

FULL PAPER Pathology

Immunohistochemical Evaluation of Canine Ovarian Cysts

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ABSTRACT. To clarify the immunohistochemical characteristics of canine ovarian cysts, 109 canine ovarian cysts (57 cysts of subsurface epithelial structures: SES, 26 graafian follicle cysts, 12 cystic rete ovarii and 14 cysts difficult to classify morphologically) were examined regarding their lining cells immunohistochemically using antibodies against placental alkaline phosphatase (PLAP), S100, inhibin α , desmin and AE1/AE3. Both cysts of SES and cystic rete ovarii had a positive immunoreaction to desmin and AE1/AE3, whereas all cysts all but graafian follicle cysts were negative for inhibin α . PLAP-positive immunoreaction was observed only in cysts of SES. Graafian follicle cysts had a positive immunoreaction to inhibin α , but were negative for PLAP, desmin and AE1/AE3. Fourteen cysts were difficult to classify morphologically because these cysts had single-squamous lining cells and lacked other morphological characteristics. However, these unclassified cysts were immunohistochemically divided into two groups, including positive and negative cysts, by the reactivity of PLAP. The PLAP-positive cysts were considered large cysts of SES. These results suggest that PLAP was a useful marker for classification of cysts of SES, although cysts originating from SES are not always positive for this antigen.

KEY WORDS: canine, immunohistochemistry, ovarian cyst.

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Cysts from various structures occur in and around the canine ovary [1, 4]. In animals, cysts that occur in the ovary include cysts of subsurface epithelial structures (SES), graafian follicle cysts and cystic rete ovarii [9, 10]. Cysts that occur around the ovary include the cysts coming from remnant of Wolffian duct or Mullerian duct [9, 10]. In the cysts of SES, the connection with the ovarian surface is clear, and from the strong cytokeratin-positive image of the cells lining the cysts, they are distinguishable from follicular cysts with no cytokeratin positivity [9]. Graafian follicle cysts are lined by a granulosa cell layer [1], while cystic rete ovarii are lined by a single cuboidal epithelium and occur in an ovary hilar region [9]. Cystic rete ovarii are distinguished from 'cysts around the ovary' because they have no smooth muscle [9]. However, it may be difficult to determine the origin of a cyst in many cases. Since expanded cysts lose their peculiar structure under pressure, the location of the cyst often becomes unclear [5, 12]. In addition, the cells lining cysts lose their original shape with the expansion of the cysts. There are few reports of immunostaining to distinguish a kind of ovarian cyst in other animals [2, 9]. In the present study, we immunohistochemically examined 109 ovarian cysts, including cysts of SES, graafian follicle cysts, cystic rete ovarii and histologically unclassified cysts in dogs.

MATERIALS AND METHODS

We studied 109 ovarian cysts from 106 canine ovaries which had been surgically removed. Specimens were fixed in 10% buffered formalin for histopathological and immunohistochemical studies. They were trimmed, embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin (HE). All cysts were examined histologically, and classified based on histomorphology. Contiguous sections were immunohistochemically examined by the avidin-biotin-peroxidase complex (ABC) procedure (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, U.S.A.). Primary antibodies used in this study were listed in Table 1. Endogenous peroxidase was blocked with 0.3% H₂O₂ in methanol for 10 min. All sections were incubated with primary antibody at 4°C for 16 hr, with biotinized secondary antibody for 30 min at room temperature, and with avidin-peroxidase conjugate for 30 min. Staining was developed in 0.05% 3,3'-diaminobenzidine solution. Ovaries from 5 dogs with no gross lesions were also used as a control. As a negative control, a section without primary antibody was investigated the same way.

The immunohistochemical results were expressed semi-quantitatively as follows: -, negative; +, 1-50% positive cells; ++, >50% positive cells.

RESULTS

Total 109 cysts were used and categorized as cysts of SES (n=57) (Fig. 1a), graafian follicle cysts (n=26) (Fig. 1b), cystic rete ovarii (n=12) (Fig. 1c) and cysts difficult to clas-

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Table 1. List of antibodies used

Target antigen	Type of antibody	Pretreatment	Dilution (1 in)	Supplier
PLAP ^{a)}	mAb ^{c)}	MW ^{e)}	50	Dakopatts, Glostrup, Denmark
S100	pAb ^{d)}	MW	2000	Dakopatts
inhibin α	mAb	MW	50	Serotec Ltd, Oxford, UK
desmin	mAb	MW	50	Progen Biotech, Heiderberg
CK ^{b)} (AE1/AE3)	mAb	MW	prediluted	Nichirei, Tokyo, Japan

a) PLAP, placental alkaline phosphatase. b) CK, cytokeratin. c) mAb, monoclonal antibody. d) pAb, polyclonal antibody. e) MW, microwave.

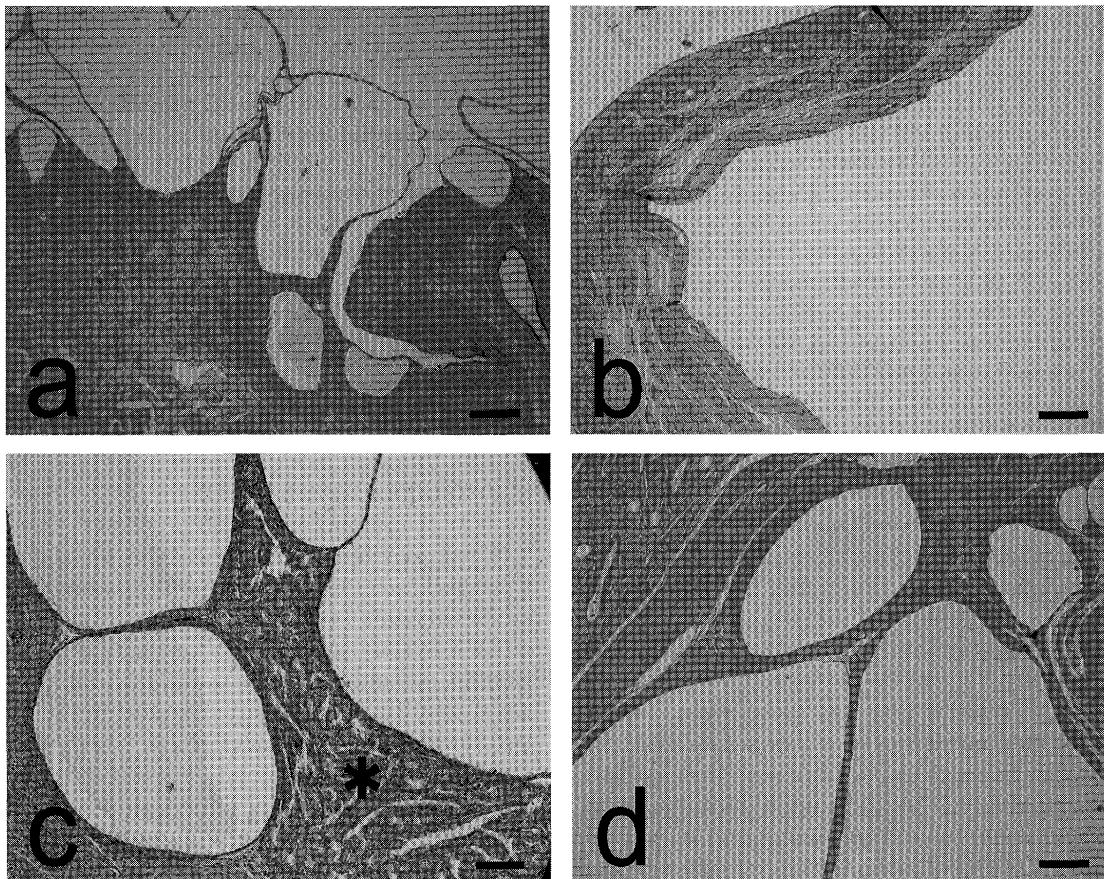


Fig. 1. Low magnification view of cysts of subsurface epithelial structures (SES). a) They were formed at shallow cortex which closely approached surface epithelium. b) Graafian follicle cyst, formed in deep cortex, is lined by granulosa cells. c) Cystic rete ovarii, surrounded with rete ovarii (asterisk). d) Cysts difficult to classify, surrounded with a collagen and smooth muscle. Hematoxylin and eosin (HE) stain. Bars= 100 μ m.

sify (n=14) (Fig. 1d) based on histological findings.

The results of the immunohistochemical study in limited structures of normal canine ovaries are summarized in Table 2. A placental alkaline phosphatase (PLAP)-positive immunoreaction was observed only in SES consisting of surface epithelium and cortical tubules. S100-positive immunoreaction was observed only in rete ovarii. Inhibin α -positive immunoreaction was observed only in the granulosa cell layer. The results of the immunohistochemical study of canine ovarian cysts are summarized in Table 3.

Cysts of SES: Most of these cysts had the simple-cuboidal lining cells (Fig. 2a). Up to 96% of the cysts had a positive immunoreaction to desmin in a lining cell. The rate of positive

immunoreaction of AE1/AE3 was 93%, and that of PLAP was 19% (Fig. 2b). Immunoreaction for S100 included positive (28%) and negative (72%). In inhibin α , all lining cells of all cysts were negative.

Graafian follicle cysts: All of these cysts had a lining of granulosa cells (Fig. 3a). All lining cells of all cysts had a positive immunoreaction to inhibin α (Fig. 3b). Positive or negative immunoreaction for S100 was observed at the same percentage. For PLAP, desmin and AE1/AE3, all lining cells of all cysts were negative.

Cystic rete ovarii: All of these cysts included the simple-cuboidal lining cells. All lining cells of all cysts had a positive immunoreaction to AE1/AE3. The rate of positive

Table 2. Immunohistochemical results in normal ovaries

Site	Target antigen				
	PLAP ^{e)}	S100	Inhibin α	desmin	AE1/AE3
SE ^{a)}	++ ^{f)}	-	-	++	++
CT ^{b)}	- ~ +	-	-	+ ~ ++	++
GCL ^{c)}	-	-	++	-	-
Rete ^{d)}	-	++	-	+ ~ ++	+ ~ ++

a) SE, surface epithelium. b) CT, cortical tubules. c) GCL, granulose cell layer. d) Rete, rete ovarii. e) PLAP, placental alkaline phosphatase. f) ++, >50% positive cells; +, 1-50% positive cells; -, Negative.

Table 3. Immunohistochemical results in ovarian cysts

Origin	Target antigen														
	PLAP			S100			Inhibin α			desmin			AE1/AE3		
	++ ^{e)}	+	-	++	+	-	++	+	-	++	+	-	++	+	-
SES ^{a)} (n=57)	0	11	46	3	13	41	0	0	57	35	20	2	46	7	4
GF ^{b)} (n=26)	0	0	26	2	11	13	14	12	0	0	0	26	0	0	26
RO ^{c)} (n=12)	0	0	12	1	2	9	0	0	12	3	7	2	7	5	0
DC ^{d)} (n=14)	0	2	12	0	7	7	0	0	14	4	10	0	12	2	0
Total (n=109)															

a) SES, subsurface epithelial structures. b) GF, graafian follicle. c) RO, rete ovarii. d) DC, difficult to classify. e) ++, >50% positive cells; +, 1-50% positive cells; -, Negative.

immunoreaction to desmin was 83%. Immunoreaction for S100 included positive (25%) and negative (75%). For PLAP and inhibin α , all lining cells of all cysts were negative.

Cysts difficult to classify: All of these cysts included the simple-rarely cuboidal lining cells (Fig. 4a). All lining cells of all cysts had a positive immunoreaction to desmin and AE1/AE3. The rate of positive immunoreaction to PLAP was 14% (Fig. 4b). Positive or negative immunoreaction for S100 was observed at the same percentage. For inhibin α , all lining cells of all cysts were negative.

DISCUSSION

In the present study, we examined 109 canine ovarian cysts and indicated their immunohistochemical characteristics. The cysts of SES characteristically indicated a PLAP-positive immunoreaction. Immunohistochemical studies of ovarian epithelial neoplastic tissue [11, 15] and examination of patient serum [11] have been reported, but the present results also indicated that PLAP is a useful marker which recognizes cysts of SES. PLAP positive tissues include syncytiotrophoblast of the placenta, an endocervix and a fallopian tube [7, 15]. PLAP is thought as hydrolytic enzyme of villus of these cells or hormonal regulator in these organizations. PLAP suggest various works, and a further study is needed for the interpretation about significance of a PLAP positive cell observed in the present study. In normal ovary, the PLAP positive immunoreaction rate was more than 50% in surface epithelium, and 1-50% in cortical tubules, which may indicate specificity in these cells. In the present study, cysts were immunohistochemically divided into two groups,

including positive and negative cysts, by the reactivity to PLAP. At least PLAP positive cysts may reflect this specificity.

Graafian follicle cysts indicated a positive immunoreaction to inhibin α , and a negative immunoreaction to AE1/AE3 and desmin. This result was in contrast to cysts of other kinds.

In cystic rete ovarii, histological and immunohistochemical results of lining cells resembled the results for cysts of SES. In normal canine ovary, an S100-positive immunoreaction at rete ovarii was indicated. However, cystic rete ovarii was not always S100-positive, and an S100-positive immunoreaction was observed in various kinds of cysts. From these findings, when ovarian rete was not observed in the vicinity, its differentiation proved impossible.

It was impossible to distinguish the cysts considered difficult to classify without clear histological information such as cyst location or the shape of their lining cells. Immunohistochemical results showed that AE1/AE3 and desmin were both positive but inhibin α was negative. Given this finding, there is little possibility that follicular cysts were among those difficult-to-classify. Cysts needing differentiation included those of SES, cystic rete ovarii, and rare cysts coming from the remnant of a Wolffian or Mullerian duct [9, 10, 14]. One of the histological characteristic findings of cysts around the ovary is the smooth muscle around them [9]. In the present study, we saw some cysts surrounded by smooth muscle. Epoophoron, said to be a remnant of Wolffian duct, exists in mesovarium rich in smooth muscle. However, part of epoophoron was also said to connect with the ovarian rete [3]. Therefore, clear distinction between a part of cystic rete ovarii and a cyst of wolfman duct was

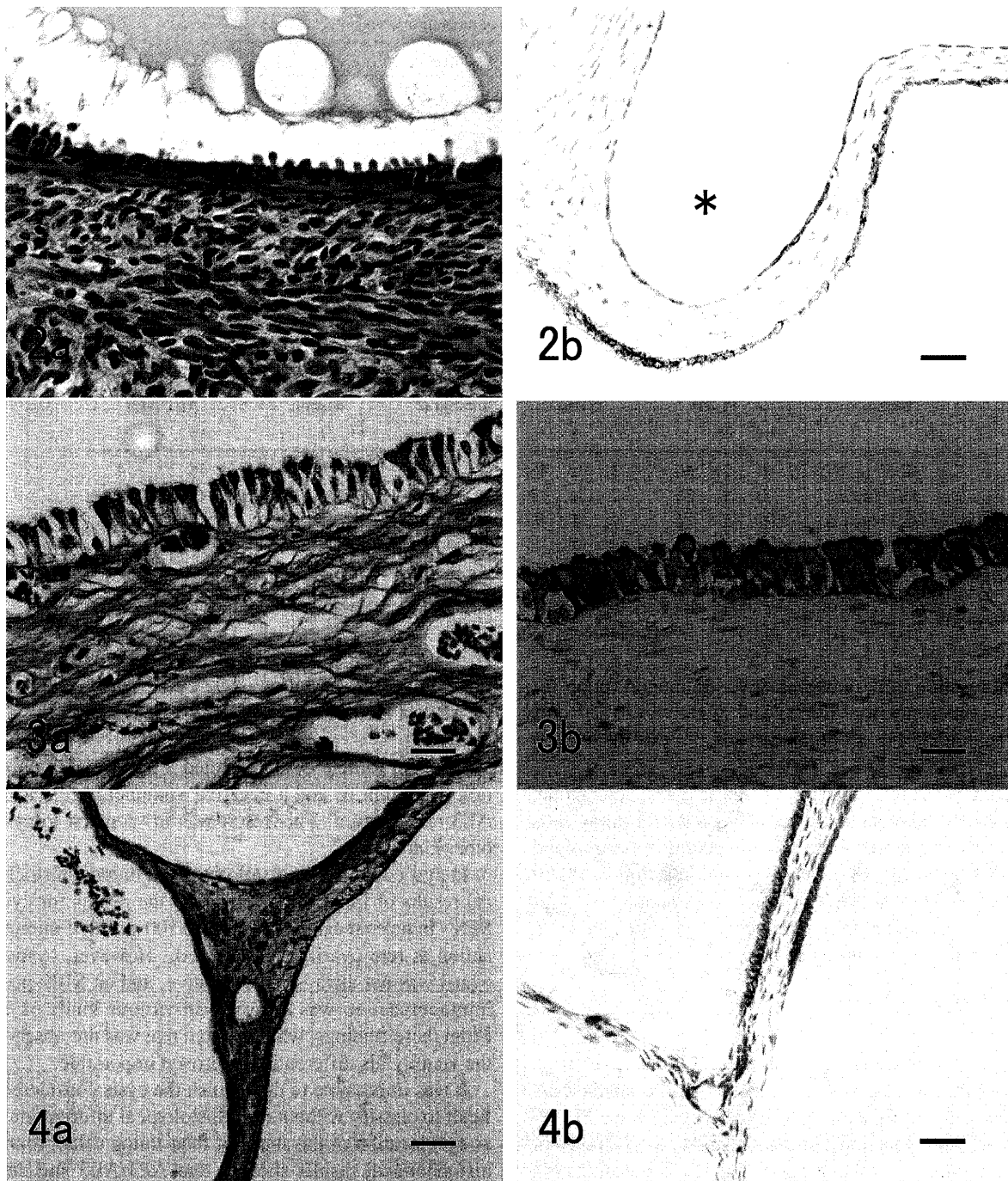


Fig. 2. a) Cysts of SES are lined by simple-cuboidal to columnar epithelium. HE stain. Bar=20 μ m. b) Part of lining cells show positive immunoreaction to placental alkaline phosphatase (PLAP). An asterisk shows the inside. Avidin-biotin-peroxidase complex (ABC) stain with hematoxylin counter stained. Bar=30 μ m.

Fig. 3. a) Graafian follicle cyst, lined by granulose cells. HE stain. b) Lining cells show positive immunoreaction to inhibin α . ABC stained with hematoxylin counter stain. Bars=20 μ m.

Fig. 4. a) Cysts difficult to classify are lined by simple-squamous or partially cuboidal epithelium. HE stain. b) Part of lining cells show positive immunoreaction to PLAP. ABC stained with hematoxylin counter stain. Bars=30 μ m.

impossible. On the other hand, Mullerian duct cysts, exist apart from ovarian parenchymal, so differentiation was possible by confirming the position by histology.

Furthermore, cells having a PLAP-positive immunoreac-

tion were also observed. From the foregoing, it appeared very likely that cysts of SES were among those difficult to classify. Besides the conventional view as to their origin in cystic rete ovarii [8, 9, 10, 13], we considered it very likely

that SES cysts fall into this category.

In conclusion, the immunohistochemical characteristics of canine ovarian cysts were demonstrated by the present results. Desmin was shown to have characteristic immunoreactivity for the differentiation of a graafian follicle cyst besides the earlier-reported AE1/AE3 and inhibin α . In addition, since the lining cells of SES and cystic rete ovarii are similar, the cell morphology is not enough to distinguish them. However, immunohistochemically, PLAP indicate a characteristic immunoreactivity for cysts of SES, which made differentiation possible. Although further examination is needed about the significance of PLAP positivity [6, 7], the possibility that cysts of SES were among those difficult to classify was indicated by immunohistochemistry using PLAP antibody, as well as the view that the origin of cysts difficult to classify was cystic rete ovarii.

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