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A case of cystic mammary adenocarcinoma in rabbit

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Abstract: Mammary adenocarcinomas are a type of malignant tumor frequently encountered in domestic animals. It is rarely seen in rabbits, most often in cats and dogs. In recent years, cases of mammary tumors in domestic rabbits have been increasing. Cases such as adenosquamous carcinomas, anaplastic carcinomas, carcinomas in situ, spindle cell carcinomas and carcinosarcomas have also been reported, most commonly in rabbits, along with variants of invasive adenocarcinomas. In this case, a biopsy sample taken from the mammary region of a 6-year-old female hybrid rabbit was examined histopathologically and immunohistochemically. As a result of the examinations, histopathologically, a large number of neoplastic cells with features of malignancy were found in the breast tissue. Cystic formations filled with proteinous material of varying sizes were found in the neoplastic tissue. Anti-cytokeratin and anti-vimentin expressions of neoplastic cells were observed in immunohistochemical findings. A diagnosis of mammary cystadenocarcinoma was made in the presence of histopathological and immunohistochemical findings. According to the authors' knowledge, the cyst adenocarcinoma variant is rarely encountered among mammary tumors observed in rabbits.
Keywords: Cyst adenocarcinoma, mammary, rabbit.

Bir tavşanda kistik meme adenokarsinomu olgusu

Özet: Meme adenokarsinomları evcil hayvanlarda sıklıkla karşılaşılan kötü huylu bir tümör çeşididir. En çok kedi ve köpeklerde görülmekte olup, tavşanlarda nadiren bildirilmektedir. Son yıllarda evcil tavşanlarda meme tümörünün teşhisi de giderek artmaktadır. Tavşanlarda en yaygın olarak invaziv adenokarsinomların çeşitleri ile birlikte bazı adenoskuamöz karsinomlar, anaplastik karsinomlar, in situ karsinomlar ve mekik hücreli karsinom ve karsinosarkom gibi olgular bildirilmiştir. Bu olguda ise 6 yaşlı, dişi, melez bir tavşanın meme bölgesinden alınan biyopsi örneği histopatolojik ve immunohistokimyasal olarak incelendi. Yapılan incelemeler sonucunda histopatolojik olarak meme dokusunda çok sayıda belirgin derecede anizositozis, anizokaryozis, makronükleoli ve polinükleoluslarla birlikte gözlenen neoplastik hücrelere rastlandı. Neoplastik hücrelerin yüksek mitotik aktiviteye sahip olduğu görüldü. Neoplastik doku içerisinde farklı büyüklüklerde proteinöz materyal ile dolu kistik oluşumlara rastlandı. İmmunohistokimyasal olarak neoplastik hücrelerde hem anti-sitokeratin hem de anti-vimentin pozitifliği gözlendi. Histopatolojik ve immunohistokimyasal bulgular eşliğinde kitleye kist adenokarsinom tanısı konuldu. Kist adenokarsinom varyantı yazarların bilgisine göre tavşanlarda gözlenen meme tümörleri içerisinde oldukça nadir olarak karşılaşılmaktadır.
Anahtar kelimeler: Kist adenokarsinom, meme, tavşan.

Introduction

Mammary gland tumors are the second most common type of tumor in female dogs after skin tumors. In cats, the most common site of tumors is the mammary glands, apart from the cutaneous, lymphoid, and hematopoietic tissues. Mammary gland tumors are rare in other pet species (Jubb et al., 2012). In domestic rabbits (*Oryctolagus cuniculus*), mammary gland neoplasia has been reported relatively less frequently than in cats and dogs. Spontaneous mammary tumors in rabbits are often associated with uterine hyperplasia or adenocarcinomas. Therefore, it is thought that there is a relationship between these conditions (Walter et al., 2010).

Mammary carcinomas are subclassified as adenocarcinomas, solid carcinomas, spindle cell carcinomas, anaplastic carcinomas, squamous cell carcinomas, and mucinous carcinomas. The most frequently reported histological types are adenocarcinomas (Baum & Hewicker-Trautwein, 2015). Adenocarcinomas are classified as solid, papillary, cystic, comedo, follicular, tubular, and trabecular (Baum, 2021), based on the appearance of the glandular tissues formed by the cells that from the tumor, or as mucinous and colloid based on the characteristics of the substance secreted by the cells that form the tumor (Hüdaverdi & Kıran, 2005). Mammary gland adenocarcinomas in rabbits were found to be tubular, papillary, solid, and tubulopapillary in appearance. Cystic forms, as well as tubular and papillary formations, have been documented in a few cases (Schöniger et al., 2014; Sikoski et al., 2008). At least 70% of mammary tumors identified in rabbits are malignant and may metastasize to the lung, liver, kidney, pancreas, adrenal gland, ovary, bone marrow, and regional lymph nodes (Schöniger et al., 2014).

In this case report, cystic mammary adenocarcinoma, which is uncommon in rabbits, was examined histopathologically and immunohistochemically.

Case Description

The case material is a biopsy sample taken from the thoracic mammary region of a 6-year-old female hybrid rabbit (Figure 1). The mass was approximately 5x4x3 (cm) in size. Macroscopically, the mass was firm in consistency and the cross-sectional surface was homogeneous. Following standard follow-up procedures, the biopsy sample was fixed in a 10% formaldehyde solution, embedded in paraffin, and cut into five micrometer-thick sections (Leica RM 2155) for examination under a light microscope. Cystic formations of various sizes filled with proteinous substances were found in the mammary tissue during the microscopic examination (Figure 2). In the neoplastic regions, cells with numerous polymorphisms were observed. These cells displayed several mitotic figures with distinct anisocytosis, anisokaryosis, macronucleoli, and multinucleoli (Figure 3). Additionally, in the immunohistochemical

examination, the sections were stained with the Avidin-Biotin Peroxidase Complex method similar to previous reports (Abdelrahman et al., 2022; Gules et al., 2019). Vimentin (Dako, clone Vim 3B4, 1:200 dilution) and cytokeratin (Dako, clone AE1-AE3, 1:100 dilution) were used as primary antibodies. 3,3′-Diaminobenzidine (DAB) was used as chromogen, and counterstaining was made with Mayer hematoxylin. Neoplastic cells were positive for both antibodies (Figure 4-5).

Figure 1: Bilateral tumoral formations in the thoracic and abdominal mammary glands

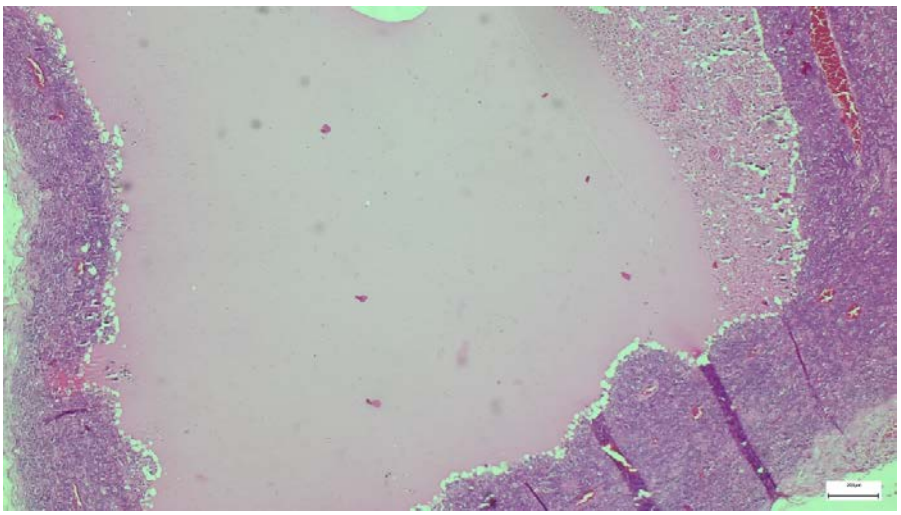

Figure 2: Cystic formation filled with proteinous material in the mammary gland tissue, H&E, Bar: 200 µm.

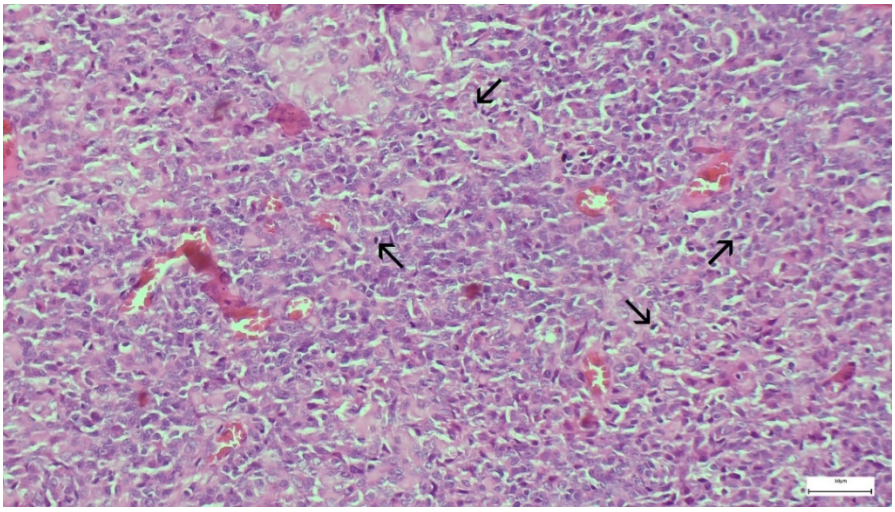

Figure 3: Multiple mitotic figures (arrows), with solid neoplastic growth areas showing distinct features of malignancy. H&E, Bar: 50 µm.

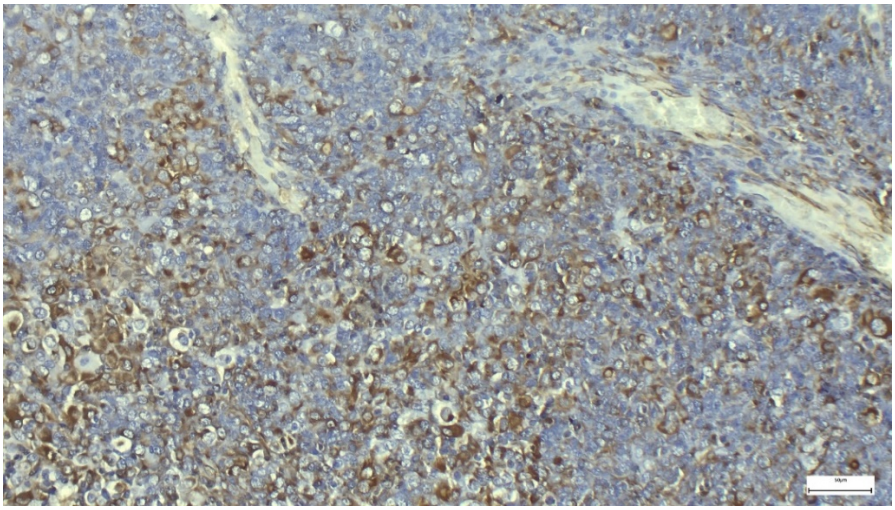

Figure 4: Anti-vimentin positivity in neoplastic cells, DAB, Bar: 50 µm.

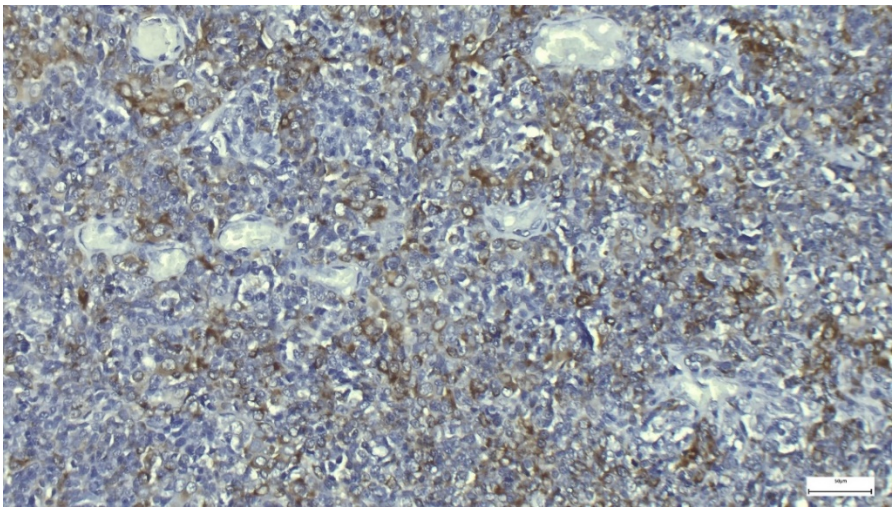

Figure 5: Anti-cytokeratin positivity in neoplastic cells, DAB, Bar: 50 µm.

Discussion

In rabbits, mammary tumors can develop at any age, with a mean of 4.9 to 5.5 years and a range of 8 months to 14 years (Sikoski et al., 2008). Additionally, according to Schöniger et al. (2014), all rabbits with mammary tumors of known sex are female or neutered females. The rabbit in our case was also 6 years old and female, which was compatible with the literature. In a study conducted by Bertram et al. (2021) on rabbits, metastasis was found in 12 necropsy cases of malignant mammary tumors and tissue samples to the lung in five cases, the liver in two cases, the regional lymph node in one case, and the spleen in one case. In our case, the presence of metastasis could not be determined because radiography and necropsy could not be performed.

It has been reported that the majority of rabbit mammary tumors are carcinomas (Schöniger et al., 2014; Baum and Hewicker-Trautwein, 2015; Degner et al., 2018). According to the study of Bertram et al. (2021), the most common tumor growth patterns are simple adenoma/carcinoma, intraductal papillary adenoma/carcinoma, and comedocarcinoma. Schöniger et al. (2014) also stated tubular, solid, and cystic growth patterns in their study. They also reported that tumors with some cystic cavities are filled with proteinaceous material. Previous studies have shown that in addition to cystic formations in tumors, tumor cells reproduce in a tubular or papillary fashion. In this present case, however, cystic formations were seen together with solid growth of epithelial tumor cells. Furthermore, the cystic formations were filled with proteinous material.

Cytoplasmic lipid droplets are frequently found in the luminal epithelial cells of normal and hyperplastic mammary glands, epithelial tumor cells, and cystic lesions (Baum & Hewicker-Trautwein, 2015; Degner et al., 2018). Due to the high fat content of rabbit milk, their presence is likely also related to lactation activity (Maertens et al., 2006). In our study, it was noted that mammary gland epithelial tumor cells showed solid growth; cell shapes varied from cuboidal to polygonal cells, but there were no vacuoles in the cytoplasm of the cells. In addition, Baum & Hewicker-Trautwein (2015) suggested that rabbit mammary carcinomas have a wide spectrum of mitotic activity, ranging from 0-36 mitotic figures at x100 high magnification. In the presented case, 4-5 mitotic activities were observed in the x100 magnification area.

Immunohistochemistry is an important method for the diagnosis of neoplasms. The diagnosis of neoplasms has been facilitated by revealing the existence of a wide variety of antibodies and using antibodies that bind to cell-specific proteins. Since all epithelial cells contain cytokeratin and all mesenchymal cells contain vimentin, cytokeratin and vimentin are

the most commonly used intermediate filaments (Jubb et al., 2012). Cytokeratin antibody has been found to be positive in the squamous and columnar epithelial cytoplasms of the skin, cervix, colon, esophagus, small intestine, and stomach, as well as in epithelial tumors (Listrom & Dalton, 1987). The vimentin antibody has been shown to strongly stain fibrocytes, lipocytes, smooth muscle cells, vascular endothelial cells, sweat, and salivary gland myoepithelial cells. However, positivity has also been reported in epithelial tumors (such as adenocarcinomas, cholangiocarcinomas) when the tumor is poorly differentiated (Azumi & Battifora, 1987). This condition is called the "epithelial-mesenchymal transition" and is a complex process involved in embryonic development, wound healing, and carcinogenesis. During this process, epithelial cells lose their defining features and acquire mesenchymal features. With the epithelial-mesenchymal transition, the cells move away from their normal structure and allow it to pass through blood and lymphatic vessels to invade different organs. Therefore, it has been seen as a cause of metastasis and tumor invasiveness (Cervantes-Arias et al., 2013; Raposo-Ferreira et al., 2018). In our case, cytokeratin positivity confirms epithelial origin, while vimentin positivity supports the presence of epithelial-mesenchymal transition and/or malignancy increase.

Conclusion

To the authors' knowledge, the cyst adenocarcinoma variant is extremely rare among mammary tumors in rabbits, and this case report may contribute to understanding the histopathology of this tumor in rabbits.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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