Case Report

# A spontaneously occurring malignant ovarian Sertoli cell tumor in a young Sprague Dawley rat

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**Abstract:** Primary ovarian tumors are generally uncommon in rats used in toxicologic studies. A malignant Sertoli cell tumor was present in the ovary of a 19-week-old female Sprague Dawley rat. Macroscopically, the mass was white and firm, 10 × 13 × 17 mm in size, and located in the right ovary. Histopathologically, the mass was composed of nests of pleomorphic cells, which formed seminiferous-like tubules separated by a thin fibrovascular stroma. The tubules were lined by tumor cells, which had basally located nuclei and abundant eosinophilic and vacuolated cytoplasm. In some areas, the tumor cells were arranged in a retiform growth pattern, mimicking a rete testis/ovarii. Disseminated metastases to the surfaces of the mesentery, spleen and liver were also present. Immuno-histochemically, many tumor cells were strongly positive for vimentin, estrogen receptor α and Ki 67. Some tumor cells were positive for pancytokeratin and inhibin α. These findings closely resemble those of an ovarian-derived human malignant Sertoli cell tumor. From our review of the literature, we believe this is the first report of a spontaneous malignant Sertoli cell tumor in the ovary of a young laboratory rat. This case might provide useful historical control information for rat toxicity studies. (DOI: 10.1293/tox.2015-0057; J Toxicol Pathol 2016; 29: 53–59)

**Key words:** immunohistochemistry, malignant tumor, ovary, Sertoli cell, spontaneous, young rat

The rat is an extremely valuable animal species used in toxicologic and carcinogenic studies. Although abundant pathological background data are available for major strains of rats, the incidence of spontaneous ovarian tumors is very low, even in aged female rats: 13/1050 (1.2%)[1], 43/3886 (1.1%)[2] and 204/39851 (0.5%)[3] in F344 rats and 158/5903 (2.7%)[4], 7/469 (1.5%)[5] and 210/7748 (2.7%)[6] in Sprague Dawley (SD) rats. Our review of the literature revealed only a few reports of spontaneously occurring ovarian tumors in rats: granulosa/theca cell tumors[7], cystoadenocarcinomas[8, 9] and malignant teratomas[10]. In young rats less than 30 weeks of age, no reports of ovarian tumors have been published with the exception reports of teratomas[10–12]. In this report, we describe the histopathological characteristics of a malignant ovarian Sertoli cell tumor in a young Crj:CD(SD) IGS rat.

For the purpose of a pharmacological study of fatty acids, 40 female Crj:CD(SD)IGS rats were purchased at 6 weeks of age from Charles River Laboratories Japan Inc. (Hino Farm, Shiga, Japan). These rats were housed in plastic cages (five rats per cage) in a room maintained at 22 ± 2°C and 60 ± 10% humidity with 12 h light and dark cycles in an animal center at Kansai Medical University. They were allowed free access to AIN-76 laboratory diet (containing 5% olive oil instead of linoleic acid, Oriental Yeast Co., Ltd., Tokyo, Japan) and distilled water. The present case was from a vehicle control group (single oral dose of sesame oil). The animal was routinely monitored for clinical signs once a day and was weighed once a week before sacrifice. At the age of 19 weeks (terminal sacrifice), this animal was anesthetized with isoflurane (Wako Pure Chemical Industries, Osaka, Japan) and sacrificed by abdominal aortic transection. A complete necropsy was conducted, and over 40 organs, including an ovarian mass detected during necropsy, were fixed in 10% neutral buffered formalin. All tissues were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin (H&E). Sequential sections from the ovarian mass and the contralateral normal ovary were labeled with antibodies for pancytokeratin (CK), vimentin (Vim), inhibin α, cancer antigen (CA) 125, anti-podoplanin (clone *D2-40*), placental alkaline phosphatase (PALP), c-kit, estrogen receptor (ER) α, progesterone receptor (PgR), androgen receptor (AR) and Ki 67. Detailed staining proto-

**Table 1.** Primary Antibodies and Reaction Conditions for Immunohistochemistry

| Primary antibody | Source[1] | Clone | Dilution[2] | Antigen retrieval[3] | Markers for main ovarian tumors[4] |
|---|---|---|---|---|---|
| Pancytokeratin | Dako | AE1/AE3 | 1:50 | 115°C for 10 min (pH 6.0 PBS) | Adenocarcinoma, embryonal carcinoma |
| Vimentin | Dako | V9 | 1:50 | 115°C for 10 min (pH 6.0 PBS) | Sex cord-stromal tumor (granulosa cell tumor, Leydig cell tumor, Sertoli cell tumor, thecoma) |
| Inhibin α | Dako | R1 | 1:50 | 115°C for 10 min (pH 9.0 PBS) | Sex cord-stromal tumor (granulosa cell tumor, Leydig cell tumor, Sertoli cell tumor, thecoma) |
| CA125 | Nichirei | 1A4 | Prediluted | 115°C for 10 min (pH 9.0 PBS) | Adenocarcinoma |
| D2-40 | Nichirei | D2-40 | Prediluted | 115°C for 10 min (pH 6.0 PBS) | Dysgerminoma, gonadoblastoma |
| Placental alkaline phosphatase | Dako | 8A9 | 1:100 | 115°C for 10 min (pH 6.0 PBS) | Dysgerminoma, embryonal carcinoma, gonadoblastoma, yolk sac carcinoma |
| c-kit | Nichirei | Polyclonal | Prediluted | 115°C for 10 min (pH 6.0 PBS) | Dysgerminoma, gonadoblastoma |
| Estrogen receptor (ER) α | Novocastra | 6F11 | 1:40 | 115°C for 10 min (pH 6.0 PBS) | ER expression of tumor cells |
| Progesterone receptor (PgR) | Abcam | Alpha PR6 | 1:50 | 115°C for 10 min (pH 6.0 PBS) | PgR expression of tumor cells |
| Androgen receptor (AR) | Santa Cruz | N-20 | 1:50 | 115°C for 10 min (pH 6.0 PBS) | AR expression of tumor cells |
| Ki 67 | Nichirei | SP6 | Prediluted | 115°C for 10 min (pH 6.0 PBS) | Proliferative activity of tumor cells |

[1]Abcam (Cambridge, UK), Dako (Carpinteria, CA, USA), Nichirei (Tokyo, Japan), Novocastra (Newcastle upon Tyne, UK), and Santa Cruz (Dallas, TX, USA). [2]Primary antibodies were incubated with tissue samples overnight at 4°C. The antigen-antibody complexes were identified using a streptavidin-biotin (LSAB) staining kit (Dako) according to the manufacturer's instructions. The reaction products were visualized with 3-3′-diaminobenzidine tetrahydrochloride. [3]Antigen retrieval was conducted by pressure-cooker heating (Pascal, Dako). [4]Sources: IARC[14], Nogales, Dulcey and Preda[15] and Kato[26].

cols for all antibodies are listed in Table 1. Briefly, sections were deparaffinized, hydrated and blocked for endogenous peroxidase. Heat-induced epitope retrieval was performed for all antibodies. The antigen-antibody complexes were identified using a streptavidin-biotin (LSAB) staining kit (Dako, Carpinteria, CA, USA) according to the manufacturer's instructions. The reaction products were visualized with 3-3′-diaminobenzidine tetrahydrochloride. Negative (1% bovine serum albumin in PBS) and positive controls for all antibodies were set. Positive controls were as follows: human ovarian adenocarcinoma for CK, Vim and CA125, human ovarian seminoma and rat testis for inhibin α and D2-40, rat placenta for PALP, human and rat gastrointestinal stromal tumors for c-kit, human and rat breast cancers for ER and PgR and human and rat prostate for AR. All positive controls were appropriately stained. An additional section from the mass was stained for reticulin using Watanabe's silver impregnation method. Histopathological examination, including immunohistochemical analysis, was conducted by two toxicologic pathologists certified by the Japanese Society of Toxicologic Pathology and/or the International Academy of Toxicologic Pathology according to the International Harmonization of Nomenclature and Diagnostic criteria (INHAND)[13]. The Ethics Committee for Animal Experiments at Kansai Medical University approved the experimental protocol, and all procedures, including animal procedures, were in accordance with the university's guidelines for animal experimentation and the Japanese Association for Laboratory Animal Science.
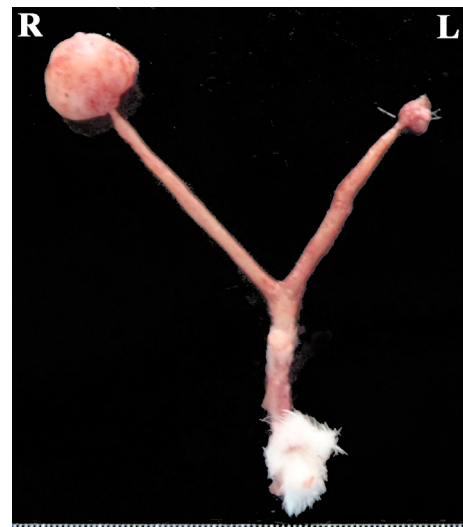


**Fig. 1.** A 10 × 13 × 17 mm firm white mass is located at the right ovary. Scale: 1 mm.

The animal had a normal growth rate and showed no clinical abnormalities before sacrifice. Necropsy revealed a large white firm mass that was round and multinodular in shape, 10 × 13 × 17 mm in size, and connected to the top of the right uterine horn, with no adhesion to the surrounding tissues (Fig. 1). The cut surface of the mass was solid without hemorrhage or cyst formation. Microscopically, this
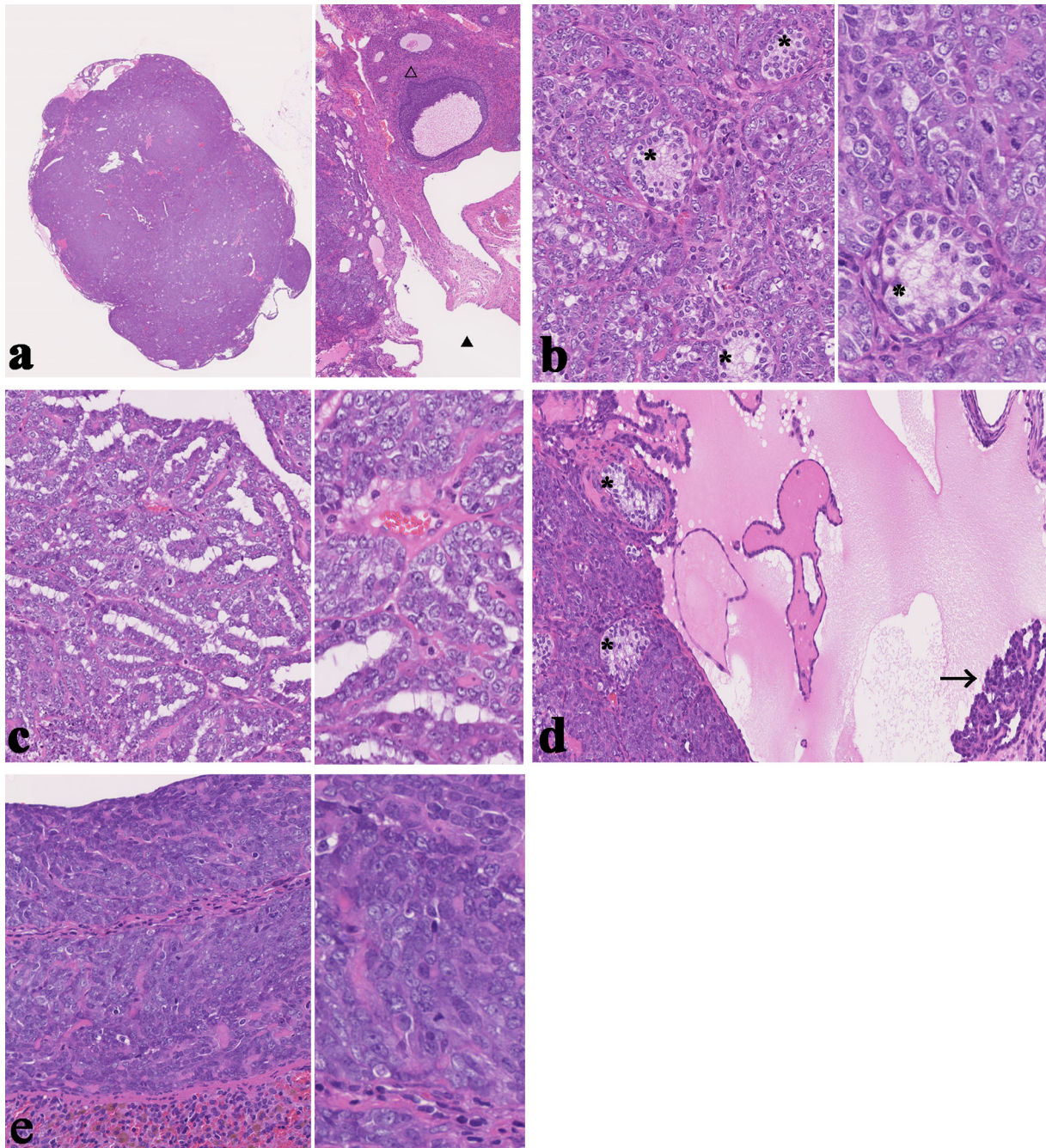
**Fig. 2.** (a) The mass has mostly replaced the ovary (×4.6). A small remnant of normal ovarian tissue was detected at the edge of the tumor (closed triangle). A dilated ovarian bursa (black triangle) was present (×50). (b) Atypical tumor cells proliferated in a solid to nested tubular pattern, separated by a fibrovascular stroma. Some irregular but more well-differentiated tubular structures were composed of tumor cells with abundant vacuolated cytoplasm, similar to Sertoli cells (asterisks) (left, ×150; right, ×400). (c) In some areas, the tumor cells proliferated in a retiform pattern, mimicking a rete testis/ovarii (left, ×150; right, ×300). (d) In the dilated ovarian bursa filled with proteinous fluid, tumor cells were present on the outer periphery of the bursa (arrow). Some irregular tubular structures composed of Sertoli-like cells (asterisks) were also present in this section (×100). (e) Disseminated metastasis of tumor cells to the surface of the spleen (left, ×200; right, ×300). H&E staining.

tumor was believed to have originated from the right ovary, since it was located at the position of the right ovary, and there was normal ovarian tissue with follicles and corpora lutea at a small peripheral part of the tumor mass (Fig. 2a, closed triangle). The tumor was characterized by the proliferation of atypical epithelioid cells in a nested to solid tubular pattern (Fig. 2b). The neoplastic cells were typically large, elongate or polyhedral with an abundant lightly eosinophilic foamy cytoplasm that contained many distinct vacuoles. More well-differentiated but irregular tubular structures were lined by tumor cells containing abundant vacuolated cytoplasm, similar to Sertoli cells (Fig. 2b, as-

**Table 2.** Immunohistochemical Expression of Antigens in this Tumor and in the Normal Ovary

| Primary antibody | This tumor | Normal ovary |
| --- | --- | --- |
| CK | Approximately 50% of tumor cells (+) | Surface epithelium (+) |
| | Ductal structure (−) | Fallopian tube epithelium (+) |
| Vim | Almost 100% of tumor cells (+) | Surface corpus luteum (+) |
| | Ductal structure (+) | Granulosa/theca cells (+) |
| | | Interstitial cells (+) |
| Inhibin a | Almost 10% of tumor cells (+) | Granulosa cells (+) |
| | Ductal structure (+) | |
| CA125 | All cells (−) | All cells (−) |
| D2−40 | All cells (−) | All cells (−) |
| PALP | All cells (−) | All cells (−) |
| c-kit | All cells (−) | All cells (−) |
| ER a | Approximately 70% of tumor cells (+) | Surface epithelium (+) |
| | Ductal structure (−) | Fallopian tube epithelium (+) |
| | | Interstitial cells (+) |
| PgR | All cells (−) | All cells (−) |
| AR | All cells (−) | All cells (−) |
| Ki 67 | Approximately 70% of tumor cells (+) | Granulosa cells (+) |
| | Ductal structure (−) | |

CK, pancytokeratin; ER, estrogen receptor; PALP, placental alkaline phosphatase; PgR, progesterone receptor; Vim, vimentin. Symbols: −, negative reaction; +, positive reaction.

terisks). These structures resembled atrophic seminiferous tubules lined by Sertoli cells. A cord-like structure consisting of similar cells was aligned along a thin fibrovascular stroma. Some tubular structures and ovarian bursa were dilated and accompanied by a proliferation of tumor cells in a retiform pattern, mimicking a rete testis/ovarii (Fig. 2c). Many mitotic figures were visible in the tumor cells except in the Sertoli-like cells lining tubular structures. Tumor cells invaded to the outer periphery of the ovarian bursa of the right ovary (Fig. 2d). Careful histopathological observation revealed a papillary growth of tumor cells with a small number of tubular formations on the surface of the mesentery, spleen and liver, suggesting disseminated metastasis of this ovarian tumor (Fig. 2e). No other organs or tissues, including the left ovary, uterus, and vagina, showed any histopathological abnormalities.

The results of the immunohistochemical staining are summarized in Table 2. The cytoplasm of approximately 50% of tumor cells was strongly positive for CK (Fig. 3a). However, tumor cells within the Sertoli-like cells lining tubular structures were negative (Fig. 3a, asterisks). Vim was strongly and diffusely expressed in the cytoplasm of almost all tumor cells (Fig. 3b), as well as the Sertoli-like cells lining tubular structures (Fig. 3b, asterisks). The tumor cells in some nests and tubular structures had cytoplasm that was positive for inhibin α (Fig. 3c). The nuclei of many tumor cells were strongly positive for ER α; however, cells within the Sertoli-like cells lining tubular structures were negative (Fig. 3d, asterisks). Nuclei of most tumor cells were strongly positive for Ki 67, suggesting high proliferating activity (Fig. 3e). No immunoreactivity was observed for CA125 as a marker for adenocarcionoma[8, 14], D2-40 as a marker for dysgerminoma and gonadoblastoma[14, 15], PALP as a marker for dysgerminoma, embryonal carcinoma, gonadoblastoma and yolk sac carcinoma[14, 15] or c-kit as a marker for dysger-

minoma and gonadoblastoma[14, 15] in any of the tumor cells. No nuclei of tumor cells were positivity for PgR or AR, which are typically expressed in human ovarian Sertoli cell tumors[16–18]. A reticulin stain emphasized the tubular pattern and also revealed nests and larger aggregates of tumor cells surrounded by reticular fibers (Fig. 3f).

This case was characterized by seminiferous-like tubules separated by a fine fibrovascular stroma and lined by atypical epithelioid cells with basally located nuclei and faintly eosinophilic and foamy cytoplasm. These cells had high proliferative activity and were strongly positive for CK, Vim and inhibin α. These results suggest that this tumor may have a sex cord stromal cell origin. Ovarian sex cord stromal cells include six types: granulosa cells, characterized by scanty cytoplasm and small, round to oval nuclei; theca cells, characterized by plump fibrous spindle cells; luteal cells, characterized by polygonal cells with abundant eosinophilic cytoplasm that contain numerous small lipid droplets; stromal cells, characterized by spindle-shaped cells arranged in whorled or storiform patterns; Leydig cells, characterized by polyhedral cells, with large prominent nuclei, eosinophilic cytoplasm and numerous lipid-filled vesicles; and Sertoli cells, characterized by tall simple columnar cells, which span from the basement membranes and form tubular structures, similar to seminiferous-like tubules[19, 20]. The morphology of the tumor cells in the present case matched that of Sertoli cells. Tubulostromal tumor was a differential diagnosis for this case. The diagnostic features of a tubulostromal tumor include delicate tubules lined by a cuboidal epithelium that resembles, or is consistent with, the surface epithelium of the ovary[13]. The tubules in a tubulostromal tumor are separated by packets of cells that may show varying degrees of luteinization. Sertoliform tubules may be present, but they would not be predominant[13]. The morphological features of the present case most closely
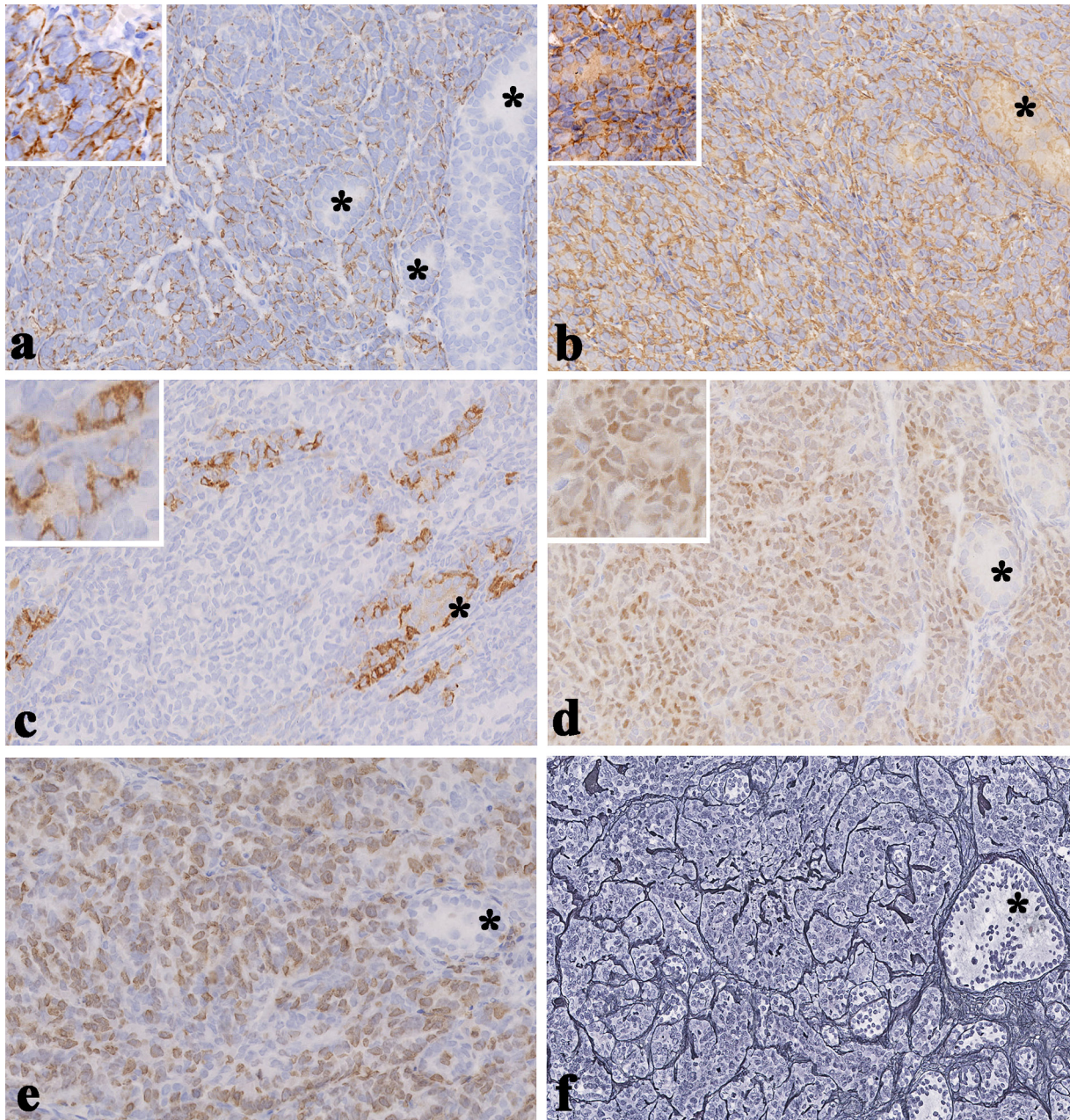
**Fig. 3.** Immunohistochemical and special stains of tumor cells. (a) Approximately 50% of tumor cells were positive for pancytokeratin. However, the more well-differentiated Sertoli-like cells lining tubular structures were negative (asterisks) (×150, insert: ×400). (b) Vimentin was diffusely expressed in the cytoplasm of almost 100% of tumor cells. Sertoli-like cells lining tubular structures were also positive (asterisk) (×150, insert: ×400). (c) Some scattered nests of inhibin α-positive cells were present. Sertoli-like cells lining tubular structures were also positive (asterisk) (×150, insert: ×400). (d) The nuclei of tumor cells were positive for estrogen receptor α; however, Sertoli-like cells lining tubular structures were negative (asterisk) (×150, insert: ×400). (e) Most tumor cells were positive for Ki 67, suggesting a high level of proliferating activity. Sertoli-like cells lining tubular structures were negative (asterisk) (×150). (f) A reticulin stain showed fibrils surrounding nests of neoplastic cells, larger aggregates of tumor cells and Sertoli-like cells lining tubular structures (asterisk) (×150).

resembled an ovarian Sertoli cell tumor, and the cells surrounding the tubules showed no evidence of luteinization. We therefore propose that this was a Sertoli cell tumor of sex cord stromal origin.

Ovarian Sertoli cell tumors and Sertoli-Leydig cell tumors have been reported in humans and other species and belong to a group of sex cord stromal tumors. They are simi-lar in morphology to a Sertoli cell neoplasm in the testis[13, 14]. They are characterized by the formation of seminiferous-like tubules lined by Sertoli cells with faintly eosinophilic or foamy cytoplasm. These tumors tend to originate from the parenchyma in the hilar region of the ovary in rodents and humans[13]. The predominant microscopic pattern is tu-bular, and other patterns are cords, trabeculae, diffuse,

pseudopapillary, retiform, islands, alveolar arrangements, and spindled[21]. The retiform type of tumor shows substantial areas of anastomosing, slit-like spaces resembling a rete testis or rete ovarii[21]. Retiform tubules are not seen in well-differentiated tumors and tend to be seen in younger human patients[14]. The diagnosis of malignancy is usually based on the presence of focal necrosis, hemorrhage, local invasion, disruption of the ovarian capsule, and/or implant on peritoneal surfaces, as well as cellular atypia of tumor cells[3, 13, 22]. In aged rodents, ovarian Sertoli cell tumors are composed predominantly (>70%) of Sertoli cells and are sometimes composed of other sex cord stromal cell types, especially granulosa cells, according to INHAND criteria[13]. When there is no specific tumor cell type (>70%), (i.e, a mixture of granulosa, luteal, theca, stromal and/or Sertoli cells), the tumor should be diagnosed as a malignant mixed sex cord stromal tumor[13].

The present case was a young adult rat that had an early onset tumor that had features of a less differentiated tumor type. The early onset of this tumor may have affected its morphology, which was different from what is typically seen in aged animals. In adult women, ovarian tumors, especially granulosa cell tumors, are diagnosed as a separate category from the granulosa cell tumors that occur in the ovaries of younger women[23]. Similarly, Sertoli cell tumors occur in young women, but a variant (Sertoli-Leydig) that occurs in women during their second decade of life has different morphologic characteristics[24]. This information, if analogous in rodents, would lend support to the present case being a Sertoli cell tumor because it did not quite fit all the criteria outlined for classical Sertoli cell tumors typically observed in aged rodents.

Only a few reports of immunohistochemical analysis of spontaneously occurring ovarian tumors in rats have been published, with the published reports including studies of S100 protein expression in malignant theca cell tumors[25]; expression of epithelial membrane antigen, cytokeratin and proliferating cell nuclear antigen in cystoadenocarcinomas[8]; and expression of ER, PgR, AR, her-2/neu, epithelial cell adhesion molecule, CA125 and β-catenin in cystoadenocarcinomas[9]. There are no published studies regarding immunohistochemical analysis of spontaneously occurring ovarian Sertoli cell tumors in rats. In the present case, the tumor cells were positive for CK, Vim, ER and inhibin α. Sertoli-like cells lining tubular structures were also positive for Vim and inhibin α. Human Sertoli-stromal cell tumors also show positive expression for CK and Vim[14]. This positivity depends on the morphologic differentiation of the tumor cells[26]. In a well-differentiated tumor with tubular formation, there is high positivity for CK and low positivity for Vim, while low positivity for CK and high positivity for Vim are seen in poorly-differentiated tumor types with sarcomatous proliferation. Inhibin α is considered a valuable marker for Sertoli-stromal tumors. In human tumors, positivity also depends on the morphologic differentiation of the tumor cells[23]; low positivity is seen in poorly differentiated types, and high positivity is seen in well-differentiated types. In the present case, many tumor cells were positive for Vim, but some tumor cells were positive for CK and inhibin α, suggesting a poorly differentiated tumor type. Additionally, many tumor cells had a high proliferating activity based on the Ki 67 immunohistochemistry results, suggesting malignant potential. In the present case, the tumor cells were positive for ERα positivity, similar to human cases[17]; in humans, Sertoli cell tumors possess estrogen reactivity in two thirds of young patients[16].

Rat ovarian tumors can be roughly divided into 3 main histological categories classified by their presumed histogenesis: gonadal (sex cord) stromal, epithelial and germ cell and others[13, 22, 27]. Malignant ovarian tumors include malignant Sertoli cell tumors, malignant granulosa cell tumors, malignant sex cord stromal tumors and malignant thecomas, which are of gonadal stromal origin; cystadenocarcinomas and tubulostromal carcinomas, which are of epithelial origin; yolk sac carcinomas, embryonal carcinomas and dysgerminomas, which are of germ cell origin; and finally choriocarcinomas and malignant teratomas, which are of other origins[13]. The majority of spontaneously occurring ovarian tumors in aged rats have been reported to be gonadal stromal in origin, primarily granulosa cell tumors in F344 rats[3, 27], and tubular adenomas, including Sertoliform types[4, 6] in SD rats. Recently, Sertoliform tubular adenoma was thought to be a synonym for a benign Sertoli cell tumor based on the INHAND criteria[13]. In F344 rats, the incidence of ovarian Sertoli cell tumors, including Sertoliform tubular adenomas, is extremely rare: 2/3886 and 20/39851[2, 3]. However, Lewis reported the incidence of Sertoli cell tumors, including Sertoliform tubular adenomas, to be 154/7748 in SD rats[6], suggesting that the Sertoli cell tumor is a major type of ovarian tumor in this strain. Although ovarian Sertoli cell tumors are reported to occur in aged rats, the present ovarian tumor was most likely congenital based on the age of the rat (19 weeks old). From our literature review, we believe that this is the first report of a spontaneous malignant Sertoli cell tumor in the ovary of a young laboratory rat. Our case might provide valuable historical control information for toxicity studies in rats.

**Disclosure of Potential Conflicts of Interest:** In connection with this paper, there is no conflict of interest with any

companies to be disclosed.

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