

## Uterine Adenocarcinoma in a Lionhead Rabbit

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**Abstract :** An 8-year-old, female lionhead rabbit with clinical sign of hematuria and vaginal discharge with/without blood was submitted to a local animal hospital. On exploratory laparotomy, three round to oval masses were observed in both uterine horns. The lumen of uterus was severely obstructed and distorted because of massive neoplastic proliferation. Histopathologically, the uterine masses revealed papillary projections along with irregular glandular structures into the lumen. The neoplastic foci were composed of numerous irregular sized neoplastic glands originated from uterine glands. These neoplastic cells showed very strong invasive tendency to muscle layer, therefore emboli of neoplastic cells were located in lymphatics. According to immunohistochemistry, the tumor cells in uterine masses demonstrated strong positive signals for cytokeratin, but negative for vimentin. Based on the gross, histopathologic and immunohistochemical features, this case was diagnosed as uterine adenocarcinoma in lionhead rabbit.

**Key words :** adenocarcinoma, cytokeratin, immunohistochemistry, rabbit, uterus.

### Introduction

Epithelial tumors of both the uterus and oviduct including adenoma and adenocarcinoma are rare in all domestic animals, except cattle and rabbits. Uterine adenocarcinoma is a tumor composed of malignant epithelial cells arising from the endometrial glands (6). In cows, this tumor is fairly frequent in older cows at slaughter, but it is not common in bovine necropsy cases. According to previous study, the incidence of uterine adenocarcinoma in does 2-3 years of age was around 4%, and in does 5-6 years of age, around 80% (7). Most cases of uterine adenocarcinoma metastasize within 12 to 24 months after the onset of clinical abnormalities (11). The incidence of this tumor in commercial rabbitries and research facilities is relatively low. This is due to the fact that these animals are usually relatively young (7).

Uterine tumors occurred in various breeds of rabbit. According to previous literatures, uterine adenocarcinoma was not found in the Belgian or Rex breeds but occurred in other breeds including Tan, Dutch and French Silver (2,4). The present case reports the occurrence of uterine adenocarcinoma in a lionhead rabbit. To our best knowledge, this is the first case report of uterine adenocarcinoma in a lionhead rabbit in Korea.

### Case

An 8-year-old, female lionhead rabbit with clinical sign of hematuria and vaginal discharge with/without blood was submitted to a local animal hospital. Complete blood count (CBC) and serum chemistry profiles were performed. Results of blood works revealed low hemoglobin level (9.6 g/dL; reference

range, 10.5-17.0), decreased lymphocytes (22.7%; reference range, 35.2-75.6), and increased granulocytes (68.3%; reference range, 20.2-59.3). Abnormalities in serum chemistry profile included increased in alanine aminotransferase (ALT, 129 U/L; reference range, 10-70), aspartate aminotransferase (AST, 116 U/L; reference range, 15-50) and creatine phosphokinase (415 U/L; reference range, 52-368). Serum alkaline phosphatase level (3 U/L; reference range, 10-160) in rabbit was decreased. Abdominal radiography showed radiopaque mass in the uterus (Fig 1). On exploratory laparotomy, three round to oval masses were observed in the uterus. These masses were pale red in color with multifocal yellowish foci (Fig 2A). One mass was about 2 × 2 × 2 cm in size and located in the right uterine horn. Two masses, 2 × 2 × 2 cm and 4 × 2 × 3 cm in size, were existed in the left uterine horn. In the three months after the surgical excision of uterine masses and ovariohysterectomy, pulmonary metastasis was observed using thoracic radiography. In the middle of anesthesia for surgical excision of pulmonary mass, the rabbit died. Unfortunately, pathologic examination for metastatic pulmonary was not performed.

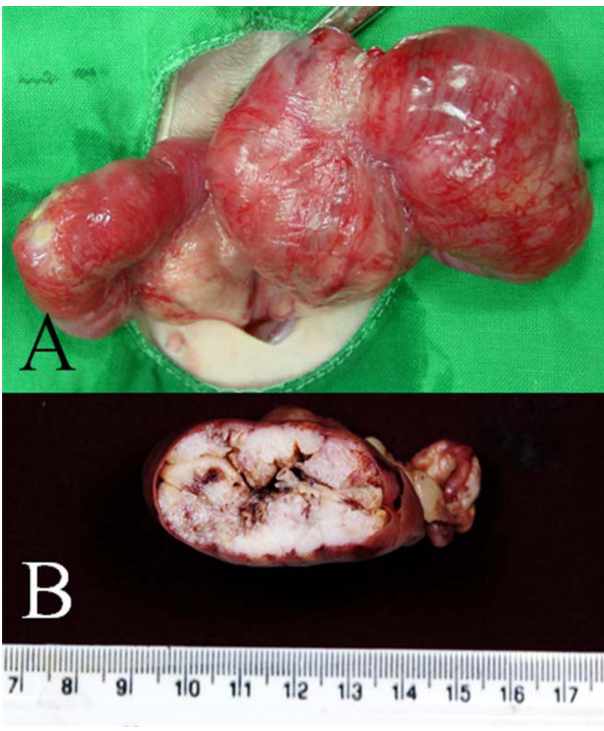
Surgically excised uterine masses were immediately fixed in 10% neutral buffered formalin, and then referred to Veterinary Pathology Laboratory in Jeju National University. The samples were processed routinely for histopathologic examination and sections were stained with hematoxylin and eosin (H&E). To reveal the origin of tumor cells, immunohistochemistry (IHC), streptavidin-biotin peroxidase complex method, was performed using several antibodies such as monoclonal mouse anti-human cytokeratin (CK, AE1/AE3, Dako, Denmark) and vimentin (Dako, Denmark).

On the cut surface of the uterine masses, the lumen of uterus was severely obstructed and distorted because of massive neoplastic proliferation (Fig 2B). Histopathologically, the uterine masses revealed papillary projections along with

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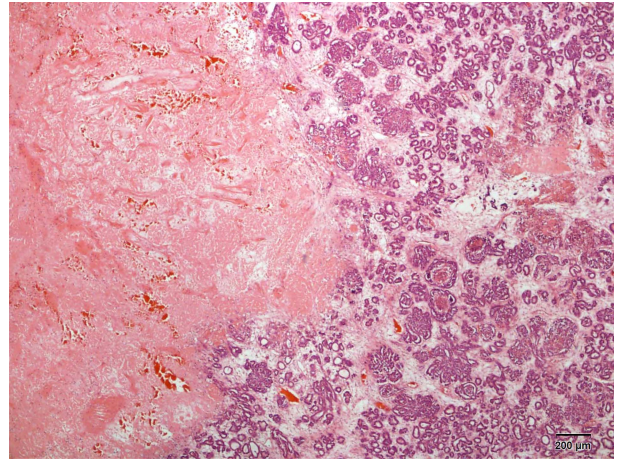


**Fig 1.** Lateral radiography of the abdomen of an eight-year-old lionhead rabbit with uterine masses (circle).

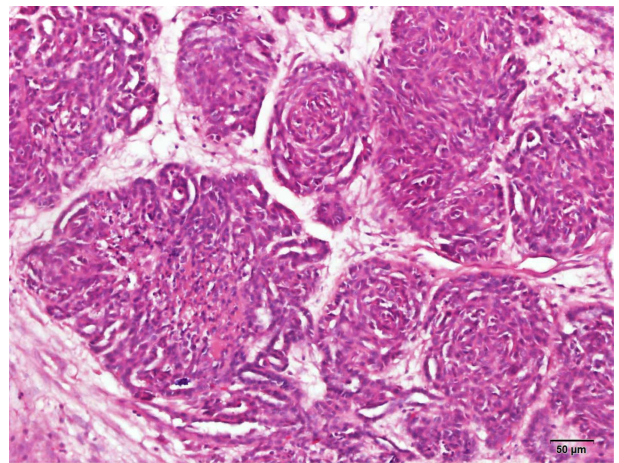


**Fig 2.** Note three round to oval masses in both uterine horns (A). Because of mild tan-colored mass, uterine lumen was severely obstructed and distorted on the cut surface (B).

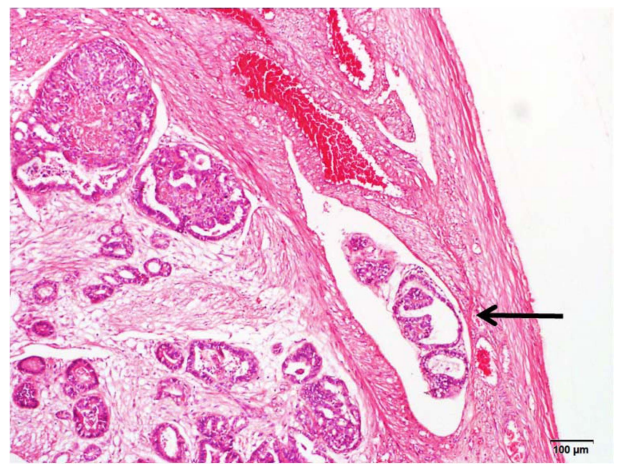
irregular glandular structures into the lumen. Very large neoplastic area occupied from mucosa to muscle layer of uterus. This neoplastic area was composed of numerous irregular sized neoplastic glands originated from uterine glands (Fig 3). Most neoplastic glands were lined by single to multiple layers of cells with low cuboidal or flattened neoplastic cells. Neoplastic glandular epithelia had dark basophilic nucleus, prominent nucleoli, scant cytoplasm, and low mitosis (0-1 per high power field). Many glands contained eosinophilic protein fluids or coagulative necrotic cellular debris in their lumens. Some neoplastic glands showed severe cystic dilatation with intra-luminal papillary growth of lining epithelium, therefore neoplastic glands sometimes formed cribriform glands with



**Fig 3.** Uterine mass was composed of numerous irregular sized neoplastic glands with adjacent large necrotic foci. H&E, Bar = 200 µm.

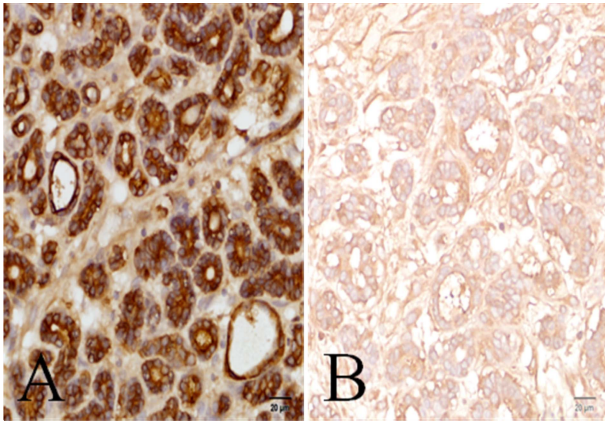


**Fig 4.** Some neoplastic glands formed cribriform glands with sieve-like arrangement. H&E, Bar = 50 µm.



**Fig 5.** Some emboli of neoplastic cells (arrow) were located in lymphatic. H&E, Bar = 100 µm.

sieve-like arrangement (Fig 4). These neoplastic cells showed very strong invasive tendency to submucosa and muscle layer, therefore emboli of neoplastic cells were located in lymphat-



**Fig 6.** Neoplastic cells showed positive reactions for CK (A), but negative for vimentin (B). IHC, Bar = 20 µm.

ics (Fig 5). Multifocal severe necrosis and hemorrhage also accompanied throughout the uterine masses.

According to IHC, the tumor cells in uterine masses demonstrated strong positive signals for CK, the epithelial cell specific marker (Fig 6). However, there were no positive reactions for vimentin, the mesenchymal cell marker.

## Discussion

Based on the gross, histopathologic and immunohistochemical features, this case was diagnosed as uterine adenocarcinoma in lionhead rabbit. In rabbits the uterine adenocarcinomas are mostly primary multiple and present in both uterine horns (3). Among three uterine masses in this case, one mass was located at the right uterine horn and other two masses were presented at the left uterine horn.

Hematuria and/or a serosanguineous vaginal discharge may indicate disorders of the genital tract such as endometrial hyperplasia or uterine adenocarcinoma (10). The blood is not truly coming from the urine but derives in the uterus and is released when the rabbit bears down urination. The major purpose to visit the animal hospital in this lionhead rabbit was also hematuria and vaginal discharge. However, in the previous study only 10 of 59 rabbits showed these signs, and they therefore cannot be considered hallmark signs of uterine disease in rabbits (10).

Generally, human endometrial carcinoma is associated with glandular hyperplasia, but rabbit carcinoma is associated with senile atrophy (2). With increasing the age of female rabbits the endometrium undergoes progressive changes, a decrease in cellularity and an increase in collagen content. These changes are associated with the development of uterine cancer (5). Constitutional abnormalities, such as dwarfism and toxemia of pregnancy, were associated with endometrial carcinoma of the rabbit. Greene (4) demonstrated that liver function of rabbit is affected by pregnancy toxemia and can last up to a year. Increased ALT and AST meant that there was a possibility of hepatic damage associated with toxemia of pregnancy in the rabbit of this study. But the association of uterine adenocarcinoma with pregnancy toxemia, pseudopregnancy, and hyperestrogenism until remains controversial (4). Mammary gland abnormalities are often associated with

endometrial hyperplasia or adenocarcinoma in rabbits (10). However, the lionhead rabbit did not show any evidences of mammary lesions.

Uterine adenocarcinoma is a slowly developing tumor with early local invasion of the myometrium and may extent through the uterine wall to adjacent organs in peritoneal cavity (3,9). Hematogenous metastasis to the lungs, liver, and sometimes bone, brain, and thyroid is reported within 1 to 2 years after the clinical onset (1,3). If there are no metastases, the prognosis after ovariohysterectomy in rabbits with uterine adenocarcinoma is fair or improved (10). However, the lionhead rabbit died in the middle of anesthesia for surgical treatment of pulmonary metastasis of tumor within 3 months following ovariohysterectomy.

Uterine adenocarcinomas in rabbits were classified into 2 types such as papillary and tubular/solid type, based on the prominent architecture of 42 neoplastic tissues (1). Typical papillary growth of the uterine glands and the foci of cribriform morphology were observed in papillary type adenocarcinoma but not in tubular/solid type. Myometrial invasion was seen in 80.8% of papillary adenocarcinomas, and 93.8% of tubular/solid adenocarcinomas. Two modes of invasion, expansive and invasive, were observed. Most of the papillary adenocarcinomas (19/21, 90.5%) showed an expansive mode. In contrast, most of the tubular/solid adenocarcinomas (14/15, 93.3%) showed an invasive mode (1). Histopathologically, the papillary growth of neoplastic foci into the lumen of uterus and the papillary growth of lining epithelia in cystic dilated glands were observed throughout the uterine masses in this case. And multifocal cribriform structures also presented in cystic dilated neoplastic glands. In contrast to previous study, strong invasion of neoplastic glands to myometrium was observed in papillary uterine adenocarcinoma in this case. Therefore some lymphatic in myometrium contained emboli of neoplastic cells in their lumens. Multifocal severe necrosis in neoplastic mass and central coagulative necrosis in cystic dilated glands also presented in this case. The occurrence of metastasis is closely associated with the occurrence of extensive necrosis in the primary tumor (3).

Uterine adenocarcinoma in the rabbit is regarded as an animal model of endometrial adenocarcinoma in women and is thought to have a relationship with hormonal stimuli, especially estrogen stimulation (3). Estrogen and progesterone are crucial to the control of endometrial activity. The development of human endometrial neoplasia is associated with the influence of steroid hormones and the expression of steroid receptors in neoplastic cells is considered a prognostic factor useful for the establishment of hormonal therapy (9). Recent study for immunohistochemical evaluations of estrogen receptor- $\alpha$  (ER- $\alpha$ ) and progesterone receptor (PR) demonstrated that the overall immunoreactivities were significantly different between histopathologic types of uterine adenocarcinomas including papillary and tubular/solid in rabbits (1). According to previous literature, PR was expressed within the nucleus of normal endometrial cells and in one of three samples of endometrial hyperplasia, each of four adenomas and in four of six adenocarcinomas in rabbit (9). These findings suggest that ER- $\alpha$  and PR expressions are not directly involved in neoplastic transformation of the endometrium and that

such expression is not a prognostic indicator (1,9).

Observation of clinical signs, abdominal palpation, and radiography are particularly helpful to the clinical diagnosis for uterine adenocarcinoma in rabbits. Several studies demonstrated that abdominal ultrasonography was more sensitive than radiography for diagnosing uterine disorders in rabbits (8,10). Lionhead rabbit is one of the newer breeds of domestic rabbits in the world. Rabbits have become popular pets and are therefore commonly seen in veterinary practice (10). In cats and dogs the incidence of uterine tumors will be diminished by the common practice of spaying (3). Because uterine disorders can occur in young rabbits and disease may be subclinical in its early stages, female pet rabbits unintended for breeding should therefore be neutered before one year of age (10). Regular ultrasonography or radiography of the genital tract is recommended for pet rabbits without spaying.

### Acknowledgments

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### References

1. Asakawa MG, Goldschmidt MH, Une Y, Nomura Y. The immunohistochemical evaluation of estrogen receptor-alpha and progesterone receptors of normal, hyperplastic, and neoplastic endometrium in 88 pet rabbits. *Vet Pathol* 2008; 45: 217-225.
2. Baba N, von Haam E. Animal model for human disease: spontaneous adenocarcinoma in aged rabbits. *Am J Pathol* 1972; 68: 653-656.
3. Elsinghorst TA, Timmermans HJ, Hendricks HG. Comparative pathology of endometrial carcinoma. *Vet Q* 1984; 6: 200-208.
4. Greene HSN. Adenocarcinoma of the uterine fundus in the rabbit. *Ann N Y Acad Sci* 1959; 75: 535-542.
5. Harkness JE, Wagner JE. Specific diseases and conditions. In: *The Biology and Medicine of Rabbits and Rodents*, 2<sup>nd</sup> ed. Philadelphia: Lea & Febiger. 1983: 143-144.
6. Kennedy PC, Cullen JM, Edwards JF, Goldschmidt MH, Larsen S, Munson L, Nielsen S. Histological classification of tumors of the genital system of domestic animals. Second series Volume III. Washington: Armed Forces Institute of Pathology. 1998: 31-39.
7. Percy DH, Barthold SW. Rabbit. In: *Pathology of Laboratory Rodents and Rabbits*, 3<sup>rd</sup> ed. Ames: Blackwell Publishing. 2007: 304-305.
8. Saito K, Nakanishi M, Hasegawa A. Uterine disorders diagnosed by ventrotomy in 47 rabbits. *J Vet Med Sci* 2002; 64: 495-497.
9. Vinci A, Bacci B, Benazzi C, Caldin M, Sarli G. Progesterone receptor expression and proliferative activity in uterine tumours of pet rabbits. *J Comp Pathol* 2010; 142: 323-327.
10. Walter B, Poth T, Böhmer E, Braun J, Matis U. Uterine disorders in 59 rabbits. *Vet Rec* 2010; 166: 230-233.
11. Weisbroth SH, Flatt RE, Kraus AL. Neoplastic disease. In: *The Biology of the Laboratory Rabbit*. New York: Academic Press. 1974: 336-339.