2. CHAPTER Ⅰ

Malignant Mesothelioma and Granulosa Cell Tumor in a Japanese-Black Cow

2.1 *INTRODUCTION*

Mesothelioma is a rare mesothermal neoplasm that arises from the mesothelial cells lining the serous of the pericardial, pleural and peritoneal cavities [5,12,22,30,31,36, 52,54,60,62]. In domestic animals, mesothelioma has been reported most frequently in cattle because they are known to arise as a congenital tumor in fetal or young cattle [22,31, 36]. However, the incidence of the tumor might be relatively low in other animal species such as dogs [22, 36]. In medical fields, Wagner et al. [60] had been proposed the close relationship between the histories of asbestos exposure and the incidence of mesotheliomas [42], while such epidemiological evidences of the tumors in domestic animals still remain unclear [12, 22,36].

On the other hand, ovary granulosa cell tumor is one of the most common tumors in cattle and they have tended to appear unilateral and usually non-malignant tumors. These biological features of the tumors are most likely consistent in any species [22,31, 36]. Granulosa · cell tumors sometimes show intra-peritoneal metastasis resulting multiple serous seeding of the tumors. Pathologically, these features sometimes make it difficult to give complete diagnosis for the tumor and as the differential diagnoses, metastastic carcinomas from the stomach, intestines, and adrenal glands, and mesotheliomas might be possible.

Although both mesothelioma and granulosa tumors can occur in calves forming multiple tumors, malignant mesothelioma also frequently involved the pleural cavities and

their morphological features were somewhat different from those of the granulosa cell tumors. The present paper describes pathological features of a Japanese black cattle involved in both malignant mesothelioma and ovarian granulosa cell tumor. The different pathological features of these tumors are also discussed.

2.2 *MATERIALS AND METHODS*

Histopathology: Tissue samples of the pleural and left ovarian masses, diaphragm, lung, heart, liver, spleen, kidney, adrenal, premediastinal lymph nodes, and right ovary were fixed with 10% neutral buffered formalin, and embedded in paraffin. Paraffin sections of 4 μm, were stained with hematoxylin and eosin (HE), Periodic acid-Schiff (PAS), Alcian blue (pH 2.5), and Toluidin blue (pH 2.5 and 7.0, respectively), and Azan methods.

Immunohistochemistry: Immunostaining was carried out by the labelled streptavidin-biotin (LSAB) method using a kit (Dako, Carpinteria, CA, USA.). The primary antibodies were rabbit sera against Cytokeratin (prediluted, Dako), monoclonal antibodies for vimentin (1:20, Dako), carcino-enbryonal antigens (CEA, prediluted, Dako). The secondary antibodies were biotinylated goat anti-sera against rabbit or mouse immunoglobulins (prediluted, Dako). The reaction products were visualized using 3,3'-diaminobenzidine (Sigma, St. Louis, MO, USA) counter-stained with Mayer's hematoxylin.

Tissue culture: The tumor tissue was minced, and digested with 4 mg/ml collagenase (232 U/mg, Wako, Tokyo, Japan) in Dulbecco's modified Eagle's medium (DMEM, Sigma) and Ham's nutrient mixture F-12 (Sigma) containing 10% fetal calf serum (FCS), 100 IU/ml penicillin, and 100 μg/ml streptomycin, for 6 hours at 37℃ in a humidified atmosphere of 5% carbon dioxide in air. The digested tissue was filtered

through nylon mesh cloth (80 μm), centrifuged at 1,000 rpm for 10 min and cultured according to Hiratsuka *et al.* [19]. Coverslips with cultured tumor cells were washed in PBS, fixed in cold acetone, and stored at -20℃ for 30 min. The cells were incubated with one or other of the primary antibodies overnight at 4℃. The reacted antibodies were visualized by the LSAB method.

2.3 *RESIULTS*

Gross findings: A 3-year-old female JBC, weighing about 650 kg, was slaughtered at Miyakonojo Meat Inspection Office. No clinical symptoms were noted by the general physical examinations. Grossly, multiple pleural masses, about 1 to 5 cm in diameter, from gray-white to yellow colored nodules, were observed (Fig.1). Some superficial nodules were also found in the superficial parts of the lungs and diaphragm. Cut surface of the pleural mass were grayish-white (Fig.2). They were consisted of hard tissues like fibrous peduncles. The premediastinal lymph nodes were grossly intact. There were no neoplastic nodules in the parenchyma of the lungs and diagram. The left ovary was enlarged to approximately $20 \times 15 \times 10$ cm in size, and its surface was reddish-white and coarsely nodular. Cut surface of the ovary mass was dark reddishbrown, serous fluid lucent and their walls were fibrous and hard with hemorrhage and necrosis (Fig.12). The right ovary and the others samples were grossly intact.

Histopathological findings: Histopathological examinations of the tumors of the pleura, diaphragm, and lungs revealed the wide-dissemination of the neoplastic lesions. The neoplastic cells proliferated at the superficial layer of the serosa, and some neoplastic foci were hard to distinguish from hyperplastic lesions of normal mesothelial cells. The histological features of the serosa were almost consistent to those in tubular pattern of the epithelial form of mesotheliomas. Most parts of the pleural tumor exhibited the lesions

of mesotheliomas of the epithelial type with tubular and papillary patterns (Fig.3). The neoplastic cells were cuboidal or round in shape with moderate cellular atypia and pleomorphism (Fig.4). The neoplastic cells had moderate amount of eosinophilic cytoplasm and round to ovoid nuclei with the defined chromatin and a distinct nucleolus. Mitotic figures of the neoplastic cells were less frequent. The cytoplasm of the neoplastic cells was positively stained with toluidn blue (pH 7.0) (Fig.5) and negative for that (pH2.5), alucian blue and PAS (Fig.6), while those in stamp specimens were stained by PAS. Metastastic lesions of the neoplastic cells were found in the premediastinal lymph nodes. In the trabecular sins, there were a small number of cell clusters consisting of abundant collagen fibers and flattened neoplastic cells. Some clusters composed of less collagen fibers and cuboidal neoplastic cells.

Besides, the left ovary was entirely replaced by the proliferation of neoplastic cells forming insular and/or follicular structures mimicking the Graafian follicle. The follicular structures sometimes contained a small amount of clear to eosinophilic fluid mimicking Call-Exner bodies that occasionally found in granulosa cell tumors (Fig.12). The tumor cells in the left ovary are round or ovoid and resemble normal follicular cells (Fig.13), and the nuclei are round or ovoid and hyperchromatic. Mitotic figures were relatively frequent as compared to those in the pleural tumors. The cytoplasm contained PAS and toluidin blue (pH 7.0 and pH 2.5)-positive materials (Fig.14).

Immunohistochemical features: Immunohistochemically, the neoplastic cells of the pleural tumors were intensely positive for cytokeratin (Fig.7), and were negative for vimentin and CEA. Although, the cultured cells of the pleural tumor were proliferated in bundles or fascicular patterns to form the meshwork architecture (Fig.8), those were positive for cytokeratin (Fig.9) and vimentin, and were negative for CEA (Fig.11). On the other hands, the neoplastic cells from ovarian tumor were positive for vimentin, and

were negative for cytokeratin and CEA (Fig.15). The results of several special-stainings and immunohistochemical-stainings of pleural and ovarian tumors were summarized in Table 1.

2.4 *DISCUSSION*

Based on the gross and histopathological findings, the present case was considered as to be involved in diffuse malignant mesothelioma of the pleural cavities and concurrent ovarian granulosa cell tumor. The morphological features of both tumors had their own specific features, respectively. The immunohistochemical and special staining results of these tumors might reflect the different natures of these tumors. There are a few possibilities that the multiple tumors in the pleural cavities appeared as the metastastic lesions of the ovarian granulosa cell tumor. However, the different morphological features may support our diagnosis for the present case.

The definite relationship between the asbestos exposure and the occurrence of mesothelioma has been established epidemiologically and experimentally [5,30,31,36], but the mechanisms of tumoregenesis associated to asbestos still remain unclear [42], especially in domestic animals [12,22,36]. In the present case, asbestos fibers were never found in the neoplastic lesions of malignant mesothelioma or the respiratory systems by routine histological examinations. In humans, malignant mesothelioma is a rare tumor, approximately 0.34-3.5 per million per year in frequency [62]. It has been estimated that the incidence of this tumor is one to two per million in the general population, although among the populations exposed occupationally to asbestos, the incidence has been increasing at a high rate [52].

In cattle, this tumor is also rare, approximately 0.22-1.4% incidence in bovine tumors [5,34]. In our experiences, tumors were detected in 328 of 139,556 of

slaughtered cattle examined during 1974-1994 at Miyakonojo Meat Inspection Office in Miyazaki prefecture (Table 2). Mesotheliomas of 60 cases (18.3%) that was approximately 4.3 per 10 thousands were observed most frequently as the previous examinations by Miyazaki University that 10 cases (18.2%) of 55 tumors in the cattle were mesotheliomas [44]. The incidence of mesotheliomas in cattle tented to be high at Southern Kyushu, especially around Miyakonojo city. In this area, many small farmers of Japanese black cow were usually used "Shirasu" for calves instead of sawdusts. The chemical composition of "Shirasu" which is volcanic ashes very likes asbestos in a main component of SiO_2 [60]. This fact may indicate the possibility of carcinogenicity of mesothelioma by "Shirasu".

On the other hand, although multiple primary tumors may be related to hereditary or iatrogenic diseases in man [25], and those are rare in cattle. The mechanisms of the occurrence of multiple primary tumors are still unclear. In our examination, highly, susceptible tumors were in the order of bovine mesothelioma and ovarian granulosa cell tumor in Miyakonojo and surrounding areas. Therefore, it is assumed that this case of multiple primary tumors is almost spontaneous generation with coincidence.

2.5 *ABSTRACT*

A 3-year-old female Japanese black cow had malignant mesothelioma on the pleura and granulosa cell tumor of the left ovary. The pleural surface was disseminated with numerous small neoplastic nodules, and the tumor cells were immunohistochemically positive for cytokeratin and negative for vimentin and CEA, while cultured epithelioid cells derived from the tumor were positive for vimentin. The left ovary was entirely replaced by the proliferation of neoplastic cells with insular and follicular patterns, and the neoplastic cell clusters with some inter cellular spaces were separated by connective tissue septa. The tumor cells were positive for vimentin and negative for cytokeratin and CEA.

KEY WORDS : granulosa cell tumor, mesothelioma, multiple tumor.

Table 1. The results of special staining for the tumors

Staining for		Pleura	Ovary
Perioidic acid-Schiff		- (+)	+
Perioidic acid-Schiff(stamp)		+	N D
Toluidin Blue	(pH2.5)	-	-
Toluidin Blue	(pH7.0)	+	-
Alcian blue		-	-
【Immunohistochemistry】			
Cytokeratin (Prediluted, Dako)		+	-
Vimentin	(1:20, Dako)	- (+)	+
CEA	(Prediluted, Dako)	-	-
【Cultured Immunohistochemistry】			
Cytokeratin (Prediluted, Dako)		+	N D
Vimentin	(1:20, Dako)	+	N D
CEA	(Prediluted, Dako)	-	N D

+ : Positive - : Negative N D : Not done ( ) : Stroma

Table 2. Incidence of tumors among spesies of cattle durig 21 years

Type of tumor	cases	Type of tumor	cases	Type of tumor	cases
Hematopoietic system	***44***	***Digestive system***	***56***	***Genital system***	***60***
lymphoma	41	hepatoma	18	malignant granulosa cell tumor	8
(adult type)	⑰	(hepatocellulor carcinoma)	⑪	granulosa cell tumor	40
(thymic type)	③	(cholangiocarcinoma)	④	leiomyoma of the uterus	6
(lymphosarucoma)	⑤	liver papilloma	1	leiomyosarcoma of the uterus	2
(reticulosarcoma)	⑥	liver cell adnoma	11	squmous cell tumor	2
hyperplasia of the spleen	3	hemangioma	2	adenoma, adenocarcinoma	2
		fibrosarcoma	1		
Cardiovascular system	***1***	fibroma	1	***Skeletal system***	***6***
aotic body tumor	1	squmous cell tumor	1	rhabdomyosarcoma	3
		papilloma of the gallbladder	4	rhabdomyoma	1
Respiratory system	***21***	myxoma	1	schwannoma	1
lung cancer	19	intestinal adenoma	4	fibroma	1
(squmous cell tumor)	⑥	papilloma of the stomach	1		
(epithelioid sarcoma)	①	stomac hyperplastic polyp	3	***Others***	***121***
fibrosarcoma	2	hyperplasia of the pancreas	4	malignant mesothelioma	54
		pancreatic carcinoma	1	mesothelioma	6
Cutaneous system	***12***	adenocarcinoma	3	adrenocortical adenoma	10
malignant melanoma	2			pheochromocytoma	1
melanoma	2			cancer of the adrenal gland	1
fibrosarcoma	3	***Urinary system***	*7*	thyroid adenoma	4
fiborma	2	nephrobalstoma	3	fibrosarcoma	1
lipoma	1	renal carcinoma	1	ameloblastoma	1
squamous cell carcinoma	1	papilloma of the urinary bladder	2	mesenchymal chondrosarcoma	1
mixed tumor	1	rhabdomyosarcoma	1	adenocarcinoma	6
				unknown	36

○ include cases

Fig.1

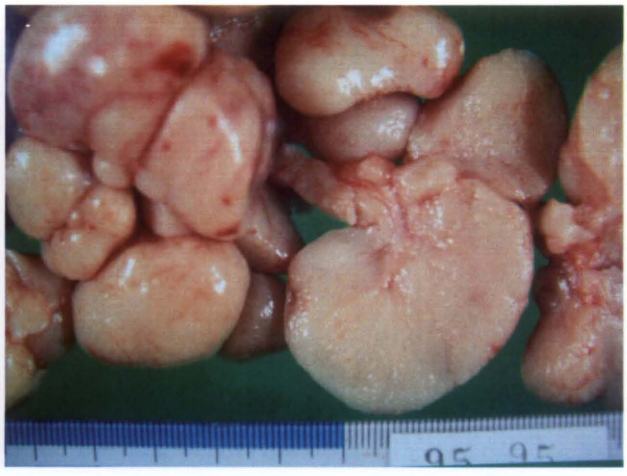

Fig.2

Fig.1 The masses of pleura were multiple about 1 to 5 cm in diameter from gray-white to yellow colored nodules which were consisted of hard tissues like fibrous peduncles.

Fig.2 Cut surface of the mass were grayish-white.

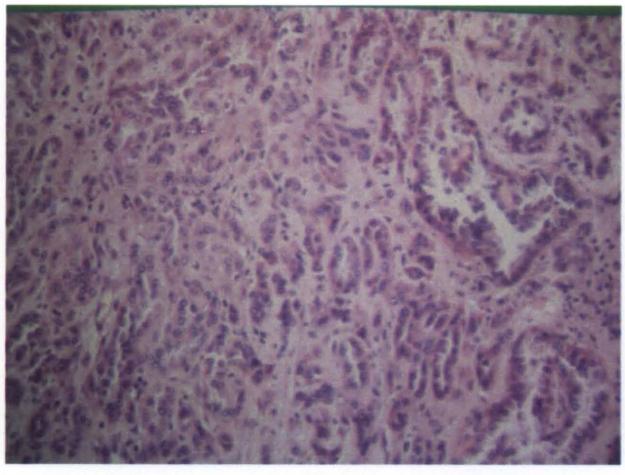

Fig.3

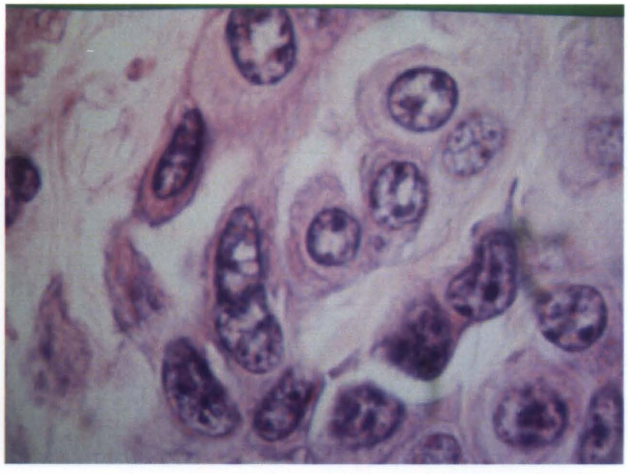

Fig.4

Fig.3 The pleura tumor cells showing tubular pattern of the epithelial form. HE stain. × 200.

Fig.4 The tumor cells were cuboidal or round in shape with moderate cellular atypia and pleomorphism. The round to ovoid nuclei had the defined chromatin and a distinct nucleolus. HE stain. × 1000.

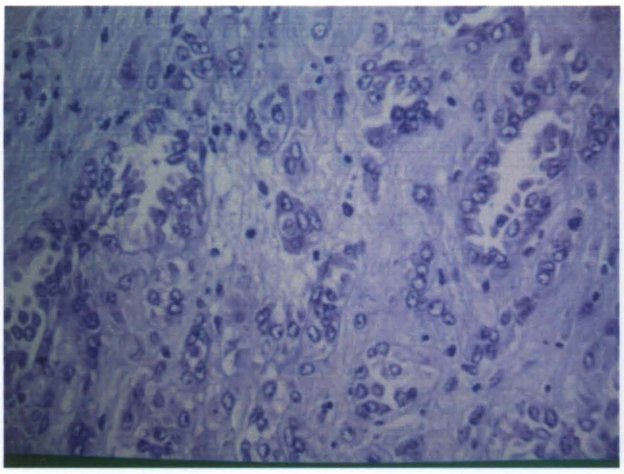

Fig.5

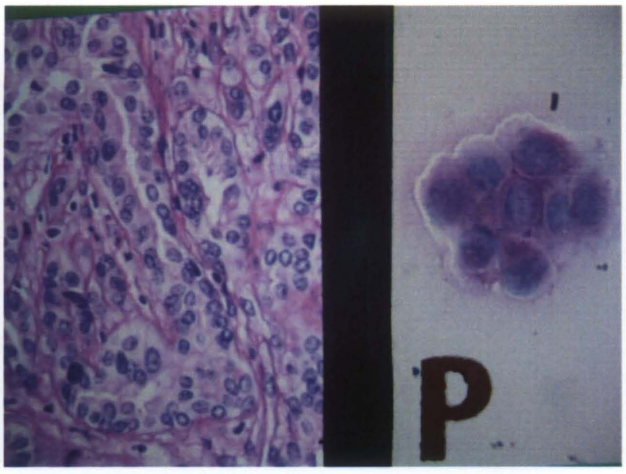

Fig.6

Fig.5 The cytoplasm of the neoplastic cells was positively stained with toluidin blue (pH 7.0). Toluidin blue stain. ×400.

Fig.6 The neoplastic cells negative for that (pH2.5), alucian blue and PAS, while those in stamp specimens (P) were stained by PAS. PAS stain ×400. ×1000.

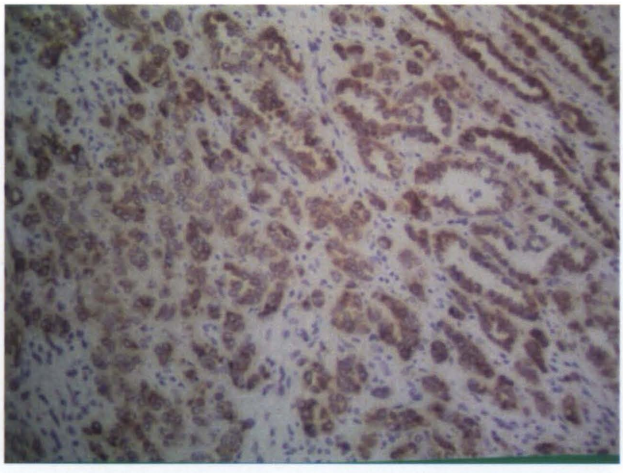

Fig.7

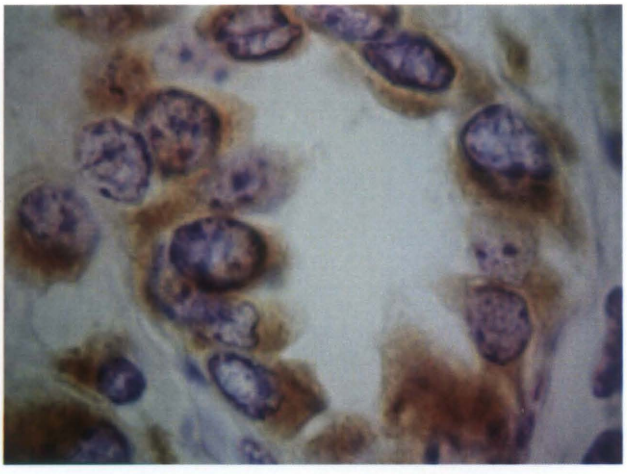

Fig.8

Fig.7 and Fig.8 The masses of pleura tumor showing tubular pattern of the epithelial form. Immunohistochemical staining for Cytokeratin. Note the stronger staining pattern in the epithelial form. Cytoketatin immunohistochemistry ×200. ×1000.

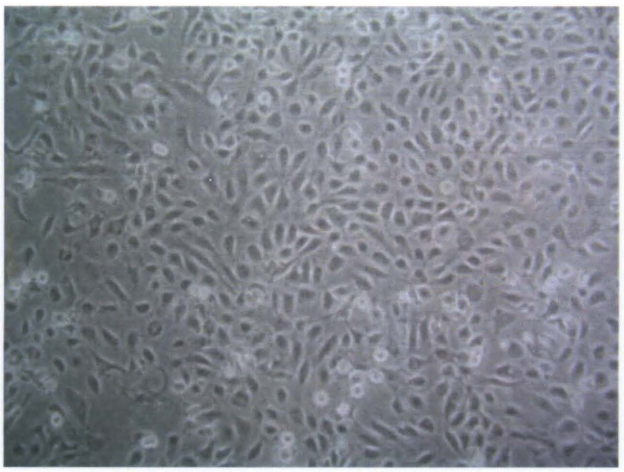

Fig.9.

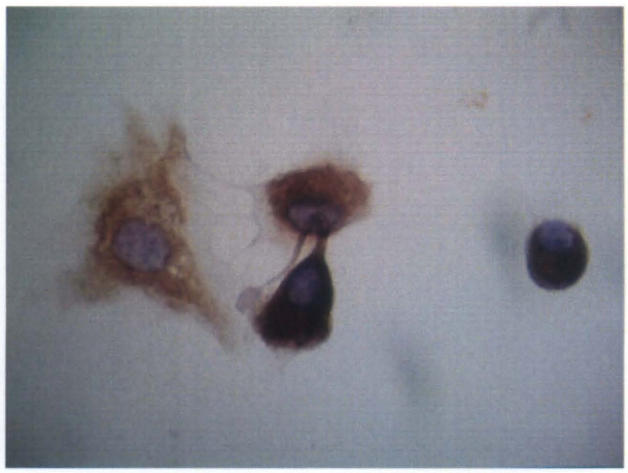

Fig.10.

Fig.9 The cultured cells were proliferated in bundles or fascicular patterns to form a meshwork architecture. Phase-contrast microscopy. × 126.

Fig.10 The cultured cells showing positive reaction for antibody against cytokeratin positive. Cytokeratin immunohistochemistry. × 1000.

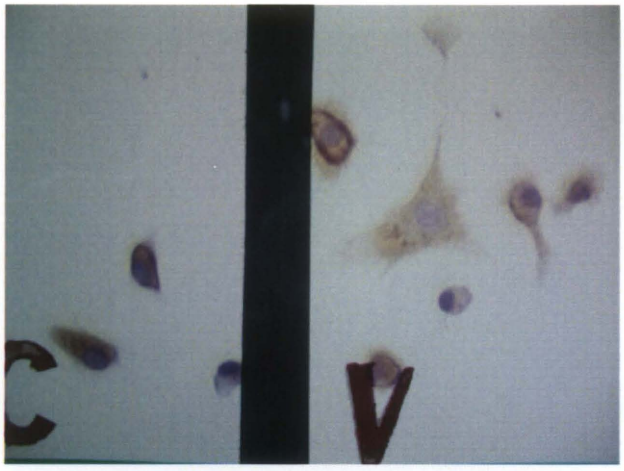

Fig.11

Fig.11 The cultured cells showing positive reaction for antibody against cytokeratin (C) and vimentin (V), and were negative for CEA. Immunohistochemistry. × 400.

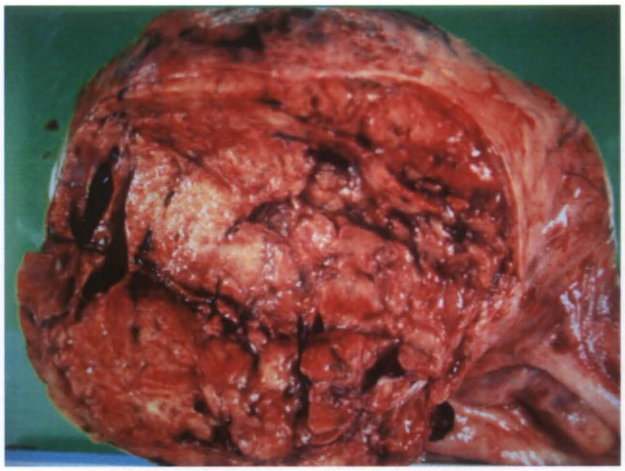

Fig.12

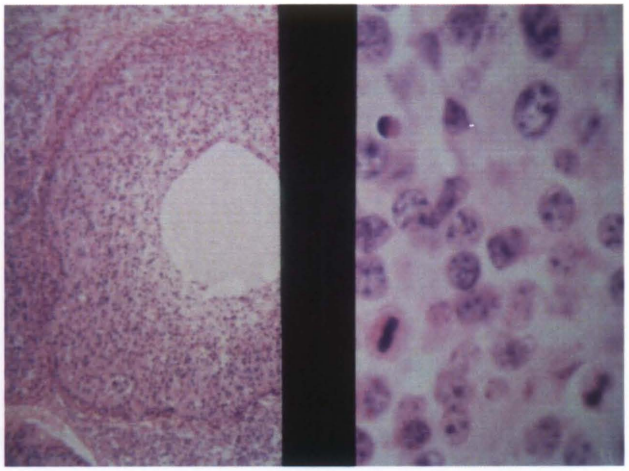

Fig.13

Fig.12 The left ovary was enlarged to approximately 15 cm in diameter the surface and cut surface was reddish-white and coarsely nodular.

Fig.13 The neoplastic cells were separated by connective tissue septa and folliclelike structure with Call-Exer body was seen in center. H.E. stain ×40. The tumor cells showing round or ovoid and and the nuclei showing round ovoid, and hyperchromatin. HE stain. ×400.

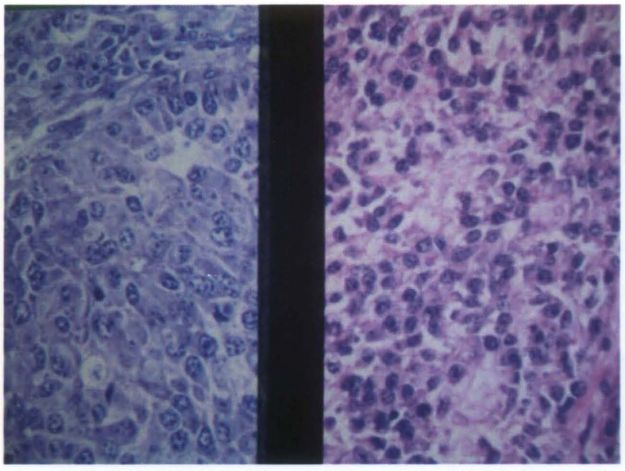

Fig.14

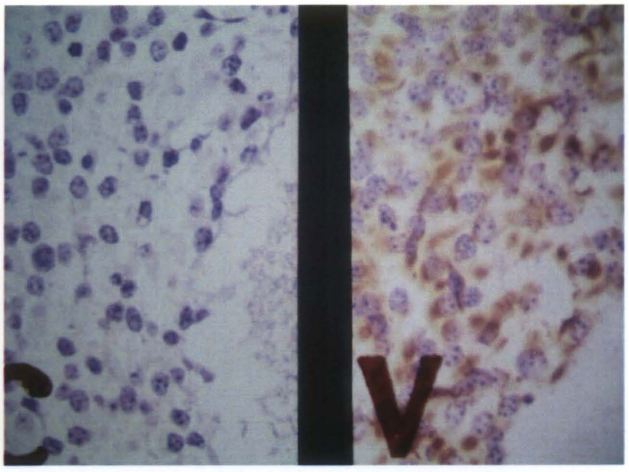

Fig.15

Fig.14 The ovarian tumor cytoplasm contained PAS(×200) and toluidin blue (pH 7.0 and pH 2.5 ×200)

Fig.15 The cells from ovarian tumor positive for vimentin (V) , and negative for cytokeratin immunohistochemistry (C). ×200.