

Original Article

# Histopathological background data of the systemic organs of CLAWN miniature swine with coronary artery stent implantation

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**Abstract:** The aim of this study was to identify potential changes that could occur during histological evaluations of CLAWN miniature swine, with potential consequences for subsequent experiments. The systemic organs from male and female CLAWN miniature swine (16.3–42.3 months old) that had been used in long-term studies of coronary stent implantation were examined histologically. Commonly observed histopathological findings were testicular/epididymal atrophy, cyst-like follicles in the ovaries, hemosiderin deposition in the spleen, lipofuscin deposition in the proximal tubular epithelia and presence of eosinophilic globules in the Bowman's space and the lumen of the proximal tubules in the kidneys, and cellular infiltration in several organs, including the eyelids, respiratory organs, and digestive tract. However, none of these changes were serious enough to indicate a significant impact on research. In conclusion, this study identified CLAWN miniature swine as a suitable animal model for various experiments. (DOI: 10.1293/tox.2016-0024; *J Toxicol Pathol* 2017; 30: 25–38)

**Key words:** minipig, CLAWN miniature swine, background data, histopathology

## Introduction

Miniature pigs (minipigs) have anatomical and physiological features that are closer to those of humans compared with those of other non-rodent species<sup>1, 2</sup>. As a result, their application as models in surgical procedures and physiological studies has increased substantially<sup>3</sup>. Moreover, minipigs are increasingly being used in research as an alternative to other non-rodent animals such as dogs and monkeys for scientific, economic, and social reasons.

We routinely use CLAWN (the name is derived from the initials of the Central Laboratory of White Nipai) miniature swine for testing the implantation of medical devices. CLAWN miniature swine originated from the F1 progeny of the Göttingen and Ohmini strains and were developed by subsequent mating with the F1 progeny of Landrace and Large Yorkshire domestic pigs. CLAWN miniature swine have been maintained in a closed colony since 1978 and were established as an inbred strain of experimental minipigs in

Japan. The strain grows to 36.7 kg in 12 months and to 58.0 kg in 24 months. The body weight of CLAWN swine eventually increases to approximate that of an adult human<sup>4</sup>. In domestic pigs, the body structure and organs grow rapidly as the animals age, which can result in variations in the size of an implanted medical device and its implantation site. In contrast, minipigs do not grow rapidly with age, making this strain highly suitable for testing of the implantation of medical devices. In addition, because the branching pattern and diameter of the coronary arteries of CLAWN miniature swine are similar to those of humans<sup>5</sup>, we have been using this animal model to examine local tissue changes caused by the implantation of stents<sup>6, 7</sup>, a medical device used to treat narrowing of the coronary arteries. Moreover, CLAWN miniature swine are a valuable animal resource because of their swine leukocyte antigen genotype, which is the major histocompatibility complex of pigs<sup>8</sup>. CLAWN miniature swine have therefore been used for research in the fields of medical transplantation, antirejection treatment, and induced pluripotent cell therapy<sup>8</sup>.

Background data regarding body weight and hematological analysis of CLAWN miniature swine are available<sup>9</sup>, but comprehensive histopathological background data have not yet been accumulated. Availability of such a data set for the systemic organs would permit extensive examinations and allow for discussions on safety evaluations. Therefore, to determine the characteristic lesion or age-related changes of CLAWN miniature pigs, we performed histopathological

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examinations of the systemic organs of CLAWN miniature swine bred in our facilities for long-term studies of coronary artery stents. These data were compared with background information of Göttingen minipigs, which are genetically closely related to CLAWN miniature swine<sup>10</sup>.

## Materials and Methods

### *Animals*

The CLAWN miniature swine is an inbred strain, as described above. In this study, CLAWN miniature swine that had been used in implantation tests for coronary artery stents (duration: 3, 9, and 15 months) were examined. CLAWN miniature swine of desired body weight (23.4–42.8 kg) were purchased from the Japan Farm CLAWN Institute (Kagoshima, Japan) in 2008. Upon arrival, there were 16 male pigs aged between 11.9 and 26.1 months and 11 female pigs aged between 12.3 and 32.0 months. The ages of the animals at necropsy were between 16.3 and 31.4 months for males and between 22.4 and 42.3 months for females. The data of each animal were divided into groups according to sex and age at the time of necropsy (10–20, 20–30, 30–40, and 40–50 months), and appropriate data were collected and summarized. The number of animals in each age group and duration of stent implantation were as follows: for males, 6 animals in the 10–20 months (6 animals, implantation for 3 months), 5 animals in the 20–30 month age group (5 animals, implantation for 9 months), and 5 animals in the 30–40 month age group (1 animal, implantation for 3 months; 1 animal, implantation for 9 months; 3 animals, implantation for 15 months), and for females, 6 animals in the 20–30 month age group (1 animal, implantation for 3 months; 5 animals, implantation for 9 months), 3 animals in 30–40 month age group (1 animal, implantation for 3 months; 2 animals, implantation for 15 months), and 2 animals in 40–50 month age group, (1 animal, implantation for 9 months; 1 animal, implantation for 15 months).

All animal experiments were approved by the Animal Care and Use Committee of Terumo Corporation and were performed in compliance with the Laboratory Animal Policy of Terumo Corporation.

### *Housing conditions*

Animals were bred in a conventional environment maintained under constant conditions: temperature of 18°C to 28°C, humidity of 30% to 70%, and a 12-hour light-dark cycle. Each animal was housed individually in a cage and fed M-16 (CLEA Japan, Tokyo, Japan) at approximately 500 g/day, with free access to water.

### *Body weight*

Body weight was measured under sedation, induced by an intramuscular injection of 0.04 mL/kg medetomidine hydrochloride (Domitor, Nippon Zenyaku Kogyo, Fukushima, Japan) and 0.04 mL/kg midazolam (SANDOZ, SANDOZ K.K., Yamagata, Japan), on the days of receipt of the animals, stent implantation, and necropsy.

### *Operating procedures*

All animals underwent human coronary artery stent implantation. The cervix was incised, a sheath was inserted into the carotid artery and accessed using a transcatheter procedure, and the stent was implanted. Animals received oral aspirin (BUFFERIN, Bristol-Myers Squibb, Princeton, NJ, USA) at a daily dose of 330 mg and 200 mg ticlopidine hydrochloride (Nichistate Fine Granules, Nichi-Iko Pharmaceutical, Toyama, Japan) from 3 days before until 8 weeks after stent implantation. At the time of implantation, pretreatment anesthesia was achieved using an intramuscular injection of 0.04 mL/kg medetomidine hydrochloride and 0.04 mL/kg midazolam. Animals were administered inhalant anesthesia of 2% to 4% sevoflurane (SEVOFRANE, Maruishi Pharmaceutical, Osaka, Japan) during surgery. A contrast medium, ioxilan (Imagenil, Terumo, Tokyo, Japan), was also injected at the time of stent implantation. Likewise, coronary angiography was performed at necropsy.

### *Necropsy and organ weight*

Animals were humanely sacrificed for necropsy by exsanguination under inhalant anesthesia of 2% to 4% sevoflurane. After necropsy, the following systemic organs were removed for histopathological examination: the cerebrum, cerebellum, spinal cord, eye, lacrimal gland, eyelid, thymus, spleen, mesenteric lymph node, heart, aorta, larynx, trachea, lung, tongue, sublingual gland, parotid gland, esophagus, stomach, duodenum, small intestine (excluding the duodenum), large intestine, liver, gallbladder, pancreas, kidney, urinary bladder, adrenal gland, pituitary gland, thyroid gland, parathyroid, testis, epididymis, prostate, seminal vesicle, ovary, uterus, vagina, skin, mammary gland, skeletal muscle, sternum, and femur. In addition, the brain, spleen, lung, sublingual gland, liver, kidney, adrenal gland, pituitary gland, testis, epididymis, prostate, seminal vesicle, ovary, uterus, vagina, and thyroid gland were weighed. The thymus was collected only from the thoracic cavity.

### *Histopathology*

The testes were placed in Bouin's fixative, and eyes were placed in Davidson's fixative. All other organs were fixed in 10% neutral buffered formalin. The organs were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE) and with other more specific types of staining such as Schmorl's staining (potassium ferricyanide and ferric chloride, Wako Pure Chemical Industries, Ltd., Osaka, Japan), Berlin blue staining (potassium ferrocyanide trihydrate, Wako Pure Chemical Industries Ltd., Osaka, Japan, and hydrochloric acid, Kanto Chemical Co., Inc., Tokyo, Japan), Hall staining (trichloroacetic acid, Kanto Chemical Co., Inc., Tokyo, Japan, and ferric chloride, Wako Pure Chemical Industries Ltd., Osaka, Japan), and Periodic acid-Schiff (PAS) staining (Schiff's reagent, Wako Pure Chemical Industries Ltd., Osaka, Japan).

## Results

### Body weight

The body weights of male pigs ranged from 20 kg to almost 30 kg in the 10–20-month age group, and the average was about 40 kg in the 20–30-month age group and 30–40-month age group (Table 1). The body weights of female pigs ranged from 30 kg to almost 40 kg in the 20–40-month age group, and the average was about 45 kg in the 40–50-month age group (Table 2).

### Organ weights

In male pigs, the spleen, lung, sublingual gland, liver, kidney, adrenal gland, and thyroid gland were heavier in animals of the 30–40-month age group than in those of the 10–20-month age group because of animal growth, whereas the testis, epididymis, and seminal vesicle were lighter at

these ages (Table 1). In female pigs, the lung, liver, kidney, adrenal gland, pituitary gland, ovary, and uterus were heavier in animals of the 40–50-month age group than in those of the 20–30-month age group (Table 2).

### Gross pathology

A thymus of reduced size was found in two male pigs in the 30–40-month age group, in two female pigs of the 30–40-month age group, and one female pig in the 40–50-month age group.

Calculi were found in the urinary bladder of one male pig in the 10–20-month age group, and of two male pigs each in the 20–30- and 30–40-month age groups.

Unilateral testicular atrophy was found in two male pigs in the 20–30-month age group and in three male pigs in the 30–40-month age group. Furthermore, one animal in the 30–40-month age group presented with undescended testes.

**Table 1.** Summary of Body and Organ Weights: Male

Groups	10–20 months			20–30 months			30–40 months		
	Number of animals	Mean	Range	Number of animals	Mean	Range	Number of animals	Mean	Range
Body weight(kg)	6	27.5	23.9–31.1	5	40.9	40.1–43.4	5	42.7	31.9–53.5
Organ weight(g)									
Brain	6	75.3	67.5–78.8	5	79.1	75.1–84.5	5	81.2	69.2–89.5
Spleen	6	41.1	37.5–43.2	5	62.1	52.4–72.4	5	63.9	46.5–82.8
Lung	6	162	141–230	5	196	170–255	5	266	193–466
Sublingual gland	6	19.5	12.6–23.3	5	36.8	23.8–64.5	5	33.7	26.1–39.4
Liver	6	405	315–479	5	449	370–482	4	724	445–946
Kidney	6	48.9	44.2–53.4	5	61.5	53.7–78.5	5	65.3	52.2–80.1
Adrenal gland	6	1.43	1.05–1.78	5	1.76	1.47–2.07	5	2.01	1.63–2.42
Pituitary gland	6	0.16	0.13–0.21	5	0.24	0.21–0.29	5	0.22	0.19–0.27
Testis	6	36.8	26.4–48.8	5	36.4	6.5–51.0	5	25.6	7.9–45.7
Epididymis	6	19.2	15.9–22.5	5	18.1	6.0–28.3	5	17.4	11.6–26.1
Prostate	5	5.72	3.69–7.25	5	7.1	5.42–9.36	5	6.44	4.05–9.02
Seminal vesicle	5	124	80–220	5	120	51–185	5	111	37–181
Thyroid gland	6	5.24	4.47–5.95	5	7.38	6.42–8.89	5	8.07	4.86–9.48

**Table 2.** Summary of Body and Organ Weights: Female

Groups	20–30 months			30–40 months			40–50 months		
	Number of animals	Mean	Range	Number of animals	Mean	Range	Number of animals	Mean	Range
Body weight (kg)	6	40.6	28.7–49.9	3	39.8	29.1–45.6	2	44.7	39.9–49.4
Organ weight (g)									
Brain	3*	72.7	71.3–75.1	3	83.2	81.7–84.7	2	81.6	80.3–83.0
Spleen	3*	64.9	47.5–75.5	3	64.3	51.6–76.4	2	58.9	50.3–67.6
Lung	3*	177	168–193	3	180	158–194	2	197	194–201
Sublingual gland	3*	12.2	10.5–13.6	3	13.6	7.3–17.3	2	14.1	13.2–14.9
Liver	3*	386	360–416	3	516	374–666	2	558	523–593
Kidney	3*	56.9	48.3–63.5	3	64.7	47.4–80.7	2	78.5	71.1–85.4
Adrenal gland	3*	1.65	1.40–1.84	3	1.76	1.57–2.13	2	2.53	2.07–2.76
Pituitary gland	3*	0.19	0.18–0.22	3	0.26	0.14–0.33	2	0.29	0.25–0.32
Ovary	3*	3.84	2.65–4.86	3	16.6	3.2–42.8	2	19.2	4.3–36.2
Uterus	3*	269	212–368	3	311	203–441	2	345	319–370
Thyroid gland	3*	5.44	4.13–6.48	3	5.37	4.91–5.71	2	6.66	5.68–7.64

\*The organ was weighed in only 3 animals at necropsy.

Among the animals with unilateral testicular atrophy, an atrophied epididymis was noted in two animals each in the 20–30- and 30–40-month age groups.

In female pigs, ovarian cysts were observed in one animal in the 20–30-month age group, two animals in the 30–40-month age group, and one animal in the 40–50-month age group.

### *Histopathology*

Incidences of histopathological findings for each organ are presented in Tables 3 and 4.

**Lacrimal gland:** In male pigs, mononuclear cell infiltration of the lacrimal gland was noted in all age groups. Neutrophilic infiltration, fibrin deposition, and hemorrhage were noted in the 10–20-month age group, while eosinophilic infiltration was noted only in the 20–30-month age group. In female pigs, mononuclear cell infiltration was found in the interstitium in the 30–40- and 40–50-month age groups. Moreover, hemorrhage, mineralization, and necrosis of acinar cells were found in the 20–30-month age group.

**Eyelid:** Mononuclear cell infiltration of the eyelid was found in both male and female pigs in all age groups. In male pigs, microgranuloma was noted in one animal in the 10–20-month age group, and eosinophilic infiltration was found in animals in the 20–30- and 30–40-month age groups. In female pigs, eosinophilic infiltration was found in the 20–30- and 30–40-month age groups, and pigment deposition was seen in basal cells in the 20–30-month age group.

**Thymus (Figs. 1 and 2):** Involution and eosinophilic infiltration of the thymus were noted in both male and female pigs in all age groups. The involution of the thymus was accompanied by fatty infiltration. The degree of involution was more severe in males in the 30–40-month age group than in the 10–20-month age group, and it was more severe in females in the 40–50-month group than in the 20–30-month age group. In male pigs, low incidences of mononuclear cell infiltration and macrophage, mainly consisting of foamy cells, infiltration were noted in the 20–30-month age group, while a low incidence of hemosiderin deposition was observed in the 30–40-month age group. In female pigs, low incidences of necrosis and mineralization were noted in the 20–30-month age group.

**Spleen (Figs. 3 and 4):** A high incidence of hemosiderin deposition was found in the spleen in male and female pigs in all age groups. In male pigs, eosinophilic infiltration was noted in the 20–30- and 30–40-month age groups, and a hematoma was observed in the 30–40-month age group. In female pigs, eosinophilic infiltration was found in all age groups, and atrophy of the white pulp was noted in the 40–50-month age group.

**Mesenteric lymph node:** In male pigs, eosinophilic infiltration of the mesenteric lymph nodes was noted in all age groups. Moreover, neutrophilic infiltration was noted in the 30–40-month age group. In female pigs, eosinophilic infiltration was seen in the 20–30- and 30–40-month age groups.

**Heart (Fig. 5):** A myocardium infarction was found in the distal area of the stent implantation site in one male pig from the 30–40 month age group; there were no other findings anywhere else. In female pigs, a low incidence of fatty infiltration was noted in the 20–30- and 30–40-month age groups.

**Larynx:** Mononuclear cell infiltration of the submucosa was found in male pigs in the 10–20- and 30–40-month age groups and in female pigs in the 20–30-month age group.

**Trachea:** In male pigs, mononuclear cell infiltration of the submucosa of the trachea was noted in all age groups, whereas in female pigs, this change was found in animals only in the 20–30- and 30–40-month age groups.

**Lung (Fig. 6):** A high incidence of mononuclear cell infiltration into the lung was noted in male and female pigs in all age groups. In male pigs, eosinophilic infiltration was noted in the 20–30- and 30–40-month age groups. Hemosiderin deposition, neutrophilic infiltration, hemorrhage, and foreign-body granuloma were found in the 30–40-month age group, while interstitial pneumonia and alveolar macrophage aggregation were found in the 20–30-month age group. In female pigs, eosinophilic infiltration was noted in all age groups. Osseous metaplasia, vascular obstruction, mineralization, fibrosis, inflammation, and an abscess were found in one animal in the 20–30-month age group. Hemorrhage was noted in the 30–40- and 40–50-month age groups, and hemosiderin deposition was observed in one animal in 40–50-month age group.

**Sublingual gland:** In male pigs, hyperplasia of the acinar cells of the sublingual gland was found in the 10–20- and 20–30-month age groups. In addition, hemosiderin deposition and mononuclear cell infiltration were found in the periductal areas in the 10–20-month age group. Eosinophilic infiltration was noted in the 20–30- and 30–40-month age groups, and fatty infiltration was observed in the 30–40-month age group. In female pigs, fatty infiltration was noted in all age groups, and mononuclear cell infiltration was observed in the periductal areas in the 20–30-month age group.

**Esophagus:** In male pigs, mononuclear cell infiltration was found in the submucosa of the esophagus in the 20–30- and 30–40-month age groups. Vacuolization of the mucosal epithelia was noted in the 10–20-month age group. Likewise, in female pigs, mononuclear cell infiltration was found in the submucosa in the 20–30- and 30–40-month age groups. Mononuclear cell infiltration was seen in the lamina propria in the 20–30- month age group, and vacuolization of the mucosal epithelia was noted in the 30–40- and 40–50 month-age groups.

**Stomach:** Mononuclear cell infiltration was noted in the lamina propria for males in all age groups, and mononuclear cell infiltration was observed in the submucosa of the stomach in the 20–30-month age group. Eosinophilic infiltration was noted in the lamina propria in the 20–30- and 30–40-month age groups. Likewise, in female pigs, mononuclear cell infiltration was noted in the lamina propria at a high incidence in the 20–30- and 30–40-month age groups,

and mononuclear cell infiltration was noted in the submucosa in the 40–50-month age group. Eosinophilic infiltration was noted in the lamina propria in female pigs in the 20–30- and 30–40-month age groups. In addition, congestion was observed in the lamina propria in the 20–30-month age group, and vacuolization was seen in the epithelium in the 30–40-month age group.

**Duodenum:** In male and female pigs of all age groups, mononuclear cell infiltration and eosinophilic infiltration were found in the lamina propria of the duodenum at a high incidence. Moreover, in male pigs, mononuclear cell infiltration was observed in the submucosa in the 30–40-month age group, and erosion was noted in the 10–20- and 20–30-month age groups.

**Small intestine (excluding the duodenum) (Fig. 7):** Mononuclear cell infiltration and eosinophilic infiltration were noted in the lamina propria of the small intestine at a high incidence in male and female pigs in all age groups. Moreover, erosion was found in male pigs in the 10–20- and 30–40-month age groups and in female pigs in the 20–30-month age group.

**Large intestine:** Mononuclear cell infiltration was found in the lamina propria at a high incidence in male pigs and female pigs in all age groups and was observed in the submucosa of the large intestine of males in the 20–30-month age group. In addition, in male pigs, eosinophilic infiltration was noted in the lamina propria in all age groups and in the submucosa in the 20–30-month age group. In female pigs, eosinophilic infiltration of the lamina propria was noted in the 20–30- and 30–40-month age groups, and erosion was also observed in the 20–30-month age group.

**Liver:** In male pigs, centrilobular hemorrhage and microgranuloma were observed in the 10–20-month age group, and focal necrosis of the liver was seen in the 20–30-month age group. Eosinophilic infiltration, hemosiderin deposition in Kupffer cells, thrombus, centrilobular congestion, and extramedullary hematopoiesis were all noted in the 30–40-month age group. Furthermore, mononuclear cell infiltration was observed in the 20–30- and 30–40-month age groups. The incidence of all of these findings was low. In female pigs, hemosiderin deposition was found in all age groups, mononuclear cell infiltration was seen in the 20–30- and 30–40-month age groups, and microgranuloma was observed in the 20–30-month age group.

**Gallbladder:** In male pigs, mononuclear cell infiltration of the submucosa of the gallbladder was found in the 10–20- and 20–30-month age groups, and mononuclear cell infiltration was noted in the lamina propria in the 20–30- and 30–40-month age groups. Moreover, eosinophilic infiltration of the submucosa and hyperplasia of the lymphoid tissues were noted in the 20–30-month age group. In female pigs, mononuclear cell infiltration of the lamina propria was noted in the 20–30-month age group, and mononuclear cell infiltration of the submucosa was seen in the 40–50-month age group. Moreover, eosinophilic infiltration was found in the 20–30-month age group, and hyperplasia of the lymphoid tissues was seen in the 20–30- and 30–40-month age

groups.

**Pancreas:** Fatty infiltration of the pancreas was observed in male and female animals in all age groups at a high incidence. In male pigs, a low incidence of reduced islets of Langerhans was seen in the 10–20-month age group. In female pigs, a low incidence of microgranulomas was observed in the 30–40-month age group.

**Kidney (Figs. 8–11):** In male pigs in all age groups, lipofuscin deposition in the proximal tubular epithelia (Figs. 8 and 9) and PAS-negative eosinophilic globules in the Bowman's space and in the lumen of the proximal tubules of the kidney (Figs. 10 and 11) were found at a high incidence. Cysts in the cortex were observed in male pigs in all age groups. Moreover, degeneration of the tubular epithelia and interstitial mononuclear cell infiltration were observed in male pigs in the 30–40-month age group. In female pigs in all age groups, a high incidence of PAS-negative eosinophilic globules in the Bowman's space and in the lumen of the proximal tubules was noted. Cysts were also observed in the cortex in female pigs in all age groups. Moreover, in female pigs, lipofuscin deposition was seen in the tubular epithelia in the 20–30- and 30–40-month age groups, mononuclear cell infiltration of the interstitium was observed in the 20–30- and 40–50-month age groups, fatty degeneration was seen in the 20–30-month age group, and degeneration of the tubular epithelia was noted in the 30–40-month age group.

**Urinary bladder (Figs. 12 and 13):** In male pigs, mucinous metaplasia was noted in the 10–20- and 20–30-month age groups, and mononuclear cell infiltration of the submucosa of the urinary bladder was seen in the 20–30-month age group. Moreover, a hematoma was found in one animal in the 30–40-month age group, and neutrophilic infiltration, hemorrhage, and hemosiderin deposition were observed in the serosa in this age group. The incidence of all of these findings was low. In female pigs, mononuclear cell infiltration of the submucosa was found in the 20–30- and 40–50-month age groups.

**Adrenal gland:** In male pigs, fatty infiltration of the adrenal gland was noted in the 20–30- and 30–40-month age groups, and accessory adrenocortical tissue was observed in the 30–40-month age group. In female pigs, fatty infiltration was noted in the 20–30-month age group.

**Pituitary gland:** In male pigs, vacuolization of the anterior lobe of the pituitary gland was found in the 10–20-month age group. In the intermediate lobe, mononuclear cell infiltration was observed in the 20–30-month age group, and eosinophilic infiltration was observed in the 30–40-month age group. In female pigs, mononuclear cell infiltration of the anterior lobe was noted in the 20–30- and 30–40-month age groups, and mineralization was seen in the intermediate lobe in the 20–30-month age group.

**Testis (Fig. 14):** Neither tubular atrophy nor Leydig cell hyperplasia was present in the testis of animals in the 10–20-month age group; however, the incidence of these findings increased with age, such that both were observed in 2/5 animals in the 20–30-month age group and all animals

**Table 3.** Incidence of Histopathological Findings: Male

Organ	Findings	The age of animals at necropsy, months			
		10–20 (n=6)	20–30 (n=5)	30–40 (n=5)	
Cerebrum	Infiltration, mononuclear cell, gray matter	0	1	0	
Eye	Infiltration, mononuclear cell, cornea	0	1	0	
Lacrimal gland	Hemorrhage	1	0	0	
	Deposit, fibrin	2	0	0	
Eyelid	Infiltration, mononuclear cell, interstitium	1	2	2	
	Infiltration, eosinophilic, interstitium	0	1	0	
	Infiltration, neutrophilic, interstitium	2	0	0	
	Microgranuloma	1	0	0	
	Infiltration, mononuclear cell	4	5	4	
Thymus	Infiltration, eosinophilic	0	1	3	
	Involution	4	2	4	
	Deposit, hemosiderin	0	0	1	
Spleen	Infiltration, mononuclear cell	0	1	0	
	Infiltration, eosinophilic	2	3	4	
	Infiltration, macrophage	0	1	0	
	Hematoma	0	0	1	
Lymph node, mesenteric	Deposit, hemosiderin	5	4	5	
	Infiltration, eosinophilic	0	5	2	
	Infiltration, eosinophilic	4	5	5	
Heart	Infiltration, neutrophilic	0	0	1	
	Infarct*	0	0	1	
Larynx	Infiltration, mononuclear cell, submucosa	2	0	1	
Trachea	Infiltration, mononuclear cell, submucosa	3	1	2	
Lung	Alveolar macrophage aggregation	0	1	0	
	Granuloma, foreign body	0	0	1	
	Pneumonitis, interstitial	0	1	0	
	Hemorrhage	0	0	3	
	Deposit, hemosiderin	0	0	2	
	Infiltration, mononuclear cell	3	5	5	
	Infiltration, eosinophilic	0	3	2	
	Infiltration, neutrophilic	0	0	1	
	Sublingual gland	Hyperplasia, acinar cell	1	2	0
		Fatty infiltration	0	0	1
Deposit, hemosiderin		2	0	0	
Infiltration, mononuclear cell, periductal		1	0	0	
Esophagus	Infiltration, eosinophilic	0	1	1	
	Vacuolization, epithelium	1	0	0	
Stomach	Infiltration, mononuclear cell, submucosa	0	1	1	
	Infiltration, mononuclear cell, lamina propria	2	3	5	
Duodenum	Infiltration, eosinophilic, lamina propria	0	2	3	
	Erosion	2	1	0	
	Infiltration, mononuclear cell, submucosa	0	0	1	
Small intestine (excluding the duodenum)	Infiltration, mononuclear cell, lamina propria	3	5	5	
	Infiltration, eosinophilic, lamina propria	5	5	4	
	Infiltration, mononuclear cell, submucosa	0	1	0	
	Infiltration, mononuclear cell, lamina propria	6	5	5	
Large intestine	Infiltration, eosinophilic, submucosa	0	1	0	
	Infiltration, eosinophilic, lamina propria	5	3	2	
	Infiltration, eosinophilic, lamina propria	5	3	2	
Liver	Thrombus	0	0	1	
	Congestion, centrilobular	0	0	1	
	Extramedullary hematopoiesis	0	0	1	
	Microgranuloma	1	0	0	
	Hemorrhage, centrilobular	1	0	0	
	Deposit, hemosiderin, Kupffer cell	0	0	2	
	Necrosis, focal	0	1	0	
	Infiltration, mononuclear cell	0	1	2	
	Infiltration, eosinophilic	0	0	1	

\*Infarct: this change occurs in downstream myocardium of the stent implantation site.

**Table 3.** Continued.

Organ	Findings	The age of animals at necropsy, months		
		10–20 (n=6)	20–30 (n=5)	30–40 (n=5)
Gallbladder	Hyperplasia of lymphoid tissue	0	1	0
	Infiltration, mononuclear cell, submucosa	1	1	0
	Infiltration, mononuclear cell, lamina propria	0	4	1
Pancreas	Infiltration, eosinophilic, submucosa	0	1	0
	Fatty infiltration	3	4	5
Kidney	Reduction of islets of langerhans	1	0	0
	Cyst, cortex	4	1	2
	Eosinophilic globules, Bowman's space/tubular lumen	6	5	5
Urinary bladder	Degeneration, tubular epithelium	0	0	3
	Deposit, lipofuscin, proximal tubular epithelium	4	4	3
	Infiltration, mononuclear cell, interstitium	0	0	3
	Mucinous metaplasia	2	1	0
	Hematoma	0	0	1
	Hemorrhage, serosa	0	0	1
	Deposit, hemosiderin, serosa	0	0	1
Adrenal gland	Infiltration, mononuclear cell, submucosa	0	2	0
	Infiltration, neutrophilic, serosa	0	0	1
Pituitary gland	Fatty infiltration	0	1	1
	Accessory adrenocortical tissue	0	0	1
Testis	Degeneration, vacuolar, pars distalis	1	0	0
	Infiltration, mononuclear cell, pars intermedia	0	1	0
	Infiltration, eosinophilic, pars intermedia	0	0	1
Epididymis	Atrophy, tubular	0	2	5
	Hyperplasia, Leydig cell	0	2	5
Prostate	Atrophy	0	2	3
	Cyst	0	0	1
Seminal vesicle	Atrophy	1	0	1
	Mineralization	1	0	2
Seminal vesicle	Hemorrhage	0	0	1
	Hyperplasia	0	3	1

in the 30–40-month age groups. These changes included a unilateral alteration in 2/2 animals in the 20–30-month group and in 3/5 animals in the 30–40-month group. Spermatogenesis was decreased in all of these animals in the 30–40-month age group. The Leydig cell hyperplasia was characterized by an increase in the number and hypertrophy of the Leydig cells.

**Epididymis (Fig. 15):** Epididymal atrophy was not seen in the 10–20-month age group; however, the incidence increased with age, and atrophy was found at a high incidence in the 30–40-month age group. This change was found unilaterally in 2/2 animals in the 20–30-month age group and in 2/3 animals in the 30–40-month age group. Sperm was not present in the atrophied epididymis. Furthermore, a low incidence of cysts was observed in the 30–40-month age group.

**Prostate:** A low incidence of atrophy and mineralization of the prostate was observed in the 10–20- and 30–40-month age groups.

**Seminal vesicle (Figs. 16 and 17):** Hyperplasia of the epithelium of the seminal vesicle was observed in the 20–30- and 30–40-month age groups, and hemorrhage was seen in the 30–40-month age group.

**Ovary (Figs. 18 and 19):** Cyst-like follicles were found at a high incidence in the ovary in all age groups. The cyst-

like follicles were lined by granulosa cells. In addition, hemorrhage and eosinophilic infiltration were observed in the 20–30-month age group, mineralization was observed in the 20–30- and 30–40-month age groups, and hemosiderin deposition was noted in the 20–30- and 40–50-month age groups.

**Skeletal muscle:** Fatty infiltration of the skeletal muscle was observed at a high incidence in female pigs of all age groups.

**Other organs:** The following were present at a low incidence in the other organs examined: mononuclear cell infiltration in the cerebrum, eye, and tongue; eosinophilic infiltration in the uterus; fatty infiltration in the parotid gland and thyroid gland; and follicular dilatation in the thyroid gland. No remarkable changes were observed in the cerebellum, spinal cord, aorta, parathyroid, vagina, skin, mammary gland, and bone marrow of the sternum and femur.

## Discussion

Numerous reports have been published related to clinical research using minipigs, including in the fields of cardiology and radiology<sup>1, 2</sup>. However, only a few reports providing histopathological background data on systemic organs in minipigs are currently available<sup>10, 11</sup>. In this study,

**Table 4.** Incidence of Histopathological Findings: Female

Organ	Findings	The age of animals at necropsy, months		
		20–30 (n=6)	30–40 (n=3)	40–50 (n=2)
Lacrimal gland	Necrosis, acinar cell	1	0	0
	Hemorrhage	1	0	0
	Mineralization	1	0	0
Eyelid	Infiltration, mononuclear cell, interstitium	0	1	1
	Deposit, pigment, basal cell	1	0	0
	Infiltration, mononuclear cell	4	3	1
Thymus	Infiltration, eosinophilic	2	2	0
	Involution	3	2	2
	Necrosis	1	0	0
Spleen	Mineralization	2	0	0
	Infiltration, eosinophilic	4	1	1
	Atrophy, white pulp	0	0	2
Lymph node, mesenteric	Deposit, hemosiderin	6	3	2
	Infiltration, eosinophilic	1	2	1
	Infiltration, eosinophilic	2	1	0
Heart	Fatty infiltration	1	1	0
Larynx	Infiltration, mononuclear cell, submucosa	2	0	0
Trachea	Infiltration, mononuclear cell, submucosa	3	1	0
Lung	Hemorrhage	0	1	1
	Deposit, hemosiderin	0	0	1
	Metaplasia, osseous	1	0	0
	Obstruction, vessels	1	0	0
	Mineralization	1	0	0
	Fibrosis	1	0	0
	Inflammation	1	0	0
	Abscess	1	0	0
	Infiltration, mononuclear cell	6	2	2
	Infiltration, eosinophilic	4	2	1
Tongue	Infiltration, mononuclear cell	1	0	0
Sublingual gland	Fatty infiltration	5	3	2
	Infiltration, mononuclear cell, periductal	1	0	0
Parotid gland	Fatty infiltration	1	0	0
Esophagus	Vacuolization, epithelium	0	2	1
	Infiltration, mononuclear cell, submucosa	1	1	0
	Infiltration, mononuclear cell, lamina propria	1	0	0
Stomach	Congestion, lamina propria	1	0	0
	Vacuolization, epithelium	0	1	0
	Infiltration, mononuclear cell, submucosa	0	0	1
Duodenum	Infiltration, mononuclear cell, lamina propria	6	3	0
	Infiltration, eosinophilic, lamina propria	5	2	0
	Infiltration, mononuclear cell, lamina propria	6	2	2
Small intestine (excluding the duodenum)	Infiltration, eosinophilic, lamina propria	5	2	2
	Erosion	1	0	0
	Infiltration, mononuclear cell, lamina propria	6	3	2
Large intestine	Infiltration, eosinophilic, lamina propria	6	3	2
	Erosion	1	0	0
	Infiltration, mononuclear cell, lamina propria	6	2	1
Liver	Infiltration, eosinophilic, lamina propria	5	2	0
	Microgranuloma	1	0	0
	Deposit, hemosiderin, Kupffer cell	2	2	1
Gallbladder	Infiltration, mononuclear cell	3	1	0
	Hyperplasia of lymphoid tissue	2	1	0
	Infiltration, mononuclear cell, submucosa	0	0	1
Pancreas	Infiltration, mononuclear cell, lamina propria	4	0	0
	Infiltration, eosinophilic, submucosa	2	0	0
	Fatty infiltration	6	3	2
Kidney	Microgranuloma	0	1	0
	Cyst, cortex	4	1	1
	Eosinophilic globules, Bowman's space/tubular lumen	4	3	1
	Degeneration, tubular epithelium	0	2	0
	Degeneration, fatty, tubular epithelium	1	0	0
	Deposit, lipofuscin, proximal tubular epithelium	1	1	0
	Infiltration, mononuclear cell, interstitium	2	0	1



**Table 4.** Continued.

Organ	Findings	The age of animals at necropsy, months		
		20–30 (n=6)	30–40 (n=3)	40–50 (n=2)
Urinary bladder	Infiltration, mononuclear cell, submucosa	1	0	1
Adrenal gland	Fatty infiltration	1	0	0
Pituitary gland	Mineralization, pars intermedia	2	0	0
Thyroid gland	Infiltration, mononuclear cell, pars distalis	2	2	0
	Fatty infiltration	1	0	0
Ovary	Follicular dilatation	0	1	0
	Cyst-like follicle	5	3	2
	Hemorrhage	4	0	0
	Deposit, hemosiderin	2	0	1
Uterus	Mineralization	2	1	0
	Infiltration, eosinophilic	2	0	0
Muscle	Infiltration, eosinophilic	1	0	0
	Fatty infiltration	5	3	2

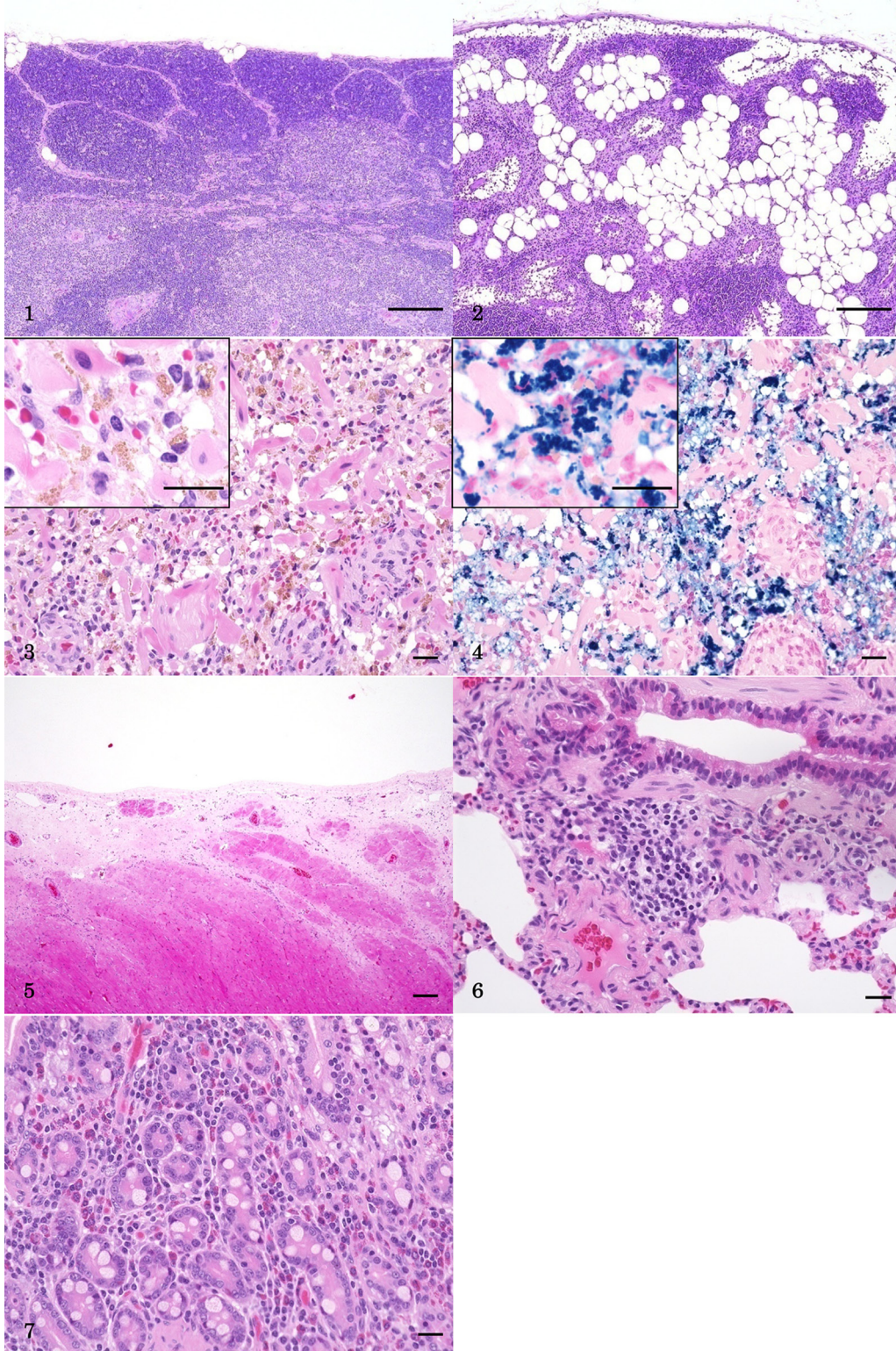
we sought to collect such histological background data and performed histopathological examinations on CLAWN miniature swine bred in our facilities for long-term studies of coronary artery stents. These data were then compared with the histopathological findings of Göttingen minipigs published previously.

In male animals older than 20 months of age, the weights of the testis and epididymis were reduced. Seminiferous tubular atrophy, Leydig cell hyperplasia, and epididymal atrophy were observed by histopathological examination in male animals older than 20 months of age. Tubular atrophy has been reported to occur in the testis with a high incidence in younger Göttingen minipigs (64.4% at 4–5 months of age, 87.0% at 6–10 months of age, and 86.4% at 10–15 months of age)<sup>12</sup>. Therefore, we consider that seminiferous tubular atrophy is a common finding in the testis of both CLAWN miniature swine and Göttingen minipigs. On the other hand, there were some differences between CLAWN miniature swine and Göttingen minipigs. The first was the difference between the two strains in the timing of the occurrence of seminiferous tubular atrophy. In the Göttingen minipig study, there was no relation between testicular tissue change and age<sup>12</sup>. In the present study with CLAWN miniature swine, an age-related difference was observed in the occurrence of seminiferous tubular atrophy. No abnormal findings were observed in the testis of animals from the youngest age group (10–20 months), while seminiferous tubular atrophy was observed in the animals older than 20 months of age. The incidence of seminiferous tubular atrophy was increased in the 30–40-month age group as compared with that in the 20–30-month age group. The CLAWN miniature swine examined in all the age groups in the present study were sexually mature, as CLAWN miniature swine become sexually mature at 6 months of age<sup>13</sup>. Furthermore, the CLAWN miniature swine in the present study were probably fertile, because the average life span for miniature swine is about 15 years<sup>14</sup>. The histological findings in the testis were not attributed to either housing

conditions or feeding, as there were no abnormal changes in general conditions or body weight caused by either aging or the stent implantation procedure. Regarding the seminiferous tubular atrophy in the testis that was observed in miniature swine, further research is needed in order to clarify the relation of atrophy with age and the causative factors. In the present study, many of the CLAWN miniature swine exhibiting seminiferous tubular atrophy also exhibited Leydig cell hyperplasia in the testis and atrophy of the epididymis, and this was second difference between CLAWN miniature swine and Göttingen minipigs. These two findings were not reported in the Göttingen minipig study<sup>12</sup>. The differences in the occurrences of Leydig cell hyperplasia and epididymal atrophy might be attributed to the low severity of seminiferous tubular atrophy in the Göttingen minipig study<sup>12</sup>. On disruption of spermatogenesis by seminiferous tubular atrophy, the reduction of testosterone levels results in increased luteinizing hormone secretion from the pituitary gland followed by Leydig cell proliferation<sup>15</sup>. Measurement of hormone levels will provide further evidence to understand Leydig cell hypertrophy in CLAWN miniature swine.

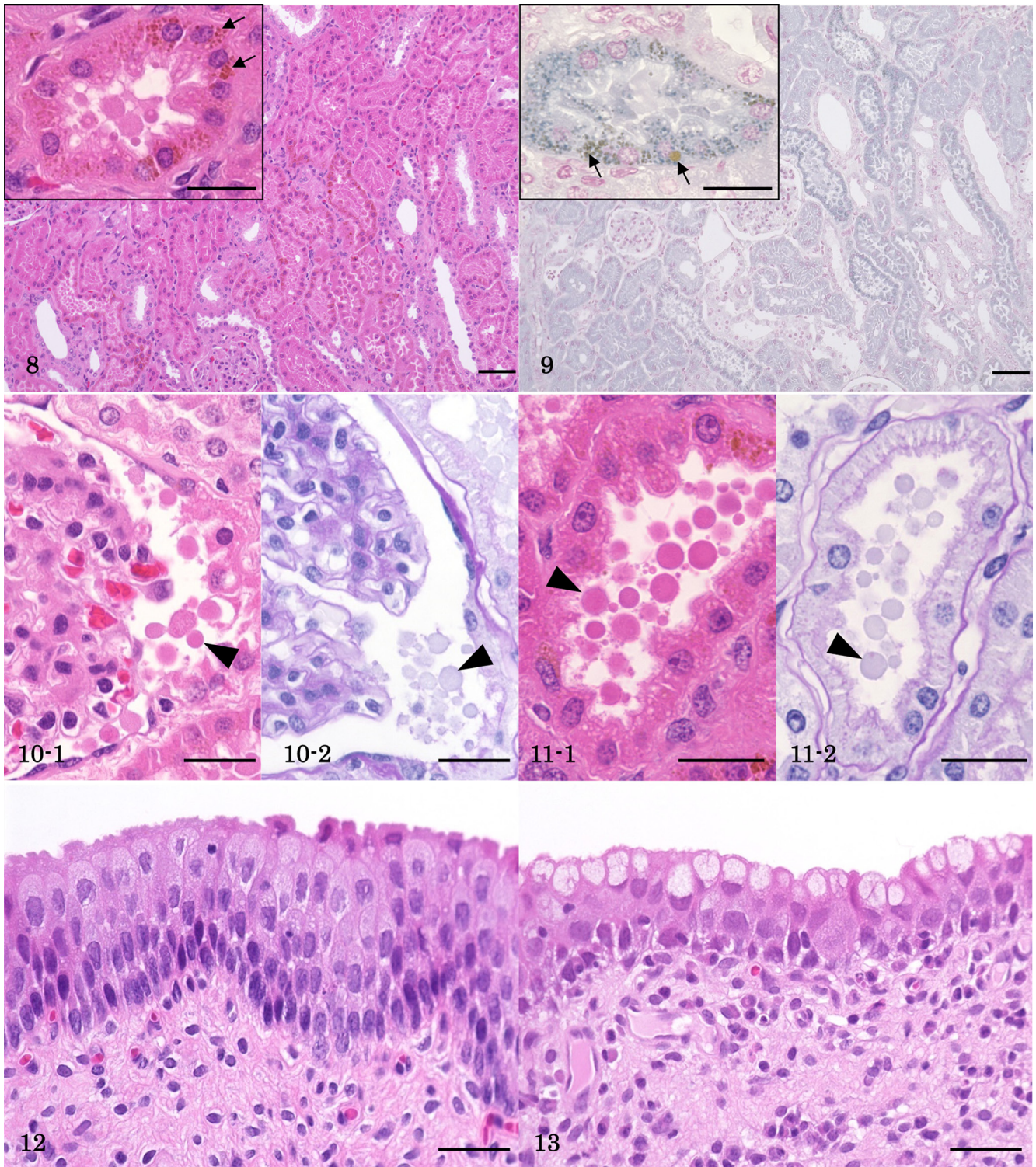
In the ovaries of female pigs, cyst-like follicles were observed in almost all animals aged 20–50 months examined in this study. A report on histological changes in the genital organs of female Göttingen minipigs (aged 5.5–21 months) indicated the presence of large follicles that were devoid of oocytes upon sexual maturation, suggesting that the large follicles could be attributed to normal cyclical changes (tertiary follicles) in swine. However, the possibility of ovarian cysts due to pathological change cannot be excluded<sup>16</sup>. The histological characteristics of the ovaries in the CLAWN miniature swine examined in the current study closely resemble those of large follicles that have been previously observed in Göttingen minipigs; therefore, the pathogenesis of these changes is quite intriguing.

In the kidneys, lipofuscin deposition was observed in the proximal tubules. In addition, eosinophilic globules were found in the Bowman's space and lumen of the proxi-



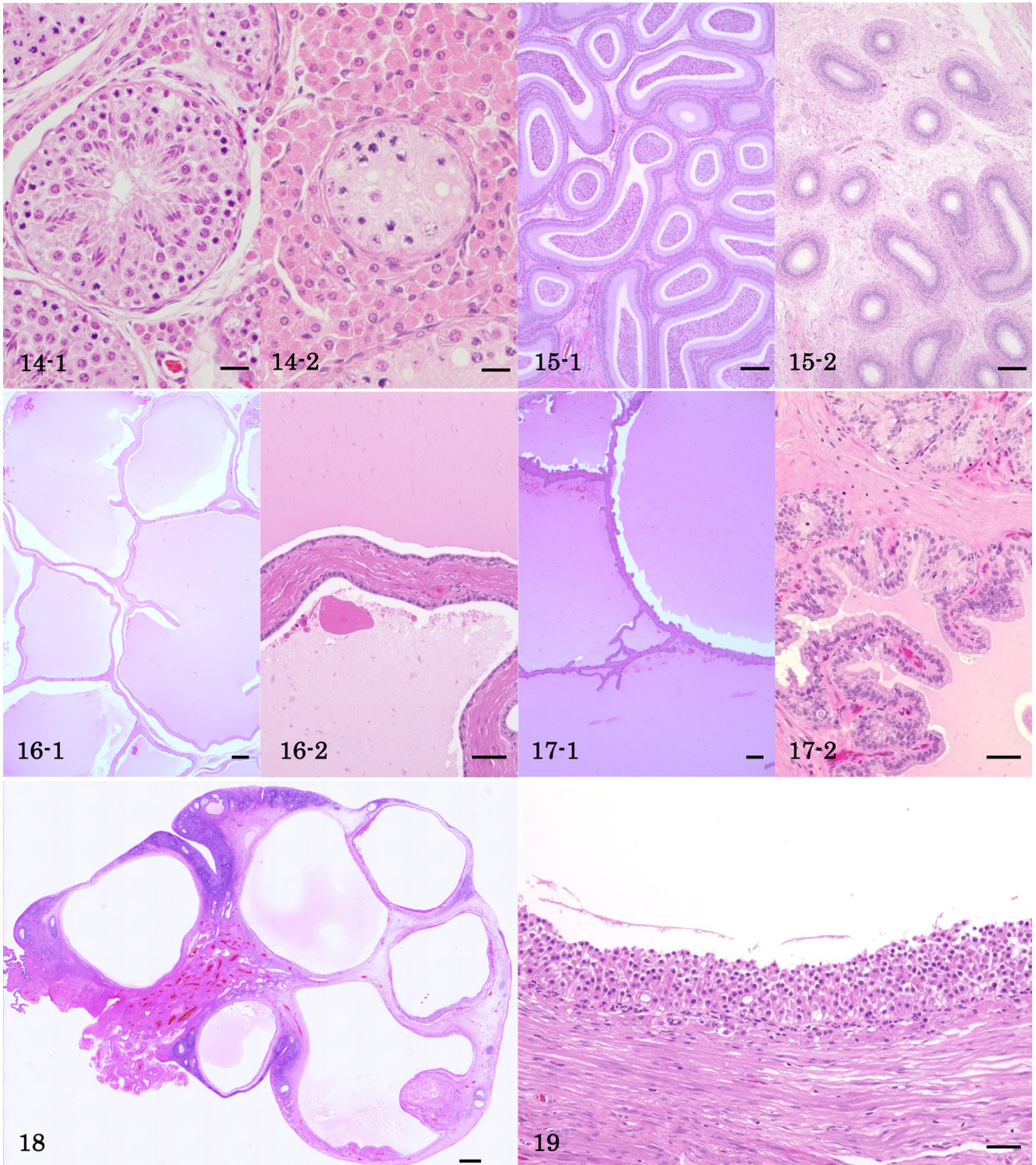
- Fig. 1.** No abnormal findings in the thymus. HE stain. Bar = 200  $\mu$ m.
- Fig. 2.** Thymus involution with fatty infiltration. HE stain. Bar = 200  $\mu$ m.
- Fig. 3.** Hemosiderin depositions in the spleen. HE stain. Bar = 20  $\mu$ m. Inset: high magnification. Bar = 20  $\mu$ m.
- Fig. 4.** Hemosiderin deposition in the spleen in the same animal as shown in Fig. 3. Berlin blue stain. Bar = 20  $\mu$ m. Inset: high magnification. Bar = 20  $\mu$ m.
- Fig. 5.** Myocardium infarction in distal area of the stent implantation site. HE stain. Bar = 200  $\mu$ m.
- Fig. 6.** Mononuclear cell infiltration in the lung. HE stain. Bar = 20  $\mu$ m.
- Fig. 7.** Eosinophilic infiltration in the lamina propria of the small intestine. HE stain. Bar = 20  $\mu$ m.





- Fig. 8.** Lipofuscin deposition in the proximal tubular epithelium of the kidney. HE stain. Bar = 50  $\mu$ m. Inset: high magnification. Arrows indicated lipofuscin pigments in the tubular cells. Bar = 20  $\mu$ m.
- Fig. 9.** Lipofuscin deposition in the proximal tubular epithelium of the same animal as shown in Fig. 8. Schmorl stain. Bar = 50  $\mu$ m. Inset: high magnification. Arrows indicate lipofuscin pigments. Bar = 20  $\mu$ m.
- Fig. 10.** Eosinophilic globules (arrowhead) in the Bowman's space of the kidney. (1) HE stain. (2) Eosinophilic globules (arrowhead) were negative with PAS stain. Bar = 20  $\mu$ m.
- Fig. 11.** Eosinophilic globules (arrowhead) in the tubular lumen of the kidney. (1) HE stain. (2) Eosinophilic globules (arrowhead) were negative with PAS stain. Bar = 20  $\mu$ m.
- Fig. 12.** No findings in the urinary bladder. HE stain. Bar = 50  $\mu$ m.
- Fig. 13.** Mucinous metaplasia in the epithelium of the urinary bladder. HE stain. Bar = 50  $\mu$ m.





- Fig. 14.** Testis. (1) No findings in the testis. (2) Tubular atrophy and Leydig cell hyperplasia in the testis. HE stain. Bar = 20  $\mu$ m.
- Fig. 15.** Epididymis. (1) No findings in the epididymis. (2) Atrophy of the epididymis; absence of sperm and ductal atrophy are indicated. HE stain. Bar = 200  $\mu$ m.
- Fig. 16.** No findings in the seminal vesicle. HE stain. (1) Low magnification. Bar = 200  $\mu$ m. (2) High magnification. Bar = 50  $\mu$ m.
- Fig. 17.** Hyperplasia of the epithelium in the seminal vesicle. HE stain. (1) Low magnification. Bar = 200  $\mu$ m. (2) High magnification. Bar = 50  $\mu$ m.
- Fig. 18.** Cyst-like follicles in the ovary. HE stain. Bar = 1,000  $\mu$ m.
- Fig. 19.** Granulosa cells lining cyst-like follicles. HE stain. Bar = 50  $\mu$ m.

mal tubules of both sexes in all age groups; these changes have not been reported in Göttingen minipigs<sup>10</sup>. Urinary excretion of various proteins and enzymes is known to increase following injection of a contrast medium. However, histopathological changes such as lipofuscin and eosinophilic globules have not reported in an animal model<sup>17</sup>. With respect to lipofuscin, its deposition in the cytoplasm of the proximal tubules has also been observed in rats, dogs, and monkeys, similar to the findings in CLAWN miniature swine. Lipofuscin can be associated with aging and with the deposition of oxidized proteins as nondegradable substances in cellular lysosomes<sup>18</sup>. In the current study, there was no correlation between the level of lipofuscin deposition in the proximal tubular epithelia and the age of the animals used. Further research will be required to elucidate the underlying pathogenesis of these observations.

Hemosiderin deposition was found in the spleen of almost all animals of both sexes. Although hemosiderin deposition in the liver has also been observed in younger Göttingen minipigs (3, 6, and 12 months), no incidence in the spleen has been reported<sup>19</sup>. In our study, hemosiderin deposition was found in the spleen in almost all animals of both sexes, indicating that this might occur at a high incidence in CLAWN miniature swine older than 20 months of age. Alternatively, this could be a unique feature of CLAWN miniature swine.

In animals of both sexes in all age groups, mononuclear cell or eosinophilic infiltration was noted at a high incidence in the eyelid, respiratory organs, and digestive tract. In the stomach and lower digestive tract, infiltration of mononuclear cells was observed in the submucosa and/or lamina propria, but eosinophilic infiltration was characteristically observed in the lamina propria. These organs are often exposed to the external environment and thus may be susceptible to cellular infiltration, as has been suggested for other animal species, including Göttingen minipigs<sup>19,20</sup>. It is therefore possible that the environment affects the incidence of cellular infiltration.

The swine thymus is present not only in the thoracic cavity but also in the cervix<sup>21</sup>. In the current study, we chose to examine only the thoracic thymus. We found that the incidence of thymus involution was higher in male pigs in the 30–40-month age group and in female pigs in the older age groups. The rat thymus undergoes a slow decrease in size with age, in both the cortex and medulla, beginning at sexual maturity. In advanced cases of involution, this is accompanied by infiltration of the adipose tissue into the cortex<sup>22</sup>. Moreover, in the human thymus, it has been proposed that the perivascular spaces expand as the thymic epithelial spaces shrink and that these expanded perivascular tissues are then infiltrated by adipocytes, which contributes to the involution<sup>23</sup>. In the CLAWN miniature swine examined in the current study, adipocytes were found throughout the perivascular tissues within the thymus and were sporadically dispersed throughout the cortex, suggesting that the process of thymus involution in CLAWN miniature swine may resemble that of humans.

In summary, the most common microscopic findings from the current study were as follows: testicular/epididymal atrophy, cyst-like follicles in the ovaries, hemosiderin deposition in the spleen, lipofuscin deposition in the proximal tubular epithelia, the presence of eosinophilic globules in the Bowman's space and the lumen of the proximal tubules in the kidneys, and cellular infiltrations in several organs, including the eyelids, respiratory organs, and digestive tract. None of the changes were serious enough to significantly affect the intended research, and it was thus concluded that CLAWN miniature swine are a suitable animal model for various types of experiments. It is our hope that this comprehensive set of background data on untreated animals will allow for a deeper understanding of the normal histology of CLAWN miniature swine.

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**Disclosure of Potential Conflicts of Interest:** The authors declare that they have no competing interests.

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