Title

Segmental aplasia of a uterine horn in two RccHan:WIST rats

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Abstract

Aplasia of the uterine horn caused by developmental defects has been reported in several species but has not been reported in RccHan:WIST rats. We encountered spontaneous segmental aplasia of a right uterine horn in two RccHan:WIST rats and detailed its pathological characteristics. The right uterine horn of both rats had similar gross and histological appearances. At necropsy, there was segmental loss of the tissues corresponding to normal right uterine horn, which consisted of a fibrous band connected to the uterine cervix. A cystic structure with clear and colorless fluid was observed in the cranial segment of the right uterine horn close to the right oviduct. The cystic structure was thought to be a partially developed tissue to the right uterine horn. The cystic structure seemed to be derived from the right uterine horn. Histologically, a single layer of cuboidal epithelium lined the luminal surface of the cystic structure, the endometrium was thin, and no uterine glands were observed. The fibrous band was composed of α-SMA positive smooth muscle cells, connective tissue, and blood vessels, but cytokeratin AE1/AE3 positive epithelium and uterine endometrium were absent. Based on these gross findings and histological features, segmental aplasia of a uterine horn was diagnosed. To our knowledge, these cases of segmental aplasia of a uterine horn are the first ones described in RccHan:WIST rats.

Key words: segmental aplasia, uterine horn, RccHan:WIST rat
Congenital malformations of the uterus have been reported in various species, including cats\textsuperscript{1, 2}, hamsters\textsuperscript{3}, rats\textsuperscript{4, 5}, dogs\textsuperscript{6}, cattle\textsuperscript{7}, and humans\textsuperscript{8}. These abnormalities result from faulty development of the paramesonephric (Müllerian) ducts during embryogenesis\textsuperscript{9}. They include bilateral agenesis, unilateral agenesis, and segmental aplasia. The diagnostic features of segmental aplasia is absence of one or more segments of the uterine horns. Although these anomalies have been reported in several species, to our knowledge, segmental aplasia of the uterine horn has not been reported in RccHan:WIST rats. Here we report the detailed pathological characteristics of 2 separate cases of spontaneous segmental aplasia of the right uterine horn in two RccHan:WIST rats.

Both of the 4-week-old female RccHan:WIST rats were purchased from Japan SLC, Inc. (Shizuoka, Japan), but they were from different lots. They were housed in suspended aluminum cages in a room kept at 24 ± 2°C, at a relative humidity of 40–70%, and on a 12-h light/dark cycle. CRF-1 pellet diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were freely available via automatic stainless steel nozzles throughout the study. The animals were observed daily and were used for experiments after a 1-week acclimation period. All experiments were performed in accordance with the Guide for Animal Care and Use of Sumitomo Chemical Co., Ltd. The two rats were 9 and 14 weeks of age, respectively, at necropsy and were euthanized under isoflurane
anesthesia before withdrawing whole blood samples from the abdominal aorta.

Tissue samples from the uterus, vagina, oviducts, and ovaries were first fixed in 10 vol% neutral buffered formalin, processed by routine methods, embedded in paraffin, sectioned into 3-μm slices, stained with hematoxylin-eosin (HE), and then examined under a light microscope.

For immunohistochemistry, 4-μm-thick sections were obtained from paraffin-embedded tissues, including tissue from the uterus, a cystic structure, and a fibrous band. After deparaffinization, the sections were heated in 10 mM citrate buffer (pH 6.0) by microwave for 25 minutes at 98°C for antigen retrieval, treated with blocking solution to inactivate endogenous peroxidase, and stained with mouse monoclonal antibody to CK AE1/AE3 (Cytokeratin AE1/AE3, ready-to-use, Nichirei Biosciences Inc., Tokyo, Japan) or α-SMA (alpha Smooth Muscle Actin, 1:100 dilution, Dako Japan Inc., Tokyo, Japan) overnight at 4°C. Immunoreactivity was detected and visualized using Histofine Simple Stain Rat MAX-PO (MULTI) (Nichirei Biosciences Inc., Tokyo, Japan) and a DAB Map kit (Ventana Medical Systems, Inc., Tucson, AZ, USA) followed by hematoxylin counterstaining.

The rat necropsied at 9 weeks was in the nontreatment group of a 4-week repeated oral dose toxicity study (Case 1). The rat necropsied at 14 weeks was used for technical training without drug administration but with determination of the estrous cycle for 2 weeks (Case 2). The number of females examined was 24 in the toxicity study and 20 in the technical training.
Neither rat showed any clinical features during the acclimation and test periods. The estrous cycle of the case 2 rat was normal for 2 weeks.

At necropsy, the gross findings were basically same in both rats. There was segmental loss of the tissues corresponding to normal right uterine horn, which consisted of a fibrous band connected to the uterine cervix. A cystic structure with clear and colorless fluid was observed in the cranial segment of the right uterine horn close to the oviduct (Fig. 1). The cystic structure was thought to be a partially developed tissue to the right uterine horn. The anatomical position of the cystic structure between the oviduct and cervix suggested that the origin was the Müllerian ducts, not the mesonephric (Wolffian) duct. The cystic structures in Case 1 and Case 2 were 10 mm and 15 mm in diameter, respectively. The left uterine horn, vagina, bilateral ovaries, and oviducts were normal.

Histologically, the tissue appearance was similar in both rats. In a cross section of the fibrous band, smooth muscle bundles were interspersed with fibrous connective tissue, and blood vessels were sometimes seen (Fig. 2). There was no lumen or endometrial glands. The luminal surface of the cystic structure was lined by a single layer of cuboidal epithelium. The epithelium was considered to be derived from the uterine endometrial epithelium, but not from the oviduct, because there were no ciliated cells. The appearance of the epithelium was similar to that of a severely dilated uterus sometimes observed in chronic studies in rats. The endometrium was thin,
and no uterine glands were observed (Fig. 3, 4). The left uterine horn, vagina, left ovary, and oviducts were microscopically normal (Fig. 5). The stage of the estrous cycle determined from histology was estrus in Case 1 and early proestrus in Case 2. It was difficult to determine the estrous cycles from the epithelium of the cystic structure, because the epithelium was dilated, the endometrium was thin, and no uterine glands were observed. The epithelium was lined by tall columnar cells, and many apoptotic epithelial cells were observed in both cases; some mitotic figures were observed in Case 1. The right ovaries in both cases contained some ovarian follicles at various developmental stages and some corpora lutea (CL). Many old CL and new CL were observed in Case 1, and vesicular follicles were observed in Case 2, suggesting that the right ovarian cycle progressed normally and was related to the estrous cycles.

Immunohistochemically, the fibrous band of Case 2 stained positively for $\alpha$-SMA, indicating the presence of smooth muscle tissue, but did not stain positively for CK AE1/AE3, indicating the absence of epithelial components (Fig. 2). The fibrous band of Case 1 also stained positively for $\alpha$-SMA, indicating the presence of smooth muscle tissue. The epithelium lining of the cystic structure in the two rats stained positively for CK AE1/AE3, and the muscle layer stained positively for $\alpha$-SMA (Fig. 4).

These histopathological and immunohistochemical results suggested that the distal segment of the uterine horn consisted of smooth muscle tissue, connective tissue, and blood
vessels, but no lumen or endometrium, and that the cystic structure was the dilated uterine horn.

Congenital malformations of the uterine horn include bilateral agenesis, unilateral agenesis, and segmental aplasia. In the condition of segmental aplasia, segments of the uterine horn may be absent, resulting in isolation of cranial segments from more distal segments of the uterine horn and body\(^9\). Due to the accumulation of secretions in blind-ended segments, uteri often become cystic and filled with necrotic cellular debris\(^9\). Generally, the ovaries, uterine tubes, and most cranial parts of the uterine horns are present and normal, except that the horns can be distended due to the accumulation of secretions, and usually exhibit typical estrous behavior\(^1\), \(^4\), \(^7\).

In our cases, the distal segment of the right uterine horn was absent and replaced by a fibrous band, and a cystic structure with clear and colorless fluid was observed in a cranial segment close to the oviduct. Additionally, a normal estrous cycle was observed. Consequently, segmental aplasia of the uterine horn was diagnosed.

Segmental aplasia with a dilated uterine horn that filled with fluid has been reported in some species\(^2\), \(^4\). In one cat, endometrial glands were observed in the dilated uterine horn, but their number was markedly reduced, and most of them were compressed\(^2\). In the present cases, a microscopic examination of multiple cross sections of the cystic structure revealed no uterine glands in the thinned endometrium. It is possible that the uterine glands were present before compression and that their atrophy was induced by the accumulation of fluid.
According to the literature, aplasia of the uterine horn is often associated with ipsilateral renal agenesis, because of the close association between the urinary and genital systems during development\textsuperscript{2, 4, 5, 8, 10, 11}. However, there were no gross abnormalities in the kidneys in the present cases. On the other hand, in most cases, the ovaries are normally positioned ipsilateral to the aplastic horn because the ovarian and genital systems have different embryologic origins\textsuperscript{1-5}. This was also true in the present cases.

Little is known about the pathogenesis of segmental aplasia, with some literature indicating a genetic basis in inbred rat strains\textsuperscript{4, 5}, shorthorn cattle\textsuperscript{7}, and humans\textsuperscript{8}. In the present cases, no genetic connection could be established between these two rats, and the pathogenesis of segmental aplasia remained unclear.

Uterine horn aplasia has been reported in two strains of rat, the ACI rat and the UUA rat\textsuperscript{4, 5}. These rats were established inbred rat strains and were characterized by unilateral renal agenesis, and sometimes complete or segmental aplasia of the uterine horn was observed associated with ipsilateral renal agenesis. In the present cases, the rats were not inbred strains, and renal agenesis was not observed.

In conclusion, we examined the detailed characteristics of the lesions in two Rcc:Han WIST rats, and the rats were diagnosed with segmental aplasia of a uterine horn based on the gross, histological, and immunohistochemical features of the affected uterine horn. The
characteristic features of this lesion were segmental loss of tissues, which consisted of a fibrous band connected to the uterine cervix, and a cystic structure in the cranial segment of the right uterine horn close to the oviduct.

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Fig. 1. Gross appearance of the female genital tract in Case 2. There was segmental loss of the tissues corresponding to the normal right uterine horn, which consisted of a fibrous band connected to the uterine cervix. A cystic structure was observed in the cranial segment of the right uterine horn close to the oviduct. (Red rectangles indicate the trimmed sections for Figs. 2, 3, and 5.)
Fig. 2. A: The fibrous band consisted of smooth muscle tissue, connective tissue, and blood vessels. HE stain. B: Epithelial components stained negative for CK AE1/AE3. C: The smooth muscle tissue and blood vessels were stained positively for α-SMA.

Fig. 3. Cystic structure, oviduct, and ovary on the right side in Case 2. The right ovary and oviducts
were microscopically normal. The ovary contained some ovarian follicles at various developmental stages and some corpora lutea. HE stain.

Fig. 4. A: The wall of the uterine cystic structure was lined by a single layer of cuboidal epithelium without cilia that looked like the uterine endometrial epithelium. The endometrium was thin, and no uterine glands were observed. HE stain. B: The epithelium lining the cystic structure stained positively for CK AE1/AE3. C: The muscle layer stained positively for α-SMA.
Fig. 5. A: Normal left uterine horn in Case 2. HE stain. B: High magnification of the endometrial epithelium.