancer [23]. It is of interest to note that the two cases of feline mammary adenocarcinoma with PRL expression survived with longer than cases of feline mammary adenocarcinoma without PRL expression. In contrast with our hypothesis, survival duration post-diagnosis is negatively correlated with PRL expression. According to this data, PRL may be a protective agent against feline mammary cancer.

Patient demographics and outcomes are shown in Figures 7 and 8, respectively. Due to the small sample size, there can not be any inferences made to the population as a whole. However, the data collected supports further study in the relationship between PRL and feline mammary cancer.
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</tr>
<tr>
<td>2</td>
<td>05-1440-1</td>
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<tr>
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Figure 7. Patient Demographic- Data collected from survivability survey conducted from June to September 2006.
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**Survival Duration (months)**

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<td>Still Alive as of 9/06</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
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**Tumor Recurrence**

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**Figure 8. Outcomes** - Data collected from survivability survey conducted from June to September 2006. Six feline cases were represented.
References:


Appendix 1: Solutions

1% Lithium Carbonate
Fisher Scientific Laboratories
AC19336-0100
Lithium carbonate 99.999%
CLi₂O₃ F.W. 73.88

30% H₂O₂
certified ACS, Hyperoxide
*store at 4°C

Antibody Diluent
DakoCytomation
S3022
0.05 mol/L Tris-HCl buffer
0.1% Tween
0.015 mol/L sodium azide
*store at 2-8°C

Alcohol
Fisher Scientific Laboratories
A962F-1GAL
Alcohol, Reagent Histological

Automation Buffer
Biomeda
M30
10X Automation Buffer
Diluted to working solution of 1X
*store at 20-25°C

Automation Hematoxylin
DakoCytomation
S3301
500ml Ready to Use
*store at 20-25°C

Mounting Media
Triangle Biomedical Sciences
SHUR/Mount
Toluene-based acrylic resin
**Nova Red Kit**
Vector Laboratories
SK-4800
NovaRed substrate kit for peroxidase
*store at 4°C and protected from light

**Protein Block**
DakoCytomation
X0909
Ready-to-use Serum-free
25% casein in PBS, stabilizing protein, and 0.015 mol/L sodium azide
*store at 2-8°C

**Xylene**
Fisher Scientific Laboratories
X3P-1GAL
Xylenes Histological
C8H10 F.W. 106.16
Appendix 2: Antibodies

**Rabbit Anti-Prolactin Polyclonal Antibody**
Chemicon International
AB960
Rabbit Anti-Prolactin Polyclonal
Antigen used purified in sheep pituitary glands
Positive control: lactotrophs
*store at -20°C

**Cytokeratin**
Dako Corp.
M3515
Anti-Human Cytokeratin, Clones AE1/AE3
*store at 2-8°C

**Universal Negative Rabbit Control**
Dako Cytomation
N1699
N-Universal Negative Control Rabbit: Ready-to-use
*store at 2-8°C
Appendix 3: Antigen Retrieval

Antigen Retrieval is a pre-treatment for the paraffin tissue sections in order to expose the epitopes, or the region of the surface antigen capable of eliciting an immune response and combining with a specific antibody produced by such a response. The paratope is the region of the epitope that is recognized by the antibody. Two antigen retrieval processes were attempted to be used in the immunohistochemistry protocol of this study, yet high nonspecific staining was the result.

Heat-Induced Epitope Retrieval

After sections were deparaffinized and rehydrated, heat-induced antigen retrieval is performed. To prepare the working solution, 2.65 ml of Vector Labs (H3300) Antigen Unmasking solution is combined with 250 ml of ddH2O. Slides were placed in a microwavable-safe container and boiled in a microwave for 5 minutes. Care was taken to not allow the tissue sections to dry out during the heating process. The sections were then cooled for 30 minutes prior to the immunohistochemistry protocol.

Proteolytic-Induced Epitope Retrieval

After sections were deparaffinized and rehydrated, proteolytic-induced antigen retrieval was performed. Dako Cytomation (S3020) ready-to-use Proteinase K improves the accessibility of the antibodies and probes to target sites within the tissue. The sections were incubated 10 minutes at room temperature prior to the immunohistochemistry protocol. Proteinase K was stored at 2-8°C.
Appendix 4: Sample Survivability Survey and Response

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**Oregon State University**
SMALL ANIMAL VETERINARY TEACHING HOSPITAL
172 Magruder Hall
Corvallis, Oregon 97331-4803
**FAX Number (541) 737-4818**

**Date:** Wednesday May 24th, 2006  **Time:** 5pm

**Veterinary Hospital:** Eastview Veterinary Clinic

**Fax Number:**

**From:** Danielle Trummel

**Number of Pages (including this page):** 3

**Voice Telephone Number:**

**E-mail address:**

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>Submission Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shadow Davis</td>
<td>8/11/2003</td>
</tr>
<tr>
<td>Freda Crumb</td>
<td>11/14/2005</td>
</tr>
<tr>
<td>Socks Harford</td>
<td>8/22/2005</td>
</tr>
<tr>
<td>Lacey Hand</td>
<td>7/26/2004</td>
</tr>
<tr>
<td>Melba Shortt</td>
<td>12/16/2005</td>
</tr>
<tr>
<td>Macy Vyverberg</td>
<td>8/13/2004</td>
</tr>
<tr>
<td>Casey Bell</td>
<td>12/29/2004</td>
</tr>
<tr>
<td>Gizmo Tousley</td>
<td>3/10/2003</td>
</tr>
<tr>
<td>Mia Rios-Northrup</td>
<td>1/20/2006</td>
</tr>
</tbody>
</table>

I am an undergraduate student working in the Bioresource Research Center. My research mentor is Dr. Michelle Kutzler, a theriogenologist on faculty in the veterinary college. I am investigating the role of prolactin on mammary cancer in bitches and queens. Specifically, I am interested in the recurrence and survival rates of dogs and cats with prolactin-positive mammary tumors. This research is described in detail in the following pages. I would greatly appreciate it if you could review your patient records regarding the histopath submission (patient name and date listed above) and answer the questions below. If you do not have this information, I would be willing to contact the pet owner directly if you would please include the client’s contact information. Thank you very much for your time and assistance in this study. Please feel free to contact me if you have any questions.

1. What was the patient’s quality of life following histologic diagnosis?
2. If the mammary tumor was removed, did it recur following excision?
3. If the patient is no longer living, was the cause of death related to mammary cancer?
4. How long following tumor removal (or histologic diagnosis) did the patient live?

*if any part of this message is missing or received poorly, please call (541) 737-4812.
May 26, 2006

Dr. Elisa Salas
Eastview Veterinary Clinic
1260 Route 14A
Penn Yan, NY 14527

Attention to: Danielle Trummel
Oregon State University
Small Animal Veterinary Teaching Hospital
172 Magrude Hall
Corvallis, Oregon 97331

Dear Danielle,

Here is the summary of our patient information for the mammary masses. We wish you the best of luck. Please do not hesitate to contact us should you need any other information.

Cheers,

[Signature]

Elisa Salas, DVM

“Shadow” Davis
D.O.B. 11/3/92 FS English Springer Spaniel D. O. D. 11/15/05
OVH performed on 9/5/01. Mammary mass noted on 7/31/03, removal on 8/6/03

What was the patient’s quality of life following histologic diagnosis? Good to excellent

If the mammary tumor was removed, did it recur following excision? Yes on 11/15/05

If the patient is no longer living, was the cause of death related to mammary cancer? Yes, recurred with anorexia and lethargy. Euthanasia was performed.

How long following tumor removal (or histologic diagnosis) did the patient live? 2 yrs 3 months.

“Freda” Cruft
DOB 4/21/93 FS Beagle mix
OVH performed on 2000. Mass noted on 10/28/05, removed 11/9/05.

What was the patient’s quality of life following histologic diagnosis? Good

If the mammary tumor was removed, did it recur following excision? Not yet

If the patient is no longer living, was the cause of death related to mammary cancer?

How long following tumor removal (or histologic diagnosis) did the patient live? Still alive to date.

“Socks” Hartford
DOB 10/9/93 FS Labrador mix
OVH performed on 5/16/03. Mass noted on 8/12/05, removed on 8/17/05.

What was the patient’s quality of life following histologic diagnosis? Good

If the mammary tumor was removed, did it recur following excision? Not yet

If the patient is no longer living, was the cause of death related to mammary cancer?
How long following tumor removal (or histologic diagnosis) did the patient live? Still alive to date.

"Lacey" Hand
DOB 6/30/90 FS West Highland Terrier DOD: approximately 1/06.
OVH performed on 2/8/99. Mass noted on 7/16/02, removed on 8/6/02.

What was the patient's quality of life following histologic diagnosis?
Fair. history of IBD like signs.
If the mammary tumor was removed, did it recur following excision?
No.
If the patient is no longer living, was the cause of death related to mammary cancer?
Unknown.
How long following tumor removal (or histologic diagnosis) did the patient live? 3.5 years

"Melba" Short
DOB 4/13/96 FS DSH
OVH performed on 9/24/96. Mass noted on 12/13/05, removed on 12/13/05.

What was the patient's quality of life following histologic diagnosis?
Good.
If the mammary tumor was removed, did it recur following excision?
Not yet.
If the patient is no longer living, was the cause of death related to mammary cancer?

How long following tumor removal (or histologic diagnosis) did the patient live? Still alive.

"Macy" Vyverberg
DOB 7/18/98 FS DSH.
OVH performed at unknown date. Mass noted on 8/9/04, removed on 8/10/04.

What was the patient's quality of life following histologic diagnosis?
Good.
If the mammary tumor was removed, did it recur following excision?
Not yet.
If the patient is no longer living, was the cause of death related to mammary cancer?

How long following tumor removal (or histologic diagnosis) did the patient live? Still alive.

"Casey" Bell
DOB 8/25/00 FS Boxer
OVH performed on 9/10/03. Mass noted on 12/15/04, removed 12/22/04.

What was the patient's quality of life following histologic diagnosis? Good
If the mammary tumor was removed, did it recur following excision? Not yet
If the patient is no longer living, was the cause of death related to mammary cancer?

How long following tumor removal (or histologic diagnosis) did the patient live? Still alive.

"Gizmo" Tousley
DOB 3/23/96 FS Lhasa Apso Mix.
OVH performed 5/9/01. Mass noted 3/3/03, removed 3/4/03.

What was the patient's quality of life following histologic diagnosis? Good.
If the mammary tumor was removed, did it recur following excision? Not yet.
If the patient is no longer living, was the cause of death related to mammary cancer?