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# Sertoli–Leydig Cell Tumor of the Testis in a Sprague-Dawley Rat

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**A rare intratubular gonadal stromal tumor was present in the testis of a 7-wk-old male Sprague-Dawley rat. The tumor comprised an intratubular mixture of 2 types of tumor cells with intercellular junctions: the predominant tumor cells were consistent with a Sertoli cell origin, and cells comprising the minor population were situated on basolateral side of the tubuli, consistent with a Leydig cell origin. The neoplastic Sertoli cells had large pleomorphic nuclei and clear cytoplasm with many tubulovesicular cristae and free ribosomes, whereas the neoplastic Leydig cells showed relatively small pleomorphic nuclei, dark cytoplasm with rich smooth endoplasmic reticulum, numerous mitochondria, and lipid droplets. Occasionally, a few transitional type neoplastic cells were observed. The presence of a thick or multilayered basement membrane was confirmed except in tumor-infiltrative lesions. The present case was considered to be a testicular mixed tubular Sertoli–Leydig cell tumor in a Sprague-Dawley rat.**

The most frequently encountered neoplasm of the rat testis is the Leydig cell tumor, the incidence of which varies greatly among strains. The rate increases with age but varies from 1% to 2% in Long-Evans rats, 1% to 5% in Sprague-Dawley rats, 4% to 7% in Wistar rats, 78% in Wistar substrain U rats, to nearly 100% in Fischer 344 rats.[4,10,12] Leydig cell tumors in rats usually are composed of round cells with dark cytoplasm, and the exact etiology of this tumor is not known.[12] In contrast, Sertoli cell tumors are rare in rats,[10] and only 2 cases in rats have been described in detail to date.[1,3] Sertoli cell tumors are composed of interdigitated rows of tubules lined with elongated palisading cells situated on a thin fibrovascular stromal basement membrane.[1] Mixed gonadal stromal tumors, Sertoli–Leydig cell tumors, are also quite rare in rats.[10] The present report describes a spontaneous testicular mixed Sertoli–Leydig cell tumor in a Sprague-Dawley rat.

## Case report

A 7-wk-old male Sprague-Dawley rat from an untreated control group of a toxicology study was presented for necropsy. All experimental procedures were conducted after approval of the Animal Care and Use Committee of the Azabu University School of Veterinary Medicine. Guidelines set by the National Institutes of Health and Public Health Service Policy on the Humane Use and Care of Laboratory Animals were followed at all times.

Cryptorchidism was not present. The left testis was 2.1 × 1.0 × 1.1 cm, and its size and weight were similar to that of the right testis and others from the same untreated group. The surface area of midsagittal sections of the left testis was white like the normal testis, and hemorrhage was not present, but the left testis felt slightly solid when incised with a scalpel blade.

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Tissue specimens including testis, adrenal glands, liver, kidney, lung, and lymph nodules were fixed in 1.2% glutaraldehyde in PBS for 2 h and then postfixed in 1.0% osmium tetroxide for 2 h. After dehydration in graded alcohols, more than 20 specimens of each organ were embedded in epoxy resin, cut into 2-µm-thick sections, stained with methylene blue, and examined under light microscopy. In addition, thin sections were cut on a Porter-Blum ultramicrotome and mounted on polyvinyl formal-coated slit grids. After double staining with uranyl acetate and lead citrate, the sections were examined by using an electron microscope (H500H, Hitachi, Tokyo, Japan).

By light microscopy, the tumor was about 6 × 8 mm, consisted mainly of variably sized and shaped expanding tubuli, and comprised 2 types of cell populations; orchitis was not noted (Figure 1). In the intratubular lesion, prominent atypical cells were randomly ordered spindle-shaped cells with relatively large nuclei, little heterochromatin, and prominent nucleoli. Nuclear grooves were seen but were generally inconspicuous. In light of the cellular structures, these cells were considered to be neoplastic Sertoli cells (Figure 1).

The other type cells were situated on the basolateral side of the tubuli. These cells were small and round to elongated methylene blue-stained cells with round, centrally located nuclei with marginally dispersed chromatin and a single nucleolus. In light of the cellular structure, these cells were considered to be neoplastic Leydig cells (Figure 1). No germ cell elements were noted. Neoplastic tissue had replaced the testis, with only a few compressed and atrophic Sertoli cell-only tubules remaining. The tumor did not possess a fibrous capsule, and some tumor cells seemed to infiltrate into beyond tubuli surrounding the Leydig cells but did not extend into the tunica albuginea of the testis. Tumor cells had no or infrequent mitotic figures. Gross morphologic and microscopic examinations did not reveal metastases of the tumor in the adrenal glands, liver, kidney, lung, or lymph node tissues, and except for the testis, the evaluated organs did not shown any pathologic alteration.

Ultrastructural observation revealed that the predominant tumor cells had large pleomorphic nuclei and clear cytoplasm

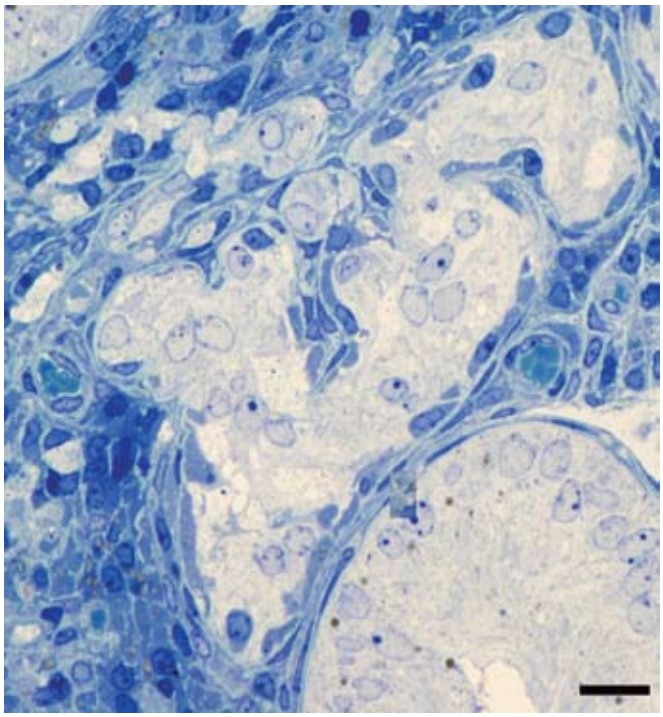

**Figure 1.** Mixed tubular Sertoli–Leydig cell testicular tumor in a Sprague-Dawley rat. Tumor consists of variously sized and shaped expanding tubuli. Neoplastic Sertoli cells are spindle-shaped and randomly ordered, whereas Leydig cells are situated on the basolateral side of tubuli and are stained by methylene blue. At the lower right, a Sertoli cell-only tubule is present. Methylene blue stain; bar, 15 µm.

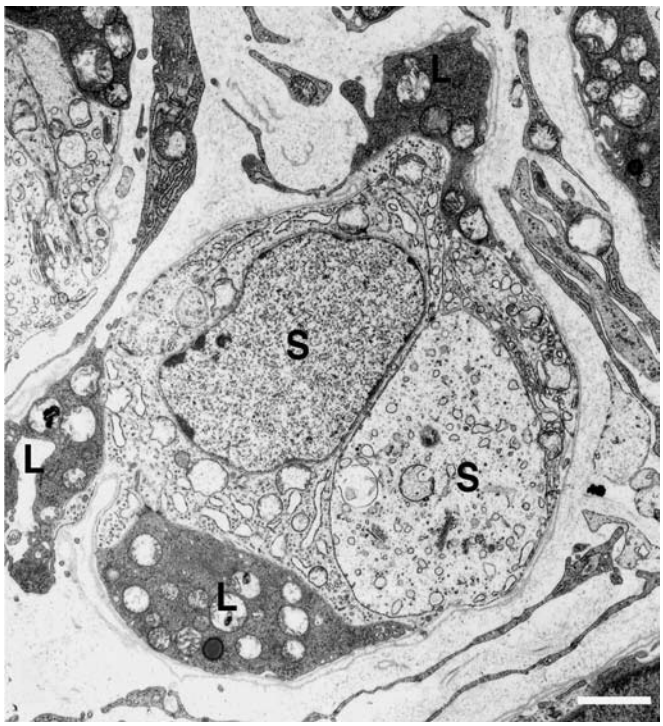


**Figure 2.** Mixed tubular Sertoli–Leydig cell testicular tumor in a Sprague-Dawley rat. Transverse section of a testicular tumor comprising neoplastic Sertoli cells (S) with clear cytoplasm , many tubulovesicular cristae and free ribosomes, and neoplastic Leydig cells (L) with relatively small pleomorphic nuclei, dark cytoplasm with rich smooth endoplasmic reticulum, numerous mitochondria, and lipid droplets. Tumor is surrounded by basement membranes. Bar, 2 µm.

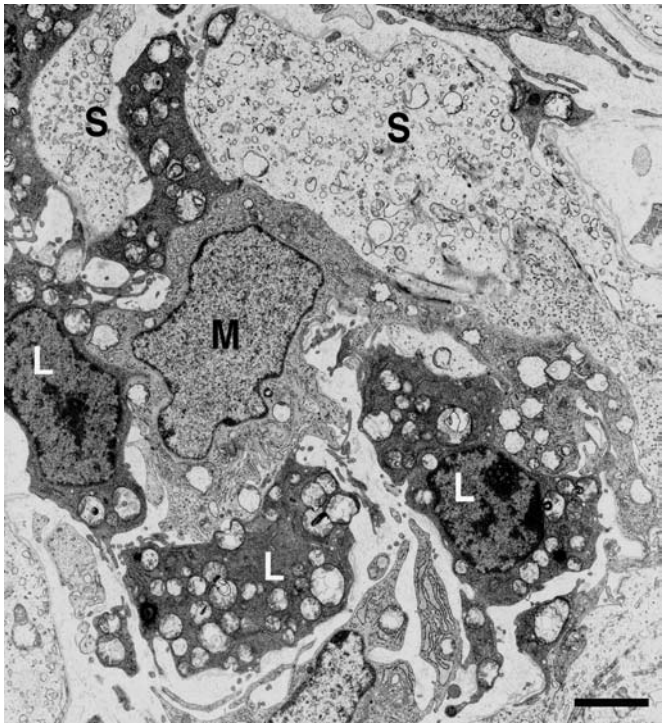


**Figure 3.** Mixed tubular Sertoli–Leydig cell testicular tumor in a Sprague-Dawley rat. Some neoplastic cells appeared to be a transitional type (M), with features of both Sertoli cells (S) and Leydig cells (L). The intratubular neoplastic Sertoli and Leydig cells are closely packed. Bar, 3 µm.

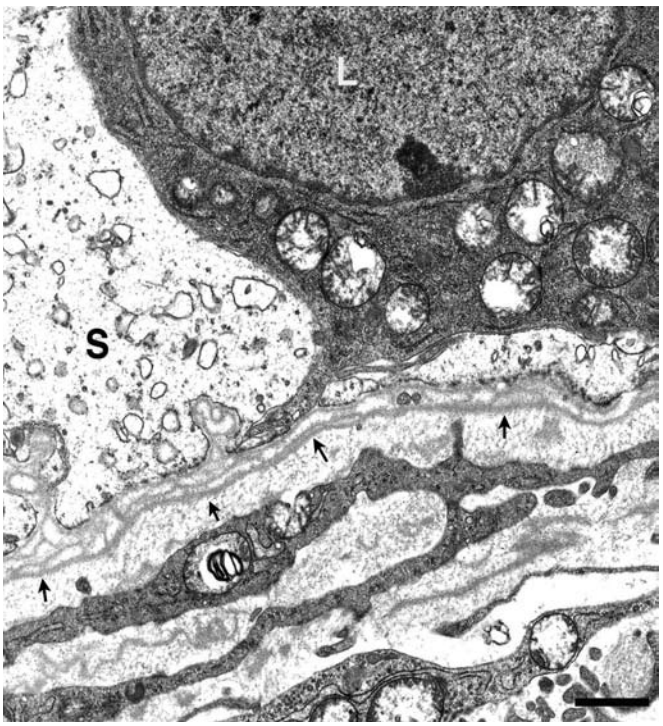


**Figure 4.** Thick, multilayered basement membrane (arrows) of the intratubular mixed Sertoli (S)-Leydig (L) cell tumor. Bar, 1 µm.

with many tubulovesicular cristae and free ribosomes, consistent with a Sertoli cell origin, and the other cells showed relatively small pleomorphic nuclei, dark cytoplasm with rich smooth endoplasmic reticulum, numerous mitochondria, and lipid droplets, consistent with a Leydig cell origin (Figures 2 to 6).

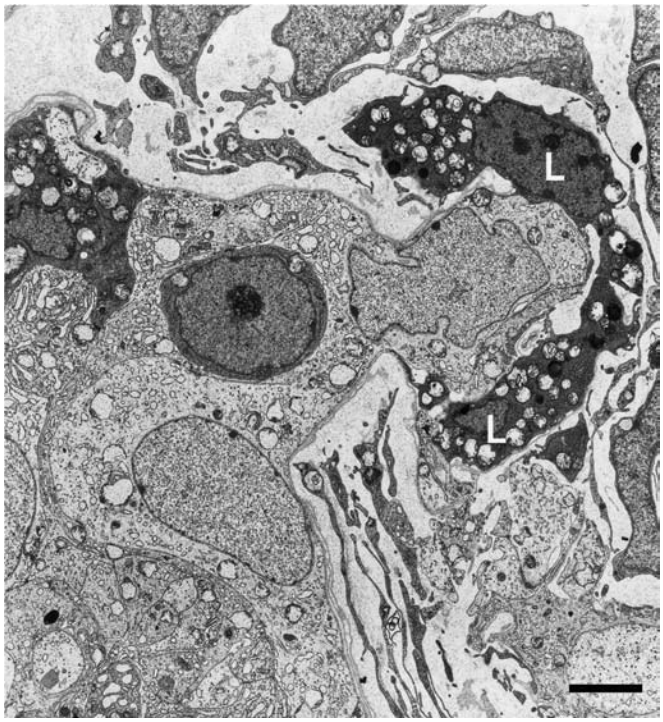


**Figure 5.** Testicular mixed tubular Sertoli–Leydig cell tumor with infiltrative neoplastic Leydig cell (L). Bar, 3 µm.

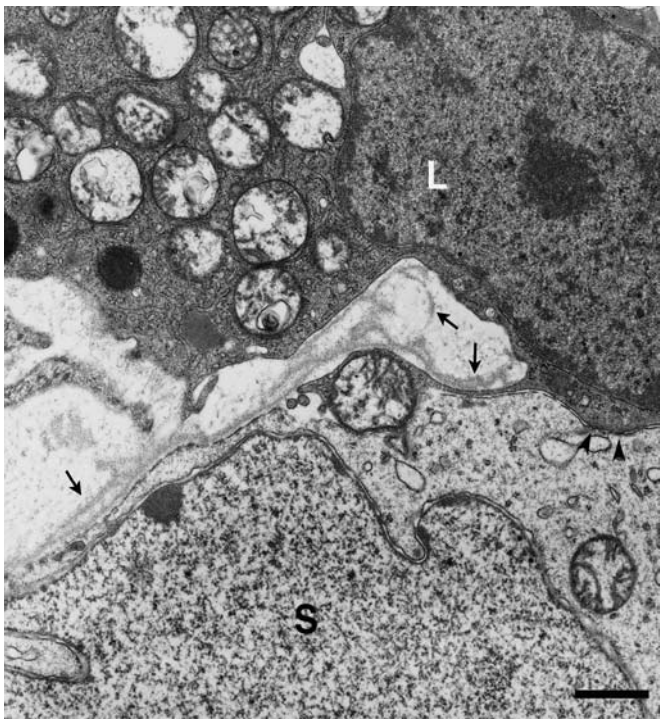


**Figure 6.** High-power view of Figure 5**.** The basement membrane (arrows) is disrupted or absent at the infiltrative tip of neoplastic Leydig cells (L), and tight junctions (arrow heads) occur between neoplastic Sertoli (S) and Leydig cells. Bar, 0.8 µm.

Occasionally, neoplastic cells that appeared to be a transitional cell type with features of both Sertoli and Leydig cells were found (Figure 3). The intratubular neoplastic Sertoli and Leydig cells were closely packed, and the tumor cells were conjugated with one another by interdigitation of cytoplasmic membranes and tight and intermediate junctions and were surrounded by thick or multilayered basement membranes, except where tumor cells infiltrated (Figures 2 to 6). A dense, fibrous capsule was not present (Figures 2 to 6).

## Discussion

Gonadal stromal tumors, Sertoli–Leydig cell tumors, are quite rare in rats, and only 1 case has been reported previously. That previous case involved a 112-wk-old Wistar (Crl:[WI]BR) rat that had been treated at 8 wk with 3 subcutaneous injections of 0.1 mmol Zn acetate and a single subcutaneous injection of 30 µmol $CdCl_2$/kg 6 h later.[11] That neoplasm consisted of an admixture of neoplastic Leydig cells and tubules of varying sizes lined with spindle-shaped cells with neoplastic Sertoli cell morphology; however, whether the tumor was induced by exposure to chemicals was not determined.[11] Sex cord stromal tumors may present with atypical morphologies and different admixtures of patterns (tubular, cystic, solid) and stromal components.[14] The present case demonstrated an admixture of 2 neoplastic cell types in a testicular tumor in a male Sprague-Dawley rat, whose lesion was considered to be a mixed tubular Sertoli–Leydig cell tumor.

In the present case, neoplastic Sertoli cells might have arisen from the seminiferous tubules, because proliferated Sertoli cells gradually replaced the tubular population of germ cells and filled the tubules. However, the question arises as to whether the neoplastic Leydig cells admixed with Sertoli cells are a proliferation of primitive gonadal precursor cells or an invasion of outer tubular neoplastic Leydig cells. A tumor with a mixture of 2 or more types of cells has a possible common embryonic origin.[11] Moreover, several previous studies have suggested that rat testicular stromal precursor cells can develop in both Sertoli and Leydig cell directions during tumor formation.[2,5,7,13] Therefore, the origin of the neoplastic Leydig cells in the present case is likely primitive gonadal stromal precursor cells.

Two phases have been described in the differentiation of Leydig cells in rats.[6,8] During the first phase, fetal-type Leydig cells develop from gonadal mesenchymal precursor cells.[6] The second phase occurs at puberty, when adult-type Leydig cells appear.[8] Although the fate of fetal-type Leydig cells is not known precisely, rat and human fetal-type Leydig cell tumor clusters are surrounded by basement membranes.[9] This shared feature suggested to us that the neoplastic Leydig cells of the present case might be differentiated from fetal-type Leydig cells.

Spontaneous testicular tumors usually occur in old rats, and they become increasingly more frequent with age.[10] However, the present case occurred at puberty, and a testicular tumor in a 7-wk-old rat is unusual. 78% of substrain U Wistar rats develop spontaneous Leydig cell tumors between the ages of 12 and 30 mo.[12] However, the first signs of tumor development, in the form of nodules of Leydig cells, may already be apparent when animals are 1 mo old.[12] The fact that the nodules are present in young rats makes it likely that the present case represented very early tumor development and may indicate that the genetic background is important in the formation of the tumor.[5,12] The present case may represent phenotypic malformation or transdifferentiation of embryonic gonadal stromal precursor cells.

## Acknowledgment

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