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A 12-month Feeding Study of Reproduction/Development in Rats Fed Meat/Milk Powder Supplemented Diets Derived from the Progeny of Cloned Cattle Produced by Somatic Cell Nuclear Transfer

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Abstract. The present 12-month feeding study was carried out with rat groups fed a diet supplemented with meat or milk (meat/milk) derived from the progeny of clones produced by somatic cell nuclear transfer (SCNT) technology. It was conducted to obtain data concerning the chronic toxicities of these edible products during the process of development and reproduction in rats fed such products. The rats were subjected to clinical observations for general health condition and examinations such as sensory/reflex function, grip strength, motor activity, body weight, food consumption, ophthalmology and urinalysis. Moreover, sexually matured rats fed the test diets were mated and examined for items such as the reproductive performances of the dams and health of their pups. After the feeding period, factors related to rat health status, based on the findings for hematology, blood biochemistry, necropsy, organ weight and histology, were examined. There were no biologically significant differences in these factors between the rat groups fed meat/milk powder supplemented diets derived from the progeny and those fed meat/milk powder supplemented diets derived from conventionally bred cattle. Therefore, the present chronic toxicity study suggests that meat and milk derived from the progeny of SCNT cattle might be equivalent to those derived from conventionally bred cattle in use as dietary supplements for rats.

Key words: Meat, Milk, 12 Month-feeding study, Progeny of cloned cattle, Rat, Somatic cell nuclear transfer (SCNT) (J. Reprod. Dev. 54: 321–334, 2008)

In 1998, the first somatic cell nuclear transfer (SCNT) cattle were produced in Japan [1]. Since the first birth of somatic cell cloned calves, many calves derived somatic cell cloning have been produced domestically. Almost all SCNT cattle of both sexes are fertile [2–4], and the first Japanese progeny derived from SCNT cattle were produced by artificial insemination (AI) in 2000 [5]. Our previous survey showed that at least 32 progeny of SCNT cattle have been used in examinations such as hematology, clinical chemistry, pathology, growth performance and meat production performance at Japanese institutions [5]. Their studies showed that there are no significant differences in the findings of these examinations between the progeny of SCNT cattle and conventionally bred cattle. Also, no abnormalities have been found in the blood parameters of the progeny of SCNT cattle produced in France [6], New Zealand [7], the United States of America (USA) [8], Brazil [8] and Argentina [8].

In January 2008, the Food and Drug Administration (FDA) of USA concluded that consumption of meat and milk derived from the clones of cattle, swine and goats and the progeny of clones from any species traditionally consumed as food is as safe as those from conventionally bred cattle [9]. At the same time, the European Food Safety Authority (EFSA) of the European Commission launched its draft opinion on animal cloning for public consultation [10]. The draft also demonstrates that healthy clones and healthy progeny do not show any significant differences from their conven-

tional counterparts.

The safety of meat and milk derived from the progeny of clones might be concluded by arguments based on science [9]; however, public controversy concerning the safety of these edible products remains based on impressions concerning reliability [11]. Thus, the present study was conducted to obtain data concerning the chronic toxicities of these products by 12-month feeding study of meat/milk powder derived from the progeny of SCNT cattle in rats as model animals for humans. The items examined here were related to the development and reproduction of rats. The data obtained will be important evidence concerning the reliability of edible products derived from the progeny of SCNT cattle.

Materials and Methods

Progeny of clones and conventionally bred cattle

Three female progeny derived from beef cattle (Japanese Black) were produced by AI of conventionally bred cows with frozen-thawed semen derived from a SCNT bull [2]. They were produced and raised at the Oita Prefectural Agriculture, Forestry and Fisheries Research Center (Oita, Japan). Three conventionally bred beef cattle (Japanese black) were also produced by AI as controls and were raised at the Shiga Prefectural Livestock Technology Promotion Center (Shiga, Japan). These cattle were fattened for meat production.

Three female progeny derived from dairy cattle (Holstein) were produced by AI of SCNT cows with frozen-thawed semen derived from a conventionally bred bull [12]. Three conventionally bred

Table 1. Nutritional analysis of meat/milk powder derived from the progeny of SCNT cattle and conventionally bred cattle

	Meat powder* derived from		Milk powder** derived from	
	Progeny of SCNT cattle	Conventionally bred cattle	Progeny of SCNT cattle	Conventionally bred cattle
Macronutrients (%; w/v)				
Crude protein	43.3	41.5	22.8	24.6
Crude fat	52.3	54.6	31.9	24.6
Carbohydrate	1.2	1.2	34.7	39.1
Crude fiber	< 0.1	< 0.1	< 0.1	< 0.1
Ash content	2.1	1.9	5.1	5.7
Water content	1.1	0.8	5.5	6.0
Vitamins (ppm)				
A (retinol)	0.08	0.04	3.9	5.4
B ₁	2.1	1.9	4.4	3.5
B ₂	4.9	6.8	9.0	10.0
B ₆	7.5	6.2	4.2	3.2
B ₁₂	0.022	0.029	0.037	0.032
D ₃	< 0.01	< 0.01	0.003	0.005
E	3	2	6	5
K ₂	0.08	0.14	0.11	0.07
Niacin	120	107	7.8	11.3
Pantothenic acid	14.4	17.7	38.4	33.1
Folic acid	0.16	0.13	0.62	0.71
Biotin	0.038	0.035	0.33	0.22
Choline	0.13 (%; w/v)	0.13 (%; w/v)	0.08 (%; w/v)	0.09 (%; w/v)
Minerals (ppm)				
Ca	95	80	7,660	8,770
P	3,930	3,830	7,180	7,860
K	5,740	5,230	15,800	17,200
Na	1,920	1,710	4,310	4,570
Mg	475	449	673	829
Fe	45.1	47.3	2.8	3.1
Zn	114	101	32.4	30.1
Cu	1.5	2.1	0.7	<0.5
S	0.31 (%; w/v)	0.30 (%; w/v)	0.21 (%; w/v)	0.23 (%; w/v)
Mn	< 0.5	< 0.5	< 0.5	< 0.5
I	0.3	0.2	0.6	0.8
Se	0.3	0.3	0.1	0.1
Mo	< 1	< 1	< 0.5	< 0.5

*:Meat derived from three progeny and three control cattle was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny meat powder and control meat powder. **:Milk derived from three progeny and three control cows was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny milk powder and control milk powder. SCNT, somatic cell nuclear transfer.

dairy cattle (Holstein) were also produced by AI as controls. These cattle were produced and raised at the National Livestock Breeding Center (Tsukuba, Japan).

Preparation of meat/milk powders

Meat derived from three progeny and three conventionally bred cattle was collected independently. Each sample was obtained as retail cuts of the carcasses. After removing excess fat from the subcutis and intermuscle, each meat sample was minced and freeze-dried below 133 Pascal for approximately 24 h. The dry matter was passed through 4-mm mesh and mixed uniformly with a mixer. The samples were prepared as two pooled powders, progeny meat powder and control meat powder.

Milk was collected independently from three progeny and three

control cows in the morning from the 3rd to 6th weeks of lactation and was stored at -25 C as frozen milk plates. The frozen milk plates were freeze-dried below 133 Pascal for approximately 24 h. The dry matter was passed through 0.85-mm mesh and mixed uniformly with a mixer. The samples were prepared as two pooled powders, progeny milk powder and control milk powder.

Preparation of test diets supplemented with meat/milk powder

Before supplementing the test diet with these pooled powders, the nutritional values of each pooled powder were analyzed as shown in our previous reports [13, 14]. The analyzed items included macronutrients (crude protein, crude fat, carbohydrates, crude fiber, ash content and water content), vitamins (A, B₁, B₂, B₆, B₁₂, D₃, E, K₂, niacine, pantothenic acid, folic acid, biotin and cho-

Table 2. Composition of test diets supplemented with milk/meat powder derived from the progeny of SCNT cattle and conventionally bred cattle

Ingredients		Meat powder* diet derived from		Milk powder** diet derived from	
		Progeny of SCNT cattle (%; w/w)	Conventionally bred cattle (%; w/w)	Progeny of SCNT cattle (%; w/w)	Conventionally bred cattle (%; w/w)
Milk/meat powder	—	5.00	5.00	10.00	10.00
Corn starch	39.75	39.71	39.73	36.48	36.08
Casein (93.5% ; w/w of protein)	20.00	17.68	17.78	17.56	17.37
Alfa-corn starch	13.20	13.19	13.19	12.12	11.98
Sucrose	10.00	10.00	10.00	10.00	10.00
Soybean oil	7.00	4.39	4.27	3.81	4.54
Cellulose	5.00	5.00	5.00	5.00	5.00
Mineral mix	3.50	3.50	3.50	3.50	3.50
Vitamin mix	1.00	1.00	1.00	1.00	1.00
L-Cystine	0.30	0.30	0.30	0.30	0.30
Choline bitartrate (41.1%; w/w of choline)	0.25	0.23	0.23	0.23	0.23
Total	100.00	100.00	100.00	100.00	100.00
Calorific value (kcal)	397	397	397	396	396
Crude protein (%; w/w)	18.7	18.7	18.7	18.7	18.7
Crude fat (%; w/w)	7.0	7.0	7.0	7.0	7.0
Carbohydrate (%; w/w)	64.7	64.8	64.8	64.6	64.6
Crude fiber (%; w/w)	5.0	5.0	5.0	5.0	5.0

*:Meat derived from three progeny and three control cattle was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny meat powder and control meat powder. **:Milk derived from three progeny and three control cows was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny milk powder and control milk powder. SCNT: somatic cell nuclear transfer.

line) and minerals (Ca, P, K, Na, Mg, Fe, Zn, Cu, S, Mn, I, Se and Mo). In accordance with the results of the analyses (Table 1), the diet was supplemented with each pooled powder to the levels equivalent to the basal diet, AIN93G6-purified diet for rodents [15], as shown in Table 2. These were high calorie diets due to 5% (w/w) meat or 10% (w/w) milk supplementation. The four test diets (meat powder diet derived from the progeny, meat powder diet derived from conventionally bred cattle, milk powder diet derived from the progeny and milk powder diet derived from conventionally bred cattle) were irradiated with γ -rays (10 kGy) and stored at -15°C until use.

Rats and their feeding conditions

Five-week-old SD rats [CrI:CD(SD)] (Charles River Japan, Yokohama, Japan) reared under specific pathogen free condition were used for this study. Each rat group, which consisted of 12 females and 12 males, was fed one of the four test diets as shown above for 12 months with water *ad libitum*. Each rat was kept in an individual stainless steel mesh cage (260 $\times$ 380 $\times$ 180 mm) in an animal room with a barrier system, controlled temperature (22 $\pm$ 3 $^{\circ}\text{C}$) /humidity (55 $\pm$ 10%) , ventilation about 10 times/h (all fresh air) and a 12-h light/dark cycle. These rats underwent the same observations and examinations (except for reproduction and development of newborns) as shown in our previous report [14].

Observations and examinations of rats during the feeding period

The animals were observed daily for clinical signs; this included

examination of outer appearance, behavior, feces and general state. Detailed clinical observations were also carried out monthly.

Moreover, grip strength of forelimbs and hindlimbs (using a grip strength measuring device: MK380R/FR; Muromachi Kikai, Tokyo, Japan), motor activity (using an auto-measuring system: Supermex; Muromachi Kikai) and sensory/reflex function (sound response, approach response, touch response, tail pinch response, pupil reflex to light, pinna reflex, eyelid reflex, ipsilateral flexor reflex and righting reflex) were examined in the 3rd, 6th, 9th and 12th months of the feeding period [16].

To confirm normal growth, the rats were weighed at the beginning of the feeding period (on day 1 of the feeding period), every 7 days during the 52-week period and the day of sacrifice. The 24-h food consumption of the rats was also measured once a week. In females, these measurements were suspended during the reproduction/development test.

During the 12th month of feeding, examinations such as ophthalmology (anterior portion of the eye, chamber, optic media and ocular fundus), urinalysis (color, pH, occult blood, protein, glucose, ketone body, bilirubin, urobilinogen, specific gravity and urine volume per 18 h) were also performed.

Hematology and blood biochemistry of rats

At the end of the feeding period, the rats were anesthetized and blood was collected from the abdominal aorta. The following blood tests were performed using a multi-item automatic blood analyzer (XT-2000iV; Sysmex, Kobe, Japan): red blood cell count (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV),

mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count, white blood cell count (WBC), differential leucocyte count, platelet count; testing for prothrombin time and activated partial thromboplastin time was performed using a blood clot automatic measurement device (KC-10A; Amelung GmbH, Lemgo, Germany). The following blood biochemistry tests were performed using an automatic biochemical analyzer (JCA-BM8 type Clinalyzer; JEOL, Tokyo, Japan), lactate dehydrogenase (LDH), aspartate aminotransferase/glutamic oxaloacetic transaminase (AST/GOT), alanine aminotransferase/glutamic pyruvic transaminase (ALT/GPT), creatine kinase (CK), alkaline phosphatase (ALP), γ -glutamyltransferase (γ -GTP), cholinesterase (ChE), total protein, albumin, globulin, A/G ratio, total cholesterol, triglyceride, phospholipid, glucose, total bilirubin, blood urea nitrogen, creatinine, calcium, inorganic phosphorus; testing for sodium, potassium and chloride was performed using automated electrolyte analyzer (NAKL-132; PKK-TOA, Tokyo, Japan).

Examinations of rats after autopsy

After the rats were sacrificed by exsanguination, necropsy was performed and their organs were weighed. The weights are shown as absolute weights and relative weights (weight/100 g body weight). The organs investigated were as follows: the brain, pituitary gland, thyroid gland, lung, heart, salivary glands (sublingual and submandibular), liver, spleen, kidney and adrenal gland for both sexes; testes, epididymis, prostate and seminal vesicle for males; ovary and uterus for females. Histological examinations of the brain, pituitary gland, eyeball, Harderian gland, thyroid gland, parathyroid, spinal cord, heart, thymus, liver, kidney, spleen, trachea, lung, adrenal gland, salivary gland, tongue, esophagus, stomach, small intestine, large intestine, pancreas, urinary bladder, testis, epididymis, prostate, seminal vesicle, ovary, uterus, vagina, aorta, sciatic nerve, lymph nodes, bone, bone marrow, skeletal muscle, mammary gland and skin were also conducted.

Reproduction of dams and observation of their pups

The estrous cycles were examined by vaginal smear for 14 days comprising the 16th to 17th weeks of feeding in the female groups fed meat powder diets and the 11th to 12th weeks of feeding in the female groups fed milk powder diets. The females were mated with the same number of males for up to three weeks. After conceiving, the following items were examined in the dams: estrous cycle, copulation index, fertility index, gestation length and gestation index.

During the period from birth until weaning at 21 days, the following items were examined in the pups: litter size, live birth index, sex ratio, body weight, viability index, lactation index, hair growth, pinna detachment, incisor eruption, eyelid opening, testicular descent, sensory response, reflex function tests, external abnormalities and visceral malformations.

Statistical analyses

For rats fed meat/milk powder diets derived from the progeny and conventionally bred cattle, statistical analyses of the origin of powders (the progeny and conventionally bred cattle) were carried

out as follows. The F-test was conducted for parametric data (body weight, body weight gain, food intake, grip strength, mortar activity, estrous cycle, hematological data, blood biochemistry and organ weight), Student's *t*-test was conducted for equal distribution data, Aspin-Welch's *t*-test was conducted for unequal distribution data, Mann-Whitney's U-test was conducted for non-parametric data (urine analyses) and Fisher's exact probability test (one sided test) [17] or χ^2 test for categorical data (clinical signs, autopsy and pathological examinations). The significance level was assumed to be 5% in all analyses.

Animal welfare and other guidelines

This study was carried out in accordance with the guidelines of animal care at RIAS, procedures based on good laboratory practice (GLP), the "Combined repeated dose toxicity study with reproduction/developmental toxicity screening test" of the Organization for Economic Co-operation and Development (OECD) [18] and the "Guidelines for designation of food additives and for revision of standards for use of food additives (recommended methods for one-year toxicity studies)" of the government of Japan [19].

Results

Clinical signs

There were no abnormal clinical signs in rats attributed to feeding meat/milk derived from the progeny of SCNT cattle. The observations described below are not unusual for long-term raising of rats.

One male rat (No. 035) in a group fed the milk powder diet derived from conventionally bred cattle exhibited no abnormal clinical signs but died on the 327th day of feeding. Pathological examinations of the rat revealed that the probable cause of their death was hepatic failure, since multifocal necrosis was found in the liver.

A subcutaneous mass was found in two female rats (Nos. 542 and 547) fed the meat powder diet derived from the progeny. The masses appeared on the 351st day of feeding in the right axillary region in No. 542 and on the 260th day of feeding in the right inguinal region in No. 547. The masses in Nos. 542 and 547 were benign adenoma and benign fibroadenoma, respectively.

Another female rat (No. 515) in a group fed meat powder diet derived from conventionally bred cattle exhibited chromodacryorrhea (from the 279th day of feeding) and pale skin (from 322nd day of feeding), and its general condition deteriorated very quickly. On the last day of feeding (the 364th day), hypothermia, an ataxic gait and accelerated respiration were observed. This rat was diagnosed with myelogenous leukemia based on a pathological examination. In addition to these symptoms, chromodacryorrhea, crushing of the incisors, crust formation and abdominal distention were also observed sporadically.

Subcutaneous masses were found in a female (No. 539) fed milk powder diet derived from the progeny (from 301st day of feeding). Pathology revealed that these masses were benign fibroadenoma of the mammary gland. Sporadic changes, such as chromodacryorrhea, crushing of teeth, alopecia, scab formation, rough fur and soiling around the anus were also observed.

Fig. 1. Body weight curves of rats fed a diet containing meat powder (Meat derived from three progeny and three control cattle was collected independently and freeze-dried). Samples were prepared as two pooled powders, progeny meat powder and control meat powder. SCNT: somatic cell nuclear transfer. —: Period of reproduction/development test.

Fig. 2. Body weight curves of rats fed a diet containing milk powder (Milk derived from three progeny and three control cows was collected independently and freeze-dried). Samples were prepared as two pooled powders, progeny milk powder and control milk powder. SCNT: somatic cell nuclear transfer. —: Period of reproduction/development test.

Sensory/reflex function

In the 3rd, 6th, 9th and 12th months of the feeding period, no abnormalities were found in sound response, approach response, touch response, tail pinch response, pupil reflex to light, pinna reflex, eyelid reflex, ipsilateral flexor reflex and righting reflex in rats fed meat/milk powder diets derived from conventionally bred cattle or the progeny of SCNT cattle. In the 12th month of feeding, one to two of the male rats fed milk powder diet derived from conventionally bred cattle showed decreased touch response, tail pinch response, eyelid reflex and righting reflex.

Grip strength and motor activity

In the 3rd, 6th, 9th and 12th months of the feeding period, the grip strengths of the forelimbs and hindlimbs and motor activities of the rat groups fed meat/milk powder diets were determined. There were no significant differences in any of the items between the rat groups fed meat/milk powder diets derived from the progeny and conventionally bred cattle.

Body weights

A fluctuating tendency for a heavier body weight was observed in females fed the meat powder diet derived from progeny of SCNT cattle compared with those fed the meat powder diet derived from conventionally bred cattle; however, there were no significant differences in body weight between the male rat groups fed the meat powder diet derived from the progeny and conventionally bred cattle (Fig. 1). In regard to the milk powder diet, there were no significant differences in body weight between the female/male rat groups fed the diets derived from the progeny and conventionally bred cattle (Fig. 2).

Food consumption

There were no significant differences in food consumption

between the rat groups fed meat/milk powder diets derived from progeny of SCNT cattle and conventionally bred cattle (Figs. 3 and 4). The average meat/milk powder consumption of rats (per kg body weight) was also examined throughout the 12-month feeding period, except for those of pregnant females. In regards to rats fed the meat powder diet derived from conventionally bred cattle, the average meat powder consumption was 2,057 mg/kg/day in males and 2,623 mg/kg/day in females. For rats fed the meat powder diet derived from the progeny, the average meat powder consumption was 2,090 mg/kg/day in males and 2,549 mg/kg/day in females. In regard to rats fed the milk powder diet derived from conventionally bred cattle, the average milk powder consumption was 4,024 mg/kg/day in males and 4,960 mg/kg/day in females. For rats fed the milk powder diet derived from the progeny, the average milk powder consumption was 4,054 mg/kg/day in males and 5,044 mg/kg/day in females. Lower values were observed for males in all diets. This was due to feeding of high calorie diets throughout the 12 month-feeding study period.

Ophthalmology

In the rats fed meat/milk powder diets derived from conventionally bred cattle or progeny, no abnormalities were found in the anterior portion of the eye, optic media or ocular fundus, regardless of meat/milk origin.

Urinalysis

There were no significant differences in color, pH, occult blood, protein, glucose, ketone body, bilirubin, urobilinogen, specific gravity or urine volume per 18 h between the rat groups fed meat/milk powder diets derived from the progeny and conventionally bred cattle.

Fig. 3. Food consumption curves of rats fed a diet containing meat powder (Meat derived from three progeny and three control cattle was collected independently and freeze-dried). Samples were prepared as two pooled powders, progeny meat powder and control meat powder. SCNT: somatic cell nuclear transfer. —: Period of reproduction/development test.

Fig. 4. Food consumption curves of rats fed a diet containing milk powder (Milk derived from three progeny and three control cows was collected independently and freeze-dried). Samples were prepared as two pooled powders, progeny milk powder and control milk powder SCNT: somatic cell nuclear transfer. —: Period of reproduction/development test.

Hematology

There were no significant differences in almost all hematological findings between the rat groups fed meat/milk powder diets derived from the progeny of SCNT cattle and conventionally bred cattle (Table 3); however, some exceptions were found. In the rats fed the meat powder diet derived from conventionally bred cattle, myelogenous leukemia was found in one female (No. 515). The blood picture in this case showed a marked increase granulated leucocytes and decreases in red blood cells, platelets and lymphocytes. In the rats fed the milk powder diet, a significantly lower WBC ($31 \times 10^2/\mu\text{l}$) was observed in females fed milk derived from progeny compared with that ($42 \times 10^2/\mu\text{l}$) of females fed milk from conventionally bred cattle. However, these values were within the range of our reference data ($19\text{--}55 \times 10^2/\mu\text{l}$).

Blood biochemistry

In the blood biochemical findings for rats fed the meat/milk powder diets, some abnormal values were observed regardless of meat/milk origin (Table 4). Significant differences were observed in some items due to origin; however, almost all values were within the reference range. The contingent findings obtained by this study are shown below.

In rats fed the meat powder diet derived from the progeny of SCNT cattle, there were significantly lower values of LDH (266 IU/l) and CK (64 IU/l) in males compared with the values (521 IU/l for LDH and 96 IU/l for CK) of rats fed meat powder from conventionally bred cattle and significantly higher values of glucose (176 mg/dl) and sodium (150 mEq/l) in males and AST (70 IU/l), blood urea nitrogen (13.3 mg/dl) and inorganic phosphorous (4.3 mg/dl) in females compared with those (161 mg/dl for glucose and 148 mEq/l for sodium in males, 56 IU/l for AST, 10.5 mg/dl for blood urea nitrogen and 3.6 mg/dl for inorganic phosphorous in females) of rats fed meat powder from conventionally bred cattle. However, when these values were compared with reference data

from our laboratory (155–810 IU/l for LDH, 57–171 IU/l for CK, 141–185 mg/dl for glucose, 144–149 mEq/l for sodium in males, 43–282 IU/l for AST, 3.00–5.34 mg/dl for inorganic phosphorous in females), the measured values were within the reference range, except for sodium in males.

In male rats fed the milk powder diet derived from progeny, inorganic phosphorous (5.7 mg/dl) was significantly higher than in males (5.1 mg/dl) fed the milk powder diet derived from conventionally bred cattle. However, these values were within our reference range (4.4–6.1 mg/dl).

Organ weights

There were no significant differences in absolute and relative organ weights between the healthy rat groups fed meat/milk powder diets derived from the progeny of SCNT cattle and conventionally bred cattle (Table 5). However, the weights of the liver, kidney and ovary were extraordinarily heavy in the leukemic female (No. 515) fed the meat powder diet derived from conventionally bred cattle.

Histology

Based on the histological findings, all lesions observed here were diagnosed as spontaneous lesions (Table 6). There were no significant differences in the occurrence rates of these lesions or the histological findings between the rat groups fed the meat/milk diets derived from the progeny of SCNT cattle and conventionally bred cattle. The observed lesions are not unusual occurrences during long-term raising of rats.

There were no distinctive lesions in the rats fed meat/milk powder diets derived from progeny. Based on the histological findings of the groups fed meat/milk powder diets derived from the progeny and conventionally bred cattle, the most common lesions found in the rats were as follows: artery mineralization and accumulation of foam cells in the lung; myocardial degeneration/fibrosis in the

Table 3. Hematological findings of rats fed milk/meat powder diets for 12 months

Parameters	Meat powder ^S diet derived from		Milk powder ^{SS} diet derived from	
	Progeny of SCNT cattle	Conventionally bred cattle	Progeny of SCNT cattle	Conventionally bred cattle
Male rats				
Number of rats	12	12	12	11 [#]
RBC (10 ⁴ /μl)	870 ± 49	881 ± 80	841 ± 52	844 ± 42
Hemoglobin (g/dl)	14.9 ± 0.8	14.8 ± 1.5	14.7 ± 0.9	14.8 ± 0.8
Hematocrit (%)	44.6 ± 2.0	44.4 ± 3.7	44.7 ± 2.7	44.5 ± 2.1
MCV (fl)	51 ± 2	51 ± 5	53 ± 3	53 ± 2
MCH (pg)	17.1 ± 0.5	16.8 ± 1.6	17.5 ± 0.9	17.6 ± 0.5
MCHC (%)	33.4 ± 0.6	33.2 ± 0.9	32.9 ± 0.5	33.3 ± 0.6
Reticulocyte	19.6 ± 8.7	23.2 ± 16.8	21.3 ± 5.5	19.1 ± 4.4
PT (sec)	13.2 ± 0.8	13.1 ± 0.5	12.7 ± 0.8	12.6 ± 0.7
APTT (sec)	17.5 ± 1.6	17.8 ± 1.8	16.6 ± 2.1	16.4 ± 1.5
Platelet (10 ⁴ /μl)	120 ± 16	128 ± 21	126 ± 18	121 ± 14
WBC (10 ² /μl)	82 ± 23	74 ± 16	87 ± 25	84 ± 22
Differential leukocyte counts (%)				
Basophil	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Eosinophil	1.4 ± 0.6	1.7 ± 0.7	1.7 ± 0.7	1.8 ± 0.9
Neutrophil	25.9 ± 8.2	27.5 ± 6.8	21.4 ± 6.9	20.7 ± 4.2
Lymphocyte	69.4 ± 8.5	67.0 ± 6.5	74.0 ± 7.6	74.8 ± 5.2
Monocyte	3.3 ± 1.1	3.8 ± 1.0	2.9 ± 1.5	2.7 ± 1.0
Female rats				
Number of rats	12	11 ^{##}	12	12
RBC (10 ⁴ /μl)	785 ± 59	803 ± 41	771 ± 40	778 ± 39
Hemoglobin (g/dl)	14.5 ± 0.6	14.9 ± 0.6	14.4 ± 0.9	14.3 ± 0.6
Hematocrit (%)	43.9 ± 1.9	44.4 ± 2.3	43.1 ± 2.7	42.5 ± 2.1
MCV (fl)	56 ± 4	55 ± 2	56 ± 2	55 ± 2
MCH (pg)	18.6 ± 1.2	18.6 ± 0.6	18.6 ± 0.5	18.4 ± 0.5
MCHC (%)	33.2 ± 0.6	33.5 ± 0.6	33.4 ± 0.3	33.7 ± 0.6
Reticulocyte	23.7 ± 7.8	19.4 ± 2.8	19.4 ± 3.9	16.6 ± 4.6
PT (sec)	12.9 ± 0.5	12.8 ± 0.3	12.4 ± 0.5	12.4 ± 0.3
APTT (sec)	16.3 ± 0.9	17.0 ± 0.6	15.8 ± 1.8	16.3 ± 1.1
Platelet (10 ⁴ /μl)	104 ± 12	107 ± 13	95 ± 14	94 ± 13
WBC (10 ² /μl)	43 ± 16	35 ± 13	31 ± 9*	42 ± 12
Differential leukocyte counts (%)				
Basophil	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Eosinophil	2.4 ± 0.9	2.1 ± 0.9	2.2 ± 0.9	2.1 ± 0.6
Neutrophil	24.6 ± 9.4	22.7 ± 4.1	27.3 ± 7.9	26.0 ± 6.5
Lymphocyte	69.5 ± 8.7	71.9 ± 4.7	67.6 ± 7.6	68.8 ± 7.0
Monocyte	3.5 ± 1.2	3.3 ± 1.3	2.9 ± 0.8	3.1 ± 1.1

Values are expressed as the mean ± SD. *: P<0.05 compared with each control group. ^S:Meat derived from three progeny and three control cattle was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny meat powder and control meat powder. ^{SS}:Milk derived from three progeny and three control cows was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny milk powder and control milk powder. [#]: One male rat (No.035) , which died on the 327th day of feeding due to hepatic failure, was omitted. ^{##}: One female rat (No. 515), which was diagnosed with myelogenous leukemia, was omitted. SCNT: Somatic cell nuclear transfer.

heart; congestion and increased extramedullary hematopoiesis in the spleen; increased hematopoiesis of bone marrow; fatty change of hepatocytes and focal necrosis in the liver; fatty change in the parotid gland; squamous hyperplasia in the forestomach, fatty change, deposit of brown pigment, fibrosis and atrophy of aciner cells in the pancreas; chronic nephrosis, pelvic inflammation and lymphocytic infiltration of the pelvis in the kidney; focal hyperplasia of anterior lobe, anterior lobe cyst and benign adenoma in the

pituitary gland; C-cell hyperplasia and remnants of the ultimobranchial body in the thyroid gland and focal hyperplasia of the cortex, hemorrhagic cyst and angioectasias in the adrenal gland. The occurrence of these lesions tended to be higher in males than females.

Other lesions were observed sporadically and were often found on histological examination of the rats. These included osseous metaplasia and congestive edema in the lung; mineralization of

Table 4. Blood biochemical findings of rats fed milk/meat powder diets for 12 months

Parameters	Meat powder ^S diet derived from		Milk powder ^{SS} diet derived from	
	Progeny of SCNT cattle	Conventionally bred cattle	Progeny of SCNT cattle	Conventionally bred cattle
Male rats				
Number of rats	12	12	12	11 [#]
LDH (IU/l)	266 ± 94*	521 ± 358	742 ± 898	343 ± 196
AST (IU/l)	91 ± 42	160 ± 155	109 ± 99	87 ± 76
ALT (IU/l)	56 ± 53	80 ± 77	37 ± 20	41 ± 46
ALP (IU/l)	193 ± 63	194 ± 48	222 ± 59	178 ± 59
γ-GTP (IU/l)	0.53 ± 0.17	0.63 ± 0.41	0.99 ± 0.44	0.72 ± 0.29
CK (IU/l)	64 ± 14**	96 ± 28	114 ± 57	82 ± 21
ChE (IU/l)	105 ± 52	78 ± 42	116 ± 75	120 ± 86
Total protein (g/dl)	6.67 ± 0.26	6.66 ± 0.33	6.61 ± 0.30	6.60 ± 0.30
Albumin (g/dl)	3.22 ± 0.21	3.16 ± 0.29	2.89 ± 0.30	3.02 ± 0.33
Globulin (g/dl)	3.45 ± 0.18	3.50 ± 0.26	3.72 ± 0.19	3.59 ± 0.19
A/G ratio	0.94 ± 0.09	0.91 ± 0.12	0.78 ± 0.10	0.85 ± 0.13
Total cholesterol (mg/dl)	109 ± 24	108 ± 28	127 ± 20	143 ± 42
Triglyceride (mg/dl)	122 ± 55	131 ± 45	148 ± 56	194 ± 66
Phospholipid (mg/dl)	160 ± 31	158 ± 32	175 ± 21	199 ± 50
Glucose (mg/dl)	176 ± 19*	161 ± 9	177 ± 17	173 ± 14
BUN (mg/dl)	10.6 ± 1.4	10.4 ± 1.4	12.5 ± 3.2	13.2 ± 2.6
Creatinine (mg/dl)	0.40 ± 0.07	0.39 ± 0.04	0.35 ± 0.04	0.36 ± 0.10
Total bilirubin (mg/dl)	0.24 ± 0.02	0.21 ± 0.04	0.30 ± 0.05	0.29 ± 0.04
Ca (mg/dl)	10.8 ± 0.3	10.7 ± 0.2	10.5 ± 0.5	10.5 ± 0.3
Inorganic phosphorus (mg/dl)	5.4 ± 0.7	5.0 ± 0.4	5.7 ± 0.6*	5.1 ± 0.3
Na (mEq/l)	150 ± 1**	148 ± 1	151 ± 4	150 ± 1
K (mEq/l)	4.51 ± 0.56	4.87 ± 0.24	4.82 ± 0.33	4.60 ± 0.35
Cl (mEq/l)	107 ± 2	107 ± 1	106 ± 2	105 ± 1
Female rats				
Number of rats	12	11 ^{##}	12	12
LDH (IU/l)	269 ± 92	240 ± 109	277 ± 125	306 ± 135
AST (IU/l)	70 ± 17*	56 ± 10	75 ± 32	77 ± 34
ALT (IU/l)	24 ± 8	20 ± 4	23 ± 8	26 ± 12
ALP (IU/l)	125 ± 76	103 ± 32	75 ± 55	58 ± 10
γ-GTP (IU/l)	0.27 ± 0.23	0.23 ± 0.15	0.65 ± 0.43	0.51 ± 0.13
CK (IU/l)	65 ± 26	63 ± 21	97 ± 30	101 ± 29
ChE (IU/l)	421 ± 131	440 ± 107	518 ± 179	533 ± 97
Total protein (g/dl)	6.91 ± 0.44	6.96 ± 0.26	7.15 ± 0.54	7.32 ± 0.36
Albumin (g/dl)	3.83 ± 0.28	3.91 ± 0.29	3.91 ± 0.55	4.14 ± 0.39
Globulin (g/dl)	3.07 ± 0.19	3.04 ± 0.20	3.24 ± 0.27	3.18 ± 0.20
A/G ratio	1.25 ± 0.07	1.29 ± 0.15	1.22 ± 0.22	1.31 ± 0.17
Total cholesterol (mg/dl)	101 ± 26	99 ± 27	101 ± 25	105 ± 14
Triglyceride (mg/dl)	105 ± 59	111 ± 87	54 ± 30	70 ± 44
Phospholipid (mg/dl)	188 ± 44	190 ± 44	178 ± 34	189 ± 30
Glucose (mg/dl)	163 ± 16	160 ± 14	146 ± 14	148 ± 14
BUN (mg/dl)	13.3 ± 2.5*	10.5 ± 2.8	11.0 ± 3.0	10.8 ± 1.5
Creatinine (mg/dl)	0.52 ± 0.08	0.48 ± 0.03	0.40 ± 0.05	0.40 ± 0.04
Total bilirubin (mg/dl)	0.22 ± 0.04	0.21 ± 0.03	0.37 ± 0.05	0.37 ± 0.06
Ca (mg/dl)	10.5 ± 0.5	10.2 ± 0.3	10.6 ± 0.4	10.6 ± 0.5
Inorganic phosphorus (mg/dl)	4.3 ± 0.8*	3.6 ± 0.5	4.6 ± 1.0	4.5 ± 0.7
Na (mEq/l)	142 ± 1	141 ± 2	143 ± 2	143 ± 1
K (mEq/l)	4.48 ± 0.50	4.47 ± 0.49	4.78 ± 0.90	4.64 ± 0.40
Cl (mEq/l)	106 ± 3	107 ± 1	110 ± 2	108 ± 2

Values are expressed as the mean ± SD. *: P < 0.05, **: P < 0.01 compared with each control group. ^S: Meat derived from three progeny and three control cattle was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny meat powder and control meat powder. ^{SS}: Milk derived from three progeny and three control cows was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny milk powder and control milk powder. [#]: One male rat (No.035), which died on the 327th day of feeding due to hepatic failure, was omitted. ^{##}: One female rat (No. 515), which was diagnosed with myelogenous leukemia, was omitted. SCNT: Somatic cell nuclear transfer.

Table 5. Absolute and relative organ weights of rats fed milk/meat powder diets for 12 months

Organs	Absolute weight						Relative weight (weight/100 g body weight)					
	Meat powder ^s diet derived from			Milk powder ^{ss} diet derived from			Meat powder ^s diet derived from			Milk powder ^{ss} diet derived from		
	Progeny of SCNT cattle	Conventionally bred cattle	12	Progeny of SCNT cattle	Conventionally bred cattle	11#	Progeny of SCNT cattle	Conventionally bred cattle	12	Progeny of SCNT cattle	Conventionally bred cattle	12
Male rats												
Number of rats	12	12	12	12	12	11#	12	12	12	12	12	12
Body weight (g)	825 ± 80	834 ± 102	825 ± 80	927 ± 126	909 ± 70	909 ± 70	825 ± 80	834 ± 102	825 ± 80	834 ± 102	825 ± 80	834 ± 102
Brain (g)	2.20 ± 0.10	2.23 ± 0.08	2.23 ± 0.10	2.23 ± 0.10	2.20 ± 0.10	2.20 ± 0.10	0.27 ± 0.02	0.27 ± 0.03	0.27 ± 0.02	0.27 ± 0.03	0.27 ± 0.02	0.27 ± 0.03
Salivary glands (g)	0.95 ± 0.09	0.97 ± 0.13	0.93 ± 0.15	0.93 ± 0.15	0.98 ± 0.13	0.98 ± 0.13	0.12 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	0.12 ± 0.03
Heart (g)	1.81 ± 0.14	1.80 ± 0.16	2.05 ± 0.24	2.05 ± 0.24	1.91 ± 0.13	1.91 ± 0.13	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02
Lungs (g)	1.92 ± 0.22	1.94 ± 0.11	2.16 ± 0.30	2.16 ± 0.30	2.08 ± 0.21	2.08 ± 0.21	0.24 ± 0.03	0.24 ± 0.03	0.24 ± 0.03	0.24 ± 0.03	0.24 ± 0.03	0.24 ± 0.03
Liver (g)	18.21 ± 2.31	17.71 ± 3.45	23.77 ± 4.82	23.77 ± 4.82	21.99 ± 2.81	21.99 ± 2.81	2.22 ± 0.26	2.12 ± 0.27	2.22 ± 0.26	2.12 ± 0.27	2.22 ± 0.26	2.12 ± 0.27
Kidneys (g)	3.83 ± 0.55	3.84 ± 0.36	4.37 ± 0.39	4.37 ± 0.39	4.77 ± 1.66	4.77 ± 1.66	0.47 ± 0.07	0.46 ± 0.05	0.47 ± 0.07	0.46 ± 0.05	0.47 ± 0.07	0.46 ± 0.05
Adrenals (mg)	64.0 ± 10.5	61.0 ± 8.3	72.6 ± 9.4	72.6 ± 9.4	68.4 ± 10.7	68.4 ± 10.7	7.8 ± 1.4	7.4 ± 1.4	7.8 ± 1.4	7.4 ± 1.4	7.8 ± 1.4	7.4 ± 1.4
Spleen (g)	0.99 ± 0.16	1.05 ± 0.35	1.23 ± 0.24	1.23 ± 0.24	1.19 ± 0.23	1.19 ± 0.23	0.12 ± 0.02	0.13 ± 0.03	0.12 ± 0.02	0.13 ± 0.03	0.12 ± 0.02	0.13 ± 0.03
Pituitary (mg)	17.7 ± 6.9	16.4 ± 2.9	17.4 ± 2.7	17.4 ± 2.7	19.1 ± 4.5	19.1 ± 4.5	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.02
Thyroids (mg)	41.4 ± 4.5	41.2 ± 4.3	45.0 ± 10.1	45.0 ± 10.1	38.5 ± 7.3	38.5 ± 7.3	0.34 ± 0.07	0.32 ± 0.07	0.34 ± 0.07	0.32 ± 0.07	0.34 ± 0.07	0.32 ± 0.07
Prostate (g)	0.43 ± 0.13	0.45 ± 0.12	0.65 ± 0.21	0.65 ± 0.21	0.71 ± 0.17	0.71 ± 0.17	0.44 ± 0.04	0.44 ± 0.06	0.44 ± 0.04	0.44 ± 0.06	0.44 ± 0.04	0.44 ± 0.06
Testes (g)	2.77 ± 0.48	2.69 ± 0.60	2.84 ± 0.47	2.84 ± 0.47	3.02 ± 0.55	3.02 ± 0.55	0.18 ± 0.03	0.18 ± 0.03	0.18 ± 0.03	0.18 ± 0.03	0.18 ± 0.03	0.18 ± 0.03
Seminal vesicle(g)	3.56 ± 0.22	3.62 ± 0.28	3.70 ± 0.27	3.70 ± 0.27	3.76 ± 0.31	3.76 ± 0.31	2.2 ± 0.8	2.0 ± 0.4	2.2 ± 0.8	2.0 ± 0.4	2.2 ± 0.8	2.0 ± 0.4
Epididymides (g)	1.51 ± 0.18	1.44 ± 0.15	1.53 ± 0.23	1.53 ± 0.23	1.56 ± 0.21	1.56 ± 0.21	5.1 ± 0.7	5.0 ± 0.7	5.1 ± 0.7	5.0 ± 0.7	5.1 ± 0.7	5.0 ± 0.7
Female rats												
Number of rats	12	12	12	12	12	12	12	12	12	12	12	12
Body weight (g)	503 ± 60	456 ± 96	450 ± 61	450 ± 61	459 ± 69	459 ± 69	503 ± 60	456 ± 96	450 ± 61	456 ± 96	450 ± 61	459 ± 69
Brain (g)	2.00 ± 0.13	1.96 ± 0.10	2.00 ± 0.09	2.00 ± 0.09	1.99 ± 0.09	1.99 ± 0.09	0.40 ± 0.05	0.45 ± 0.10	0.45 ± 0.07	0.45 ± 0.10	0.45 ± 0.07	0.44 ± 0.07
Salivary glands (g)	0.59 ± 0.06	0.57 ± 0.05	0.55 ± 0.06	0.55 ± 0.06	0.59 ± 0.07	0.59 ± 0.07	0.12 ± 0.01	0.13 ± 0.03	0.13 ± 0.02	0.13 ± 0.03	0.13 ± 0.02	0.13 ± 0.02
Heart (g)	1.29 ± 0.14	1.21 ± 0.15	1.23 ± 0.10	1.23 ± 0.10	1.24 ± 0.18	1.24 ± 0.18	0.26 ± 0.04	0.28 ± 0.08	0.28 ± 0.03	0.28 ± 0.08	0.28 ± 0.03	0.27 ± 0.04
Lungs (g)	1.37 ± 0.17	1.37 ± 0.19	1.44 ± 0.16	1.44 ± 0.16	1.43 ± 0.14	1.43 ± 0.14	0.28 ± 0.04	0.32 ± 0.11	0.32 ± 0.03	0.32 ± 0.11	0.32 ± 0.03	0.32 ± 0.05
Liver (g)	11.45 ± 2.58	10.19 ± 3.12	9.73 ± 1.71	9.73 ± 1.71	9.85 ± 1.19	9.85 ± 1.19	2.28 ± 0.44	2.34 ± 1.10	2.16 ± 0.23	2.34 ± 1.10	2.16 ± 0.23	2.17 ± 0.23
Kidneys (g)	2.66 ± 0.68	2.59 ± 1.11	2.42 ± 0.21	2.42 ± 0.21	2.56 ± 0.24	2.56 ± 0.24	0.53 ± 0.14	0.62 ± 0.43	0.54 ± 0.06	0.62 ± 0.43	0.54 ± 0.06	0.57 ± 0.07
Adrenals (mg)	70.9 ± 14.6	63.7 ± 17.9	65.9 ± 11.1	65.9 ± 11.1	66.8 ± 10.3	66.8 ± 10.3	14.3 ± 3.3	14.7 ± 6.1	14.9 ± 3.2	14.7 ± 6.1	14.9 ± 3.2	15.0 ± 3.8
Spleen (g)	0.74 ± 0.29	2.00 ± 4.85	0.81 ± 0.59	0.81 ± 0.59	0.64 ± 0.10	0.64 ± 0.10	0.15 ± 0.06	0.59 ± 1.59	0.18 ± 0.12	0.59 ± 1.59	0.18 ± 0.12	0.14 ± 0.02
Pituitary (mg)	29.9 ± 9.8	24.1 ± 8.1	38.3 ± 36.0	38.3 ± 36.0	44.6 ± 40.4	44.6 ± 40.4	14.7 ± 5.0	33.0 ± 57.8	15.9 ± 5.7	33.0 ± 57.8	15.9 ± 5.7	13.1 ± 3.1
Thyroids (mg)	32.9 ± 7.0	33.5 ± 4.8	31.2 ± 4.7	31.2 ± 4.7	31.4 ± 7.5	31.4 ± 7.5	0.18 ± 0.06	0.19 ± 0.05	0.23 ± 0.10	0.19 ± 0.05	0.23 ± 0.10	0.18 ± 0.04
Ovaries (mg)	73.8 ± 26.4	124.7 ± 171.1	70.8 ± 23.4	70.8 ± 23.4	60.2 ± 17.4	60.2 ± 17.4	6.1 ± 2.2	5.5 ± 2.2	8.4 ± 7.4	5.5 ± 2.2	8.4 ± 7.4	10.3 ± 10.7
Uterus (g)	0.87 ± 0.26	0.87 ± 0.25	0.99 ± 0.37	0.99 ± 0.37	0.82 ± 0.18	0.82 ± 0.18	6.6 ± 1.4	7.5 ± 1.3	7.0 ± 1.0	7.5 ± 1.3	7.0 ± 1.0	6.8 ± 1.2

Values are expressed as the mean ± SD. ^s:Meat derived from three progeny and three control cattle was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny meat powder and control meat powder. ^{ss}:Milk derived from three progeny and three control cows was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny milk powder and control milk powder. #: One male rat (No.035), which died on the 327th day of feeding due to hepatic failure, was omitted. SCNT: Somatic cell nuclear transfer.

Table 6. Histopathological findings of rats fed milk/meat powder diets for 12 months

Organs	Findings	Meat powder ^S diet derived from				Milk powder ^{SS} diet derived from				
		Progeny of SCNT cattle		Conventionally bred cattle		Progeny of SCNT cattle		Conventionally bred cattle		
		Sex of rats No. of rats	Female 12	Male 2	Female 12	Male 12	Female 12	Male 12	Female 12	Male 12
Lung	Mineralization, artery		6	11	5	10	5	8	3	8
	Accumulation, foam cell		1	3	0	2	1	3	2	3
	Metaplasia, osseous		1	1	0	0	0	0	1	0
	Congestive edema		0	0	0	0	0	0	0	1
Heart	Myocardial degeneration/fibrosis		4	4	5	1	4	9	4	8
Aorta	Mineralization, tunica media		0	0	0	0	0	0	1	0
Spleen	Congestion		1	0	2	3	1	1	1	2
	Increased extramedullary hematopoiesis		2	1	0	0	2	0	2	0
	Increased deposit, brown pigment		0	0	0	0	1	0	1	0
	Necrosis		0	0	1	0	0	0	0	0
Bone Marrow	Increased hematopoiesis		1	0	0	0	1	0	0	0
	Leukemia, myelogeneous		0	0	1	0	0	0	0	0
Liver	Fatty change, hepatocyte		6	9	4	8	4	8	5	7
	Necrosis, focal		0	3	0	3	0	0	0	1
	Angiectasis		0	0	0	1	0	0	0	0
	Hyperplasia, bile duct		0	0	1	0	0	0	0	0
	Hematoma		0	0	0	0	1	0	0	0
Parotid gland	Fatty change		2	9	2	12	1	10	2	12
	Lymphocytic infiltration		0	1	0	0	0	0	1	0
Forestomach	Squamous hyperplasia		0	3	0	2	1	2	1	0
Glandular stomach	Dilatation, gastric gland		0	0	0	1	1	1	0	0
Cecum	Mineralization, mucosa		0	0	0	1	0	0	0	0
	Fibrosis, lumina propria		0	0	0	1	0	0	0	1
	Inflammation, suppurative		0	0	0	0	1	0	0	0
Pancreas	Fatty change		2	9	1	6	2	8	2	7
	Deposit, brown pigment		0	4	1	4	0	6	1	6
	Fibrosis		1	2	0	2	0	5	0	8
	Atrophy, aciner cell, focal		0	4	0	7	0	2	0	2
	Lymphocytic infiltraion		0	0	0	1	0	0	0	0
Kidney	Cyst, solitary		0	0	0	0	0	0	2	0
	Cystic kidney, unilateral		0	0	0	0	0	0	0	1
	Mineralization, tubular		0	0	0	0	2	0	1	0
	Degeneration, hyaline droplet		0	0	1	0	0	0	0	0
	Fibrosis, cortex		0	0	0	1	0	0	0	0
	Lymphocytic infiltration, cortex		0	1	0	0	0	0	1	0
	Chronic nephrosis		1	8	1	9	4	8	4	12
	Lymphocytic infiltration, pelvis		1	1	1	0	0	0	0	2
	Inflammation, pelvis		1	2	1	4	3	2	2	1
Urinary bladder	Lymphocytic infiltration, submucosa		0	0	0	0	1	0	1	0
Testis	Atrophy, seminiferous tubule		–	0	–	1	–	0	–	0
Prostate	Lymphocytic infiltration		–	0	–	0	–	1	–	0
	Inflammation		–	0	–	1	–	0	–	0

tunica media in the aorta; increased deposit of brown pigmentation and necrosis in the spleen; increased hematopoiesis in the bone marrow; angiectasis, hyperplasia of the bile duct and hematoma in the liver; dilatation of the gastric gland in the glandular stomach; mucosal mineralization, fibrosis of the lumina propria and suppurative

inflammation in the cecum; solitary cyst, cystic kidney (unilateral), tubular mineralization, hyaline droplet degeneration and cortical fibrosis in the kidney; lymphocytic infiltration in the parotid gland, pancreas, kidney, urinary bladder, prostate, thyroid gland and Harderian gland; atrophy of seminiferous tubules in the

Table 6. Continued

Organs	Findings	Sex of rats No. of rats	Meat powder ^S diet derived from				Milk powder ^{SS} diet derived from			
			Progeny of SCNT cattle		Conventionally bred cattle		Progeny of SCNT cattle		Conventionally bred cattle	
			Female 12	Male 2	Female 12	Male 12	Female 12	Male 12	Female 12	Male 12
Uterus	Hyperplasia, endometrial		0	–	0	–	1	–	0	–
	Inflammation, endometrium		0	–	1	–	0	–	0	–
	Polyp, endometrial stromal		0	–	0	–	1	–	0	–
Pituitary gland	Focal hyperplasia, anterior lobe		4	2	4	2	1	2	4	5
	Cyst, anterior lobe		1	1	1	0	0	0	1	2
	Aberrant craniopharyngeal tissue		0	0	0	0	0	0	0	1
	Angioectasias, anterior lobe		0	0	0	0	0	0	2	0
	Adenoma		1	2	2	2	2	2	2	2
Thyroid gland	Remnant, ultimobranchial body		0	1	3	3	2	1	0	3
	C-cell hyperplasia		4	2	3	2	1	2	2	1
	Lymphocytic infiltration		0	0	1	1	0	1	0	0
	Adenoma, follicular cell		0	0	0	0	0	1	0	0
	Adenoma, C-cell		0	0	0	1	0	0	0	0
Adrenal gland	Focal hyperplasia, cortex		0	3	1	3	0	3	0	3
	Angioectasias		1	0	1	0	1	0	3	0
	Cyst, hemorrhagic		4	0	3	0	0	1	0	0
	Fatty change, focal		0	1	0	0	0	0	0	0
Harderian gland	Lymphocytic infiltration		0	0	1	0	0	0	0	1
Mammary gland	Inflammation		0	0	0	1	0	0	0	0
	Fibroadenoma		1	0	0	0	1	0	0	0
	Adenoma		1	0	0	0	0	0	0	0

No abnormalities were detected in the brain, spinal cord, sciatic nerve, trachea, sublingual and submandibular glands, parathyroid, lymph node, thymus, tongue, esophagus, small intestine, eye ball, skeletal muscle, skin, testis, epididymis and seminal vesicle. ^S:Meat derived from three progeny and three control cattle was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny meat powder and control meat powder. ^{SS}:Milk derived from three progeny and three control cows was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny milk powder and control milk powder. SCNT: Somatic cell nuclear transfer.

testis; inflammation in the prostate; endometrial hyperplasia, inflammation of the endometrium and benign endometrial stromal polyp in the uterus; angioectasias and aberrant craniopharyngeal tissue in the pituitary gland; C-cell hyperplasia and benign follicular cell adenoma in the thyroid gland; and inflammation in the mammary gland.

In the died rats that had been fed the milk powder diet derived from conventionally bred cattle, the histological findings from examination of gross lesions revealed multifocal necrosis in the liver. A euthanized rat fed meat powder derived from conventionally bred cattle was diagnosed with myelogenous leukemia based on pathological examination. Benign fibroadenoma and adenoma in the mammary gland were observed in rats fed the milk powder diet and in two rats fed the meat powder diet. Both powders were derived from progeny.

Reproductive function of dams and observation of their pups

There were no significant differences in estrous cycle, copulation index, fertility index, gestation length and gestation index between the dam groups fed meat/milk powder diets derived from the progeny of SCNT cattle and conventionally bred cattle (Table 7). There were also no significant differences in body weight and food consumption during pregnancy and lactation between the

dams fed meat/milk powder diets from the progeny and conventionally bred cattle.

The results of the sensory response/reflex function test were normal for all pups delivered from dams fed meat/milk powder diets. No pups exhibited any external abnormalities or visceral malformations. There were no significant differences in litter size, sex ratio, viability index (day 4 of lactation), lactation index (day 21 of lactation), hair growth, pinna detachment, incisor eruption, eyelid opening and testicular descent between pups delivered from dams fed meat/milk powder diets derived from the progeny and conventionally bred cattle (Table 7).

Discussion

To assume the normality of edible products (such as meat and milk) derived from SCNT cattle and their progeny, composition analyses have been carried out in amino and fatty acids [13, 20–22]. The data for the different kinds of edible products has demonstrated that there are no biologically significant differences in composition among clones, their progeny and conventionally bred cattle; however, it has been suggested that a toxicity test, such as a feeding study using rodents, should be included in the main safety evaluation of edible products derived from SCNT cattle and their

Table 7. Reproductive function of dams fed milk/meat powder diets and observation of their pups

Items	Unit	Sex	Meat powder [§] diet derived from		Milk powder ^{§§} diet derived from	
			Progeny of SCNT cattle	Conventionally bred cattle	Progeny of SCNT cattle	Conventionally bred cattle
<i>Reproductive function of dams</i>						
Day of the estrous cycle ^a (Number of rats)	days	—	4.1 ± 0.2 (12)	4.1 ± 0.2 (12)	4.1 ± 0.2 (11)	4.2 ± 0.2 (12)
Copulation index (Number of rats)	%	—	91.7 (11/12)	100 (12/12)	100 (12/12)	100 (12/12)
Fertility index (Number of rats)	%	—	100 (11/11)	100 (12/12)	91.7 (11/12)	83.3 (10/12)
Gestation length ^a (Number of rats)	days	—	22.5 ± 0.7 (11)	22.6 ± 0.5 (12)	22.4 ± 0.7 (11)	22.8 ± 0.4 (10)
Gestation index (Number of rats)	%	—	90.9 (10/11)	100 (12/12)	100 (11/11)	100 (10/10)
<i>Observation of pups</i>						
Litter results						
On day 0 of lactation						
Litter size ^a	—	—	10.9 ± 3.6	11.3 ± 3.3	13.3 ± 1.9	12.4 ± 4.1
Live birth index	%	—	96.7	97.1	99.3	99.2
Sex ratio; male/female	—	—	1.000	1.345	1.246	1.138
Body weights ^a	g	Male	6.8 ± 0.7	6.8 ± 0.7	6.8 ± 0.9	6.9 ± 0.5
	g	Female	6.4 ± 0.7	6.6 ± 0.7	6.5 ± 0.9	6.5 ± 0.6
On day 4 of lactation						
Viability index	%	—	94.0	97.7	97.9	98.4
Body weights ^a	g	Male	11.3 ± 1.5	11.4 ± 2.2	11.1 ± 2.0	11.6 ± 1.1
	g	Female	10.8 ± 1.6	11.2 ± 2.0	10.9 ± 1.9	10.7 ± 1.1
On day 7 of lactation						
Body weights ^a	g	Male	19.0 ± 2.5	18.6 ± 3.4	18.2 ± 2.6	19.2 ± 1.7
	g	Female	18.1 ± 2.5	18.3 ± 2.9	18.0 ± 2.4	18.2 ± 2.4
On day 14 of lactation						
Body weights ^a	g	Male	39.7 ± 3.2	39.0 ± 4.2	39.1 ± 3.8	40.2 ± 3.4
	g	Female	38.5 ± 2.8	38.3 ± 3.7	38.4 ± 3.5	39.4 ± 3.9
On day 21 of lactation						
Lactation index	%	—	98.7	98.9	100	100
Body weights ^a	g	Male	70.7 ± 4.6	69.5± 8.1	69.8 ± 5.3	72.2 ± 5.0
	g	Female	67.5 ± 4.1	67.0 ±6.2	67.2 ± 4.7	69.3 ± 5.0
Developmental observation						
Hair growth ^a	days	—	4.0 ± 0.0	4.1 ± 0.3	4.0 ± 0.0	4.0 ± 0.0
Pinna detachment ^a	days	—	4.0 ± 0.1	4.1 ± 0.2	4.0 ± 0.0	4.0 ± 0.0
Incisor eruption ^a	days	—	9.7 ± 0.8	9.5 ± 0.8	10.3 ± 0.7	10.1 ± 1.2
Eyelids opening ^a	days	—	13.7 ± 0.8	13.1 ± 1.3	13.3 ± 0.7	12.9 ± 0.7
Testicular descent ^a	days	—	18.1 ± 0.5	18.1 ± 0.7	17.8 ± 1.1	18.0 ± 0.9
Sensory response/reflex function test ^b			NAD	NAD	NAD	NAD
Teratological examination						
External abnormalities (Number of pups)	%	—	0.0 (0/120)	0.7(1c /136)	0.0 (0/146)	0.0 (0/124)
Visceral malformations (Number of pups)	%	—	0.0 (0/120)	0.0 (0/136)	0.0 (0/146)	0.0 (0/124)

[§]: Meat derived from three progeny and three control cattle was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny meat powder and control meat powder. ^{§§}: Milk derived from three progeny and three control cows was collected independently and freeze-dried. Samples were prepared as two pooled powders, progeny milk powder and control milk powder. ^a: Values are expressed as the mean ± SD. ^b: Responses to sound, approach, touch and tail pinch, pupil reflex to light, and pinna, ipsilateral flexor, eyelid and righting reflexes. ^c: One pup had a external abnormality (rudimentary tail). NAD: No abnormalities were detected. SCNT: Somatic cell nuclear transfer. Copulation index = (number of pairs with successful copulation / number of pairs mated) × 100. Fertility index = (number of pregnant females / number of pairs with successful copulation) × 100. Gestation index = (number of females with live pups / number of pregnant females) × 100. Live birth index = (number of live pups on day 0 / number of pups born) × 100. Viability index = (number of live pups on day 4 / number of pups on day 0) × 100 and Lactation index = (number of live pups on day 21 / number of pups on day 4) × 100.

progeny and in determination of the suitability of these kinds of animals as a food source [9].

In a previous study, we carried out a 14-week feeding study of rats with meat/milk derived from SCNT cattle and demonstrated that the edible products tested did not affect the physiological con-

ditions of the rats [14]. Since few such feeding studies have been reported, a raw data set of our previous report [14] was reproduced in the final report for the FDA as a 50-page appendix, Appendix I [9]. The data supports the conclusion of the FDA showing that meat and milk derived from clones is as safe to eat as those derived

from conventionally bred cattle.

The present 12-month feeding study is a “progeny version” of our previous study [14]. To our knowledge, this is the first report on the chronic toxicity of edible products derived from the progeny of SCNT cattle. We developed this feeding study using rats fed diets containing meat/milk powder derived from cattle during a previous feeding study [14]. The highest diet supplementation that does not negatively affect food consumption was estimated to be 5% for meat powder and 10% for milk powder. In diets supplemented with 5% meat powder and 10% milk powder, the calorific values of these powders were 8.2% for meat and 13% for milk. The average meat/milk powder consumption of rats (per kg body weight) in this feeding study was comparable to human (60 kg for men, 50 kg for women) daily intake of 300 g for meat (65% water content) and 1600 g for milk (89% water content). Although these amounts were still below those for conventional toxicological tests using a 100-fold dose of a single substance, they exceeded normal human dietary intake.

In the present 12-month feeding study, there were no biologically significant differences in most of the growth and reproduction related factors between the rat groups fed the meat/milk diet derived from the progeny and conventionally bred cattle; however, abnormal findings were observed in clinical signs and histology. These included sporadic symptoms that are not uncommon during long-term raising of rats. As for the lesions found in the histological examinations, these were presumed to be spontaneous [23]; therefore, we concluded that the symptoms and lesions observed in the rats were not due to feeding of the meat/milk powder diets derived from the progeny. These data concerning chronic toxicity show that meat and milk derived from the progeny of SCNT cattle would be equivalent to those derived from conventionally bred cattle in use as dietary supplements for rats. These findings, which are inferred from the data obtained by composition analysis of meat/milk derived from the progeny of SCNT cattle [5, 24], strongly support the conclusions of the FDA [9]/EFSA [10] and will be helpful for further risk assessments of these kinds of edible products.

When morbidity and mortality were compared among SCNT cattle, their progeny and conventionally bred cattle, no differences were observed between the progeny and conventionally bred cattle [9]. These empirical facts can be explained using a mouse model system; it is assumed that there is no risk increase in terms of inducement of abnormalities due to epigenetic errors in the progeny of cloned animals, since any epigenetic errors existing in a clone’s genome would be erased during the process of gamete genesis and fertilization [25]. It should be noted that other empirical evidence collected by observation of progeny, such as the calf production rate with artificial insemination, gestation period, birth weight, growth performance, blood parameters and production performance, are also consistent with this assumption [2, 5, 26]. Therefore, this evidence strongly suggests that the progeny of SCNT cattle can be produced for consumption in a manner similar to conventionally bred cattle.

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