NOTE *Pathology*

Cystic Rete Testis with Testicular Dysplasia in a Rabbit

James K. CHAMBERS[1)], Kazuyuki UCHIDA[1)]*, Yousuke MURATA[1)], Ken-ichi WATANABE[1)], Kenichiro ISE[2)], Yasutsugu MIWA[3)] and Hiroyuki NAKAYAMA[1)]

[1)]*Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo, Japan*
[2)]*Yoshizuka Pet Clinic, 5–2–3 Yoshizuka, Hakata-ku, Fukuoka, Japan*
[3)]*Miwa Exotic Animal Hospital, 1–25–5 Komagome, Toshima-ku, Tokyo, Japan*

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ABSTRACT. An 8-year-old intact rabbit was presented to a veterinary hospital with a complaint of enlarged left scrotum. Histological examination revealed a single large cyst adjacent to an efferent ductule-like tissue. The cyst wall was composed of monolayer cuboidal cells surrounded by dysplastic testicular tissue, and the seminiferous tubules were not developed at all. The epithelial cells of the cyst possessed the same properties as the epithelial cells of the rete testis that were positive for CD 10 and cytokeratin 18, negative for p63 and lacked desmin-positive muscular layer. The dysplastic testicular tissue was composed of two types of cells: small pleomorphic cells with a condensed nucleus (sex cord-like cells) and large round cells with cytoplasmic lipid droplets (Leydig cells). Both of these cells were positive for vimentin and melan A that are consistent with the staining pattern of Sertoli cells and Leydig cells. This is the first report to demonstrate cystic rete testis with testicular dysplasia in animals.
KEY WORDS: cystic rete testis, rabbit.

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Intratesticular cystic lesions, including simple cysts, tunica albuginea cysts and epidermoid cysts, are very rare in humans and also in animals [4]. Tunica albuginea cysts and epidermoid cysts are believed to derive from mesothelium and germ cells, respectively [13]. Simple cysts can occur anywhere in the testis, and its originating tissue is still disputed. In animals, there have been four case reports on cystic rete testis (in a cat, a fox, a horse and an alpaca) and one case report on efferent ductule cyst (in a dog) [5, 8, 11, 14]. However, immunohistochemical analysis of rete testis has never been demonstrated.

An 8-year-old intact rabbit was presented to a veterinary hospital with a complaint of enlarged left scrotum. On palpation, the left scrotum was swollen measuring 12 × 7 cm and was filled with liquid (Fig. 1A). Both testes were surgically removed and fixed in 10% neutral buffered formalin. Gross examination revealed a single large cyst in the left scrotum containing serous fluid. The right testis was slightly atrophied comparable to the age. The tissues were routinely embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin (HE).

Histological examination revealed a single cyst adjacent to the efferent ductules-like tissue in the left scrotum (Fig. 1B and 1C). Aspermatogenesis due to a complete defect of seminiferous tubules was confirmed. The cyst wall was lined with monolayer cuboidal epithelial cells and surrounded by tunica vasculosa, tunica albuginea and visceral tunica vaginalis (Fig. 1D). The epithelia invaginated outwards from the cyst, forming a short tubular structure. Microvilli and occasional cilia were observed on the apical surface of the epithelial cells (Fig. 1E). Beneath the epithelial layer, clusters of small pleomorphic cells with a condensed nucleus surrounded the cyst, accompanied by abundant microvessels (Fig. 1E). Admixed with these cells, large round-shaped cells with cytoplasmic lipid droplets, similar to Leydig cells, were also found (Fig. 1E). The right testis had normal seminiferous tubules with normal spermatogenic maturation.

Additional stainings including PAS, PTAH and immunohistochemistry were performed in order to further characterize the epithelium of the cyst wall. The details of the primary antibodies used for immunohistochemistry are listed in Table 1. The labeling was visualized using the anti-mouse EnVision+ System[d](Dako, Tokyo, Japan) as described. First, we analyzed normal testis and epididymal tissues collected from a 7-year-old rabbit, which had been castrated at a veterinary hospital accordingly to the owner's request (Figs. 2A, 2E, 2I, 2M and 3A). Epithelial cells of the rete testis, efferent ductules and the head and the tail of epididymis were all positive for CD 10 (Table 2). Cytokeratin 18 was expressed in the epithelial cells of rete testis, efferent ductules and the head of epididymis, but not in the tail of epididymis (Fig. 2B, 2F, 2J and 2N). The duct of epididymis had basal cells that were positive for p63, and some of the epithelial cells of the efferent ductules were weakly positive for p63, whereas rete testis had no basal cells (Fig. 2C, 2G, 2K and 2O). The efferent ductules and the epididymis possessed desmin-positive

*CORRESPONDENCE TO: UCHIDA, K., Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo, Japan.
e-mail: auchidak@mail.ecc.u-tokyo.ac.jp

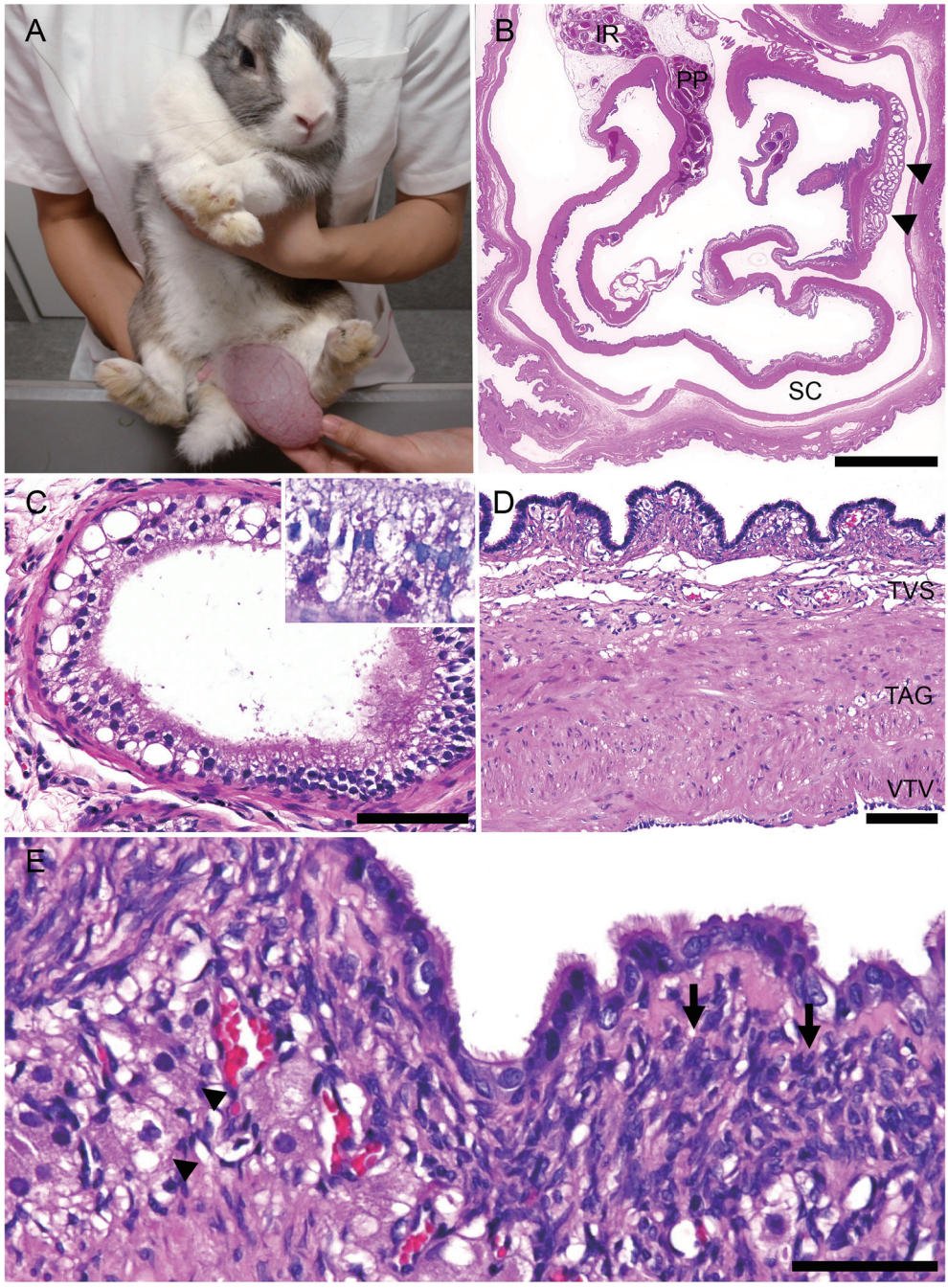

Fig. 1. (A–E) Gross and histological findings of the cyst. (A) The left scrotum is severely swollen. (B) A large cyst in the scrotal cavity is observed adjacent to an efferent ductules-like tissue (arrowheads). IR, inguinal ring; PP, pampiniform plexus, SC, scrotal cavity. HE. Bar, 5 mm. (C) The efferent ductules-like tissue is composed of pseudostratified columnar cells and is surrounded by a thin muscular layer. HE. Bar, 100 μm. The epithelial cells possess abundant PAS-positive granules in the cytoplasm (inset). PAS. (D) The wall of the cyst is composed of monolayer cuboidal epithelial cells, tunica vasculosa, tunica albuginea and visceral tunica vaginalis. TVS, tunica vasculosa; TAG, tunica albuginea; VTV, visceral tunica vaginalis. HE. Bar, 100 μm. (E) Beneath the epithelial layer of the cyst, clusters of small pleomorphic cells with a condensed nucleus (arrows) and large round-shaped cells with cytoplasmic lipid droplets, similar to Leydig cells (arrowheads), are observed. HE. Bar, 100 μm.

Table 1. Primary antibodies used in the present study

Antibody against	Clone (mouse)	Dilution	Antigen retrieval	Source
CD 10	56C6	1: 100	autoclave	Invitrogen, Camarillo, CA
CK 18	Ks18.04	Ready to use	proteinase K	Progen, Heidelberg, Germany
p63	BC4A4	1: 100	autoclave	Biocare Medical, Concord, CA
Desmin	D33	1: 50	autoclave	Dako, Tokyo, Japan
Vimentin	V9	1: 100	autoclave	Dako, Tokyo, Japan
Melan A	A103	1: 50	autoclave	Dako, Tokyo, Japan

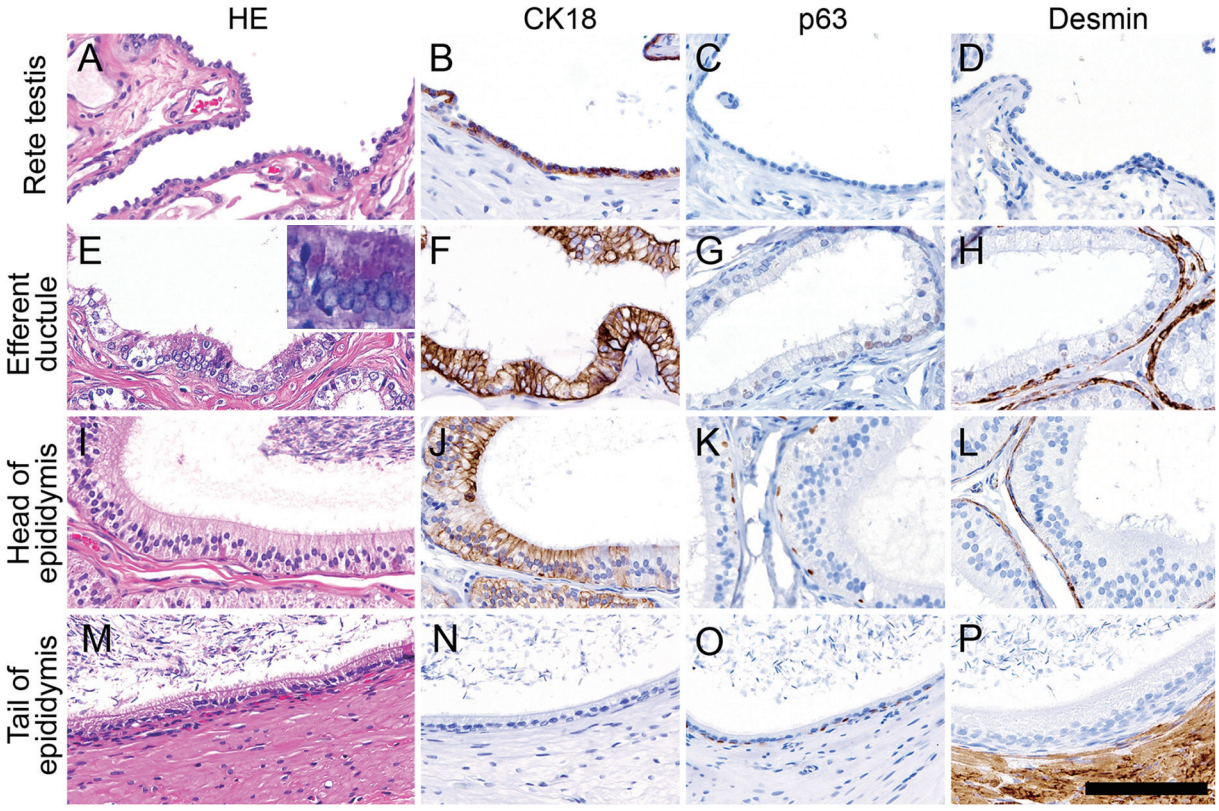

Fig. 2. (A–D) Rete testis of normal rabbit testis. (A) The rete testis is composed of monolayer cuboidal epithelial cells. (B) The epithelial cells are positive for CK18. (C) The epithelial cells lack p63-positive basal cells. (D) No desmin-positive muscular layer is observed. (E–H) Efferent ductules of normal rabbit testis. (E) The efferent ductules are composed of pseudostratified columnar cells with cytoplasmic PAS-positive granules (inset). (F) The epithelial cells are positive for CK18. (G) Some of the epithelial cells are weakly positive for p63. (H) Desmin-positive muscular layer surrounds the efferent ductules. (I-L) Head of epididymis of normal rabbit. (I) The head of epididymis is composed of ducts with tall columnar cells. (J) The epithelial cells are positive for CK18. (K) The basal cells are positive for p63. (L) Desmin-positive muscular layer surrounds the ducts. (M–P) Tail of epididymis of normal rabbit. (M) The tail of epididymis is composed of ducts with columnar cells. (N) The epithelial cells are negative for CK18. (O) The basal cells are positive for p63. (P) Desmin-positive muscular layer surrounds the ducts. (A, E, I and M) HE. (E, inset) PAS. (B–D, F–H, J–L and N–P) Immunoperoxidase reaction with DAB chromogen and hematoxylin counterstain. Bar, 100 μm.

muscular layer surrounding the epithelial cells, whereas the rete testis had no muscular layer (Fig. 2D, 2H, 2L and 2P). In the epithelial cells of efferent ductules, PAS-positive granules were observed in the cytoplasm (Fig. 2E, inset), as well as in the efferent ductules-like structure adjacent to the cyst (Fig. 1C, inset). Leydig cells and Sertoli cells were positive for vimentin (Fig. 3B). Leydig cells were also positive for melan A, and Sertoli cells were weakly positive for melan A (Fig. 3C). Vimentin was also expressed in the epithelial cells of rete testis (Fig. 3B), but not in the epididymis. The right testis and epididymis of the present case showed the same immunohistochemical characteristics with normal tissues.

The epithelial cells of the cyst wall in the present case were positive for CD 10 and CK 18 and negative for p63 (Fig. 4A, 4B and 4C). The cilia of the epithelial cells were stained in blue with PTAH-staining. No muscular layer

Table 2. Results of immunohistochemistry for epithelial layer of normal rabbit tissue and cyst

Antigen	Normal tissue				Cyst
	Rete testis	Efferent ductule	Head of epididymis	Tail of epididymis	
CD 10	+	+	+	+	+
CK 18	+	+	+	−	+
Vimentin	+	−	−	−	−
p63	−	+	+	+	−
Desmin (muscular layer)	−	+	+	+	−

+, positive; −, negative.

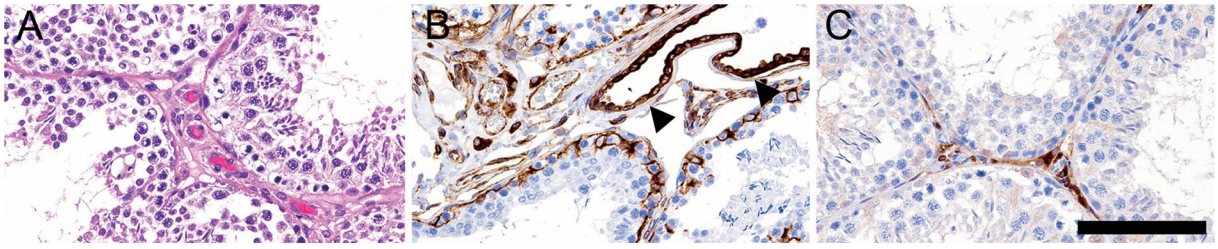

Fig. 3. (A–C) Seminiferous tubules of normal rabbit. (A) Normal spermatogenesis in the seminiferous tubules. (B) Sertoli cells, Leydig cells and the epithelial cells of rete testis (arrowheads) are positive for vimentin. (C) Leydig cells are positive for melan A, and the Sertoli cells are weakly positive for melan A. (A) HE. (B and C) Immunoperoxidase reaction with DAB chromogen and hematoxylin counterstain. Bar, 100 μm.

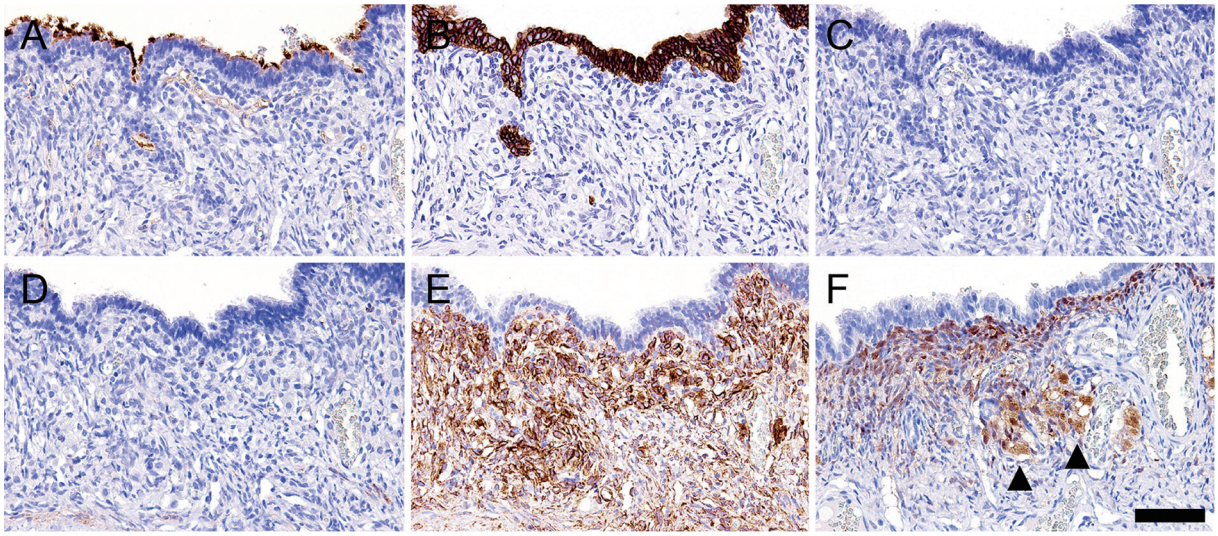

Fig. 4. (A–F) Immunohistochemistry of the cyst wall. (A) The apical surfaces of the epithelial cells are positive for CD 10. (B) The epithelial cells are positive for CK 18. (C) No p63-positive basal cells are seen in the epithelial layer. (D) There is no desmin-positive muscular layer beneath the epithelial layer. (E) Pleomorphic cells with a condensed nucleus and Leydig cells that surround the cyst wall are both positive for vimentin. (F) Leydig cells are positive for melan A (arrowheads), and the pleomorphic cells with a condensed nucleus are weakly positive for melan A. (A–F) Immunoperoxidase reaction with DAB chromogen and hematoxylin counterstain. Bar, 100 μm.

was observed surrounding the epithelial layer of the cyst wall (Fig. 4D). The clusters of cells beneath the epithelial layer were positive for vimentin, although the epithelial cells were negative (Fig. 4E). The Leydig cells observed in HE stained sections (Fig. 4E) were positive for melan A, and the pleomorphic cells with a condensed nucleus were weakly positive for melan A (Fig. 4F).

In the present study, we revealed the immunohistochemi-

cal characteristics of ducts in rabbit testis and epididymis (Table 2). As it has been demonstrated in human tissues, the epithelial cells from rete testis to epididymis express low-molecular weight CK (CK18) with decreased intensity of immune-staining in the distal part of the normal epididymis (Fig. 2B, 2F, 2J and 2N) and in the cryptorchid testis [2, 3, 7]. The efferent ductules and epididymal ductules possess basal cells and muscular layer surrounding the ductules that were positive for p63 (Fig. 2G, 2K and 2O) and desmin (Fig. 2H, 2L and 2P), respectively [12]. It has been reported that CD10 is expressed in the human rete testis and Wolffian-derived epithelium, but not in Müllerian-derived epithelium [9]. In the present case, the wall of the cyst was composed of monolayer cuboidal cells that were positive for CD 10 and CK 18, negative for p63 and vimentin and lacked desmin-positive muscular layer (Fig. 3A, 3B, 3C and 3D). These results correspond to the characteristics of rete testis, except for the negative staining of vimentin (Table 2). Vimentin expression in the human rete testis can be first identified at 12 weeks of fetal life [3]. In addition, vimentin expression diminishes in hyperplastic lesion of the rete testis [6]. In the present case, negative staining of vimentin in the epithelial cells of the cyst may be explained by its origin (id. est. remnant tissue of disordered testis development) and/or by hyperplastic proliferation of rete testis.

The testis arises from the gonadal ridge during embryogenesis, whereas the epididymis arises from the Wolffian duct. The testis is comprised of three segments: the sex cord (seminiferous tubules), the sex cord stromal cells (Leydig cells) and the connecting tubes (rete testis and efferent ductules). The seminiferous tubules are composed of Sertoli cells and germ cells that migrate from primitive sex cords and wall of yolk sac, respectively [15]. In the present case, small clusters of sex cord-like cells and Leydig cells were observed around the cyst wall, although the seminiferous tubule was not developed (Fig. 1E). As it has been demonstrated in the dog, Sertoli cells and Leydig cells in the rabbit testis were positive for vimentin and melan A (Fig. 3B and 3C), as well as the sex cord-like cells and Leydig cells around the cyst wall (Fig. 4E and 4F) [10]. In human, rete testis and primitive sex cords are developed in the 6th weeks of development, and in the 8th weeks, Leydig cells begin to secrete testosterone which influences the Wolffian ducts to differentiate into epididymis [1]. As for the present case of the rabbit, we conclude that dysplastic tissue of the left testis had developed cystic rete testis in its later life.

This is the first report to demonstrate cystic rete testis in a rabbit. The immunohistochemical analysis performed in the present study may also be applied to differentiating intratesticular cystic lesions in other animal species.

REFERENCES

1. Cerilli, L. A., Sotelo-Avila, C. and Mills, S. E. 2003. Glandular inclusions in inguinal hernia sacs: morphologic and immunohistochemical distinction from epididymis and vas deferens. *Am. J. Surg. Pathol.* **27**: 469–476. [Medline] [CrossRef]
2. De Miguel, M. P., Mariño, J. M., Gonzalez-Peramato, P., Nistal, M. and Regadera, J. 2001. Epididymal growth and differentiation are altered in human cryptorchidism. *J. Androl.* **22**: 212–225. [Medline]
3. Dinges, H. P., Zatloukal, K., Schmid, C., Mair, S. and Wirnsberger, G. 1991. Co-expression of cytokeratin and vimentin filaments in rete testis and epididymis. An immunohistochemical study. *Virchows Arch. A Pathol. Anat. Histopathol.* **418**: 119–127. [Medline] [CrossRef]
4. Dogra, V. S., Gottlieb, R. H., Rubens, D. J. and Liao, L. 2001. Benign intratesticular cystic lesions: US features. *Radiographics* **21**: S273–S281. [Medline] [CrossRef]
5. Gelberg, H. B. and McEntee, K. 1983. Cystic rete testis in a cat and fox. *Vet. Pathol.* **20**: 634–636. [Medline] [CrossRef]
6. Hartwick, R. W., Ro, J. Y., Srigley, J. R., Ordoñez, N. G. and Ayala, A. G. 1991. Adenomatous hyperplasia of the rete testis. A clinicopathologic study of nine cases. *Am. J. Surg. Pathol.* **15**: 350–357. [Medline] [CrossRef]
7. Kasper, M. and Stosiek, S. 1989. Immunohistochemical investigation of different cytokeratins and vimentin in the human epididymis from the fetal period up to adulthood. *Cell Tissue Res.* **257**: 661–664. [Medline] [CrossRef]
8. Kutzler, M. A., Shoemaker, M., Valentine, B. A. and Bildfell, R. J. 2006. Bilateral cystic rete testis in an alpaca (Lama pacos). *J. Vet. Diagn. Invest.* **18**: 303–306. [Medline] [CrossRef]
9. Ordi, J., Nogales, F. F., Palacin, A., Marquez, M., Pahisa, J., Vanrell, J. A. and Cardesa, A. 2001. Mesonephric adenocarcinoma of the uterine corpus: CD10 expression as evidence of mesonephric differentiation. *Am. J. Surg. Pathol.* **25**: 1540–1545. [Medline] [CrossRef]
10. Owston, M. A. and Ramos-Vara, J. A. 2007. Histologic and immunohistochemical characterization of a testicular mixed germ cell sex cord-stromal tumor and a leydig cell tumor in a dog. *Vet. Pathol.* **44**: 936–943. [Medline] [CrossRef]
11. Schumacher, J., Lenz, S. D. and Walker, W. 1994. Cystic rete testis associated with cryptorchidism in a horse. *Vet. Pathol.* **31**: 115–117. [Medline] [CrossRef]
12. Trainer, T. D. 2007. Testis and excretory duct system. pp. 943–963. *In*: Histology for Pathologists, 3rd ed. (Mills, S. E. ed.), Lippincott Williams and Wilkins, Philadelphia.
13. Wakui, S., Furusato, M., Nomura, Y., Iimori, M., Kano, Y., Aizawa, S. and Ushigome, S. 1992. Testicular epidermoid cyst and penile squamous cell carcinoma in a dog. *Vet. Pathol.* **29**: 543–545. [Medline] [CrossRef]
14. Wakui, S., Furusato, M., Yokoo, K. and Ushigome, S. 1997. Testicular efferent ductule cyst of a dog. *Vet. Pathol.* **34**: 230–232. [Medline] [CrossRef]
15. Woodward, P. J., Sohaey, R., O'Donoghue, M. J. and Green, D. E. 2002. From the archives of the AFIP: tumors and tumorlike lesions of the testis: radiologic-pathologic correlation. *Radiographics* **22**: 189–216. [Medline] [CrossRef]