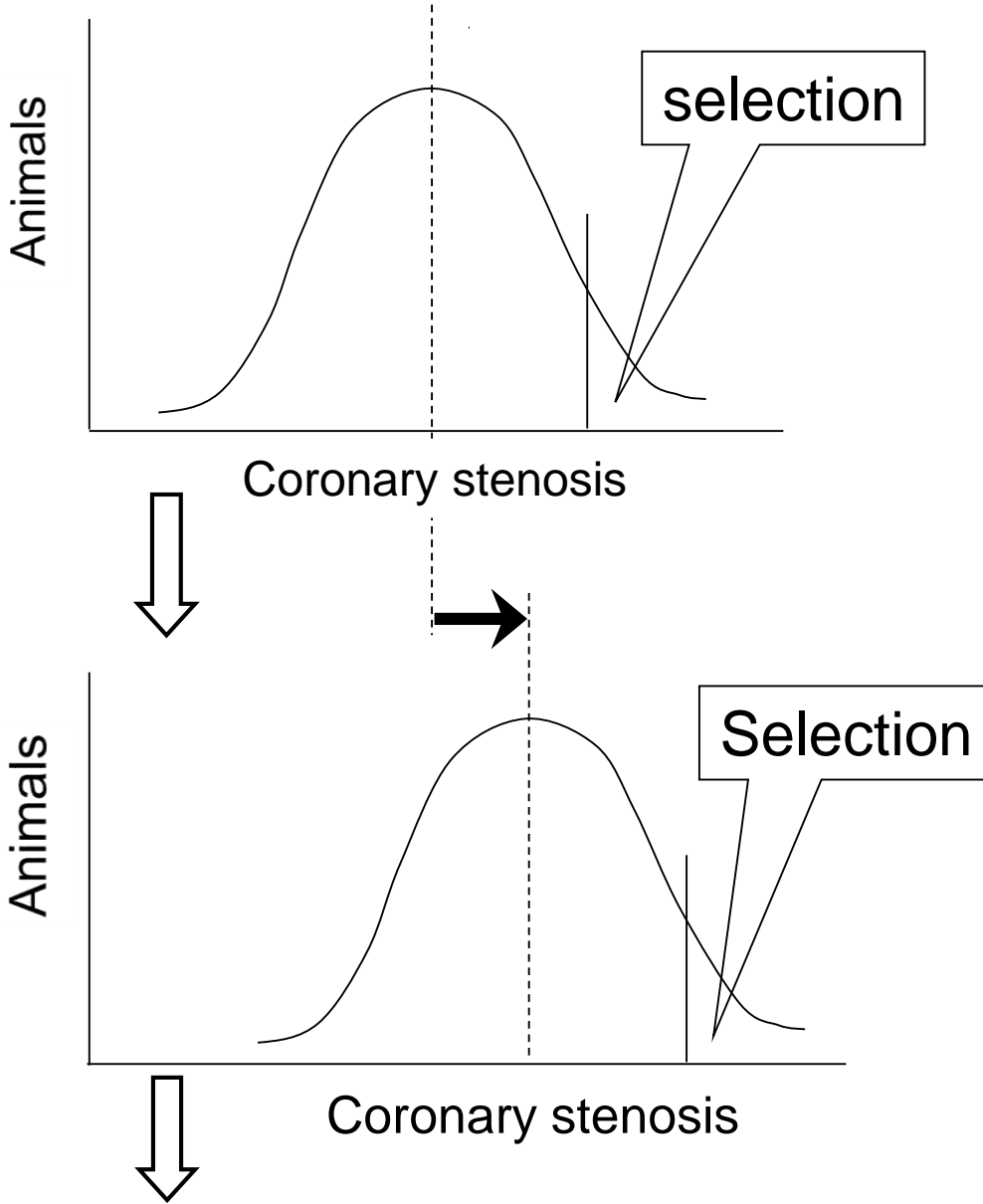


Reproduction of WHHLM rabbits

Selective breeding is necessary to keep the characteristics of WHHLM rabbits.

Development of coronary lesions and myocardial lesions is controlled by plural genes.

We select rabbits in mating, whose **parents** suffered from myocardial infarction and/or thin-capped fibroatheroma in the coronary plaques.



To keep the characteristics of WHHLMI rabbits, we have to analyze and record followings.

- The **serum lipid levels** periodically
- The degree of **coronary atherosclerosis**

死亡・実験死・安楽死 []
 最終生存確認日時 2013.6.4, 13:30 剖検日時 2013.6.4, 16:45
 固定液 [10%NBF・その他()]
 固定方法 (浸漬) 全身灌流・臓器灌流 ()] 山田

[Prep.No. 2307]
 ID No. 24-117-7 ♂・♀ Age: 18 mos, wks.

Sec.No.	LOX	病変分類	Sec.No.	LCX	病変分類	Block No.
1	95		I-1	95		I-1
2	95		2	95		I-2
3	95		3	95 95		I-3
4	95		4	40		
5	95 85		5	70		
6	95 85		6	70 85		
7	95		7			
8	95		8			
9	95 60		9			
10	95		10			
11	95		11			
12	95 95 90		12			
13	95 95		13			
14	95 95		14			
15	95 95		15			
Sec.No.	LAD	LSP	RCA			
17	70	0	0			
18	10	0	0			
19	80 85	0	0			
20	85	0	0			
21	0	0	0			
22	0	95 95	0			
23						
24						
25						

薄切間隔: 100, 250, 500(um)

薄切間隔: 250, 500, 1000(um)

MI (+)

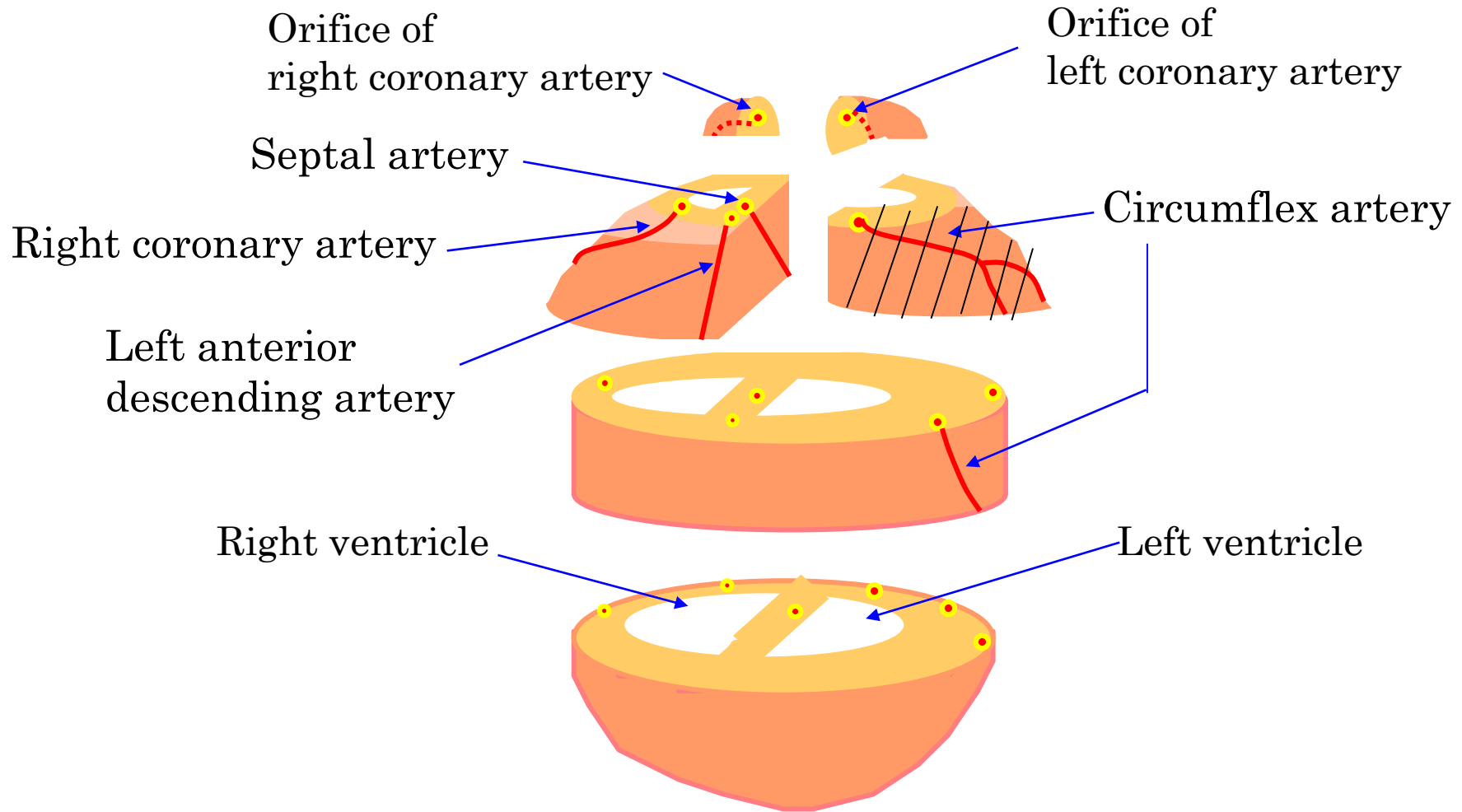
- 1/4面 Anterior, Lateral, Posterior wall, Septum, Circumferential, Papillary, Right wall
 Subendocardial, Intramuscular, Transmural, Periarterial, Pericardial, Subtotal
 Focal, Patchy, Massive, Confluent
 ≒ 10 %LV, 初期, 前期, 中期, 後期
- 2/4面 Anterior, Lateral, Posterior wall, Septum, Circumferential, Papillary, Right wall
 Subendocardial, Intramuscular, Transmural, Periarterial, Pericardial, Subtotal
 Focal, Patchy, Massive, Confluent
 ≒ %LV, 初期, 前期, 中期, 後期
- 3/4面 Anterior, Lateral, Posterior wall, Septum, Circumferential, Papillary, Right wall
 Subendocardial, Intramuscular, Transmural, Periarterial, Pericardial, Subtotal
 Focal, Patchy, Massive, Confluent
 ≒ %LV, 初期, 前期, 中期, 後期

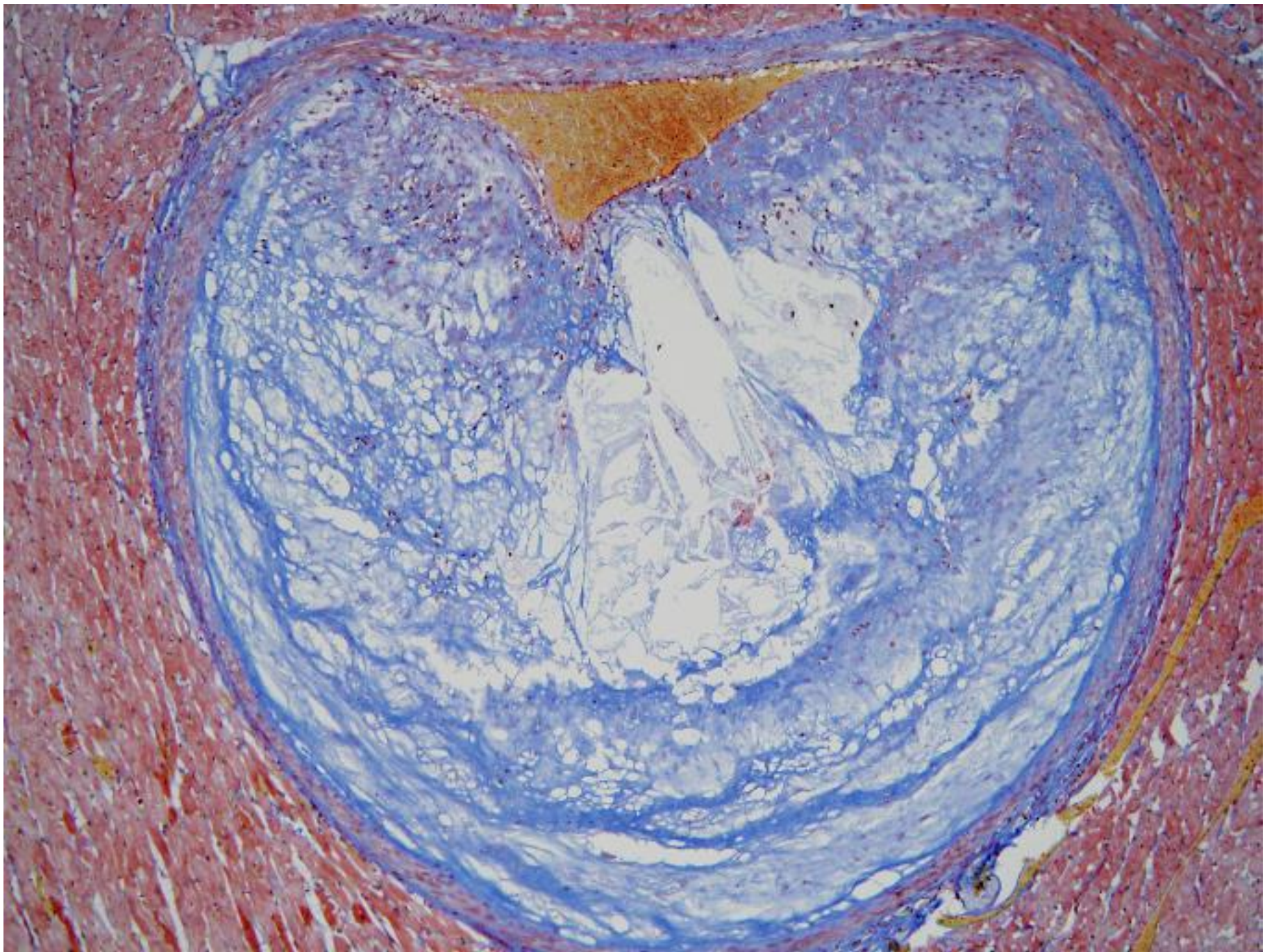


To keep the characteristics of WHHLM I rabbits, we have to analyze and record followings.

- The **serum lipid levels** periodically
- The degree of **coronary atherosclerosis**
- Development of **thin-capped fibroatheroma** in the coronary plaques

Preparation of Paraffin Blocks for Histopathological Analysis of Coronary Atherosclerosis

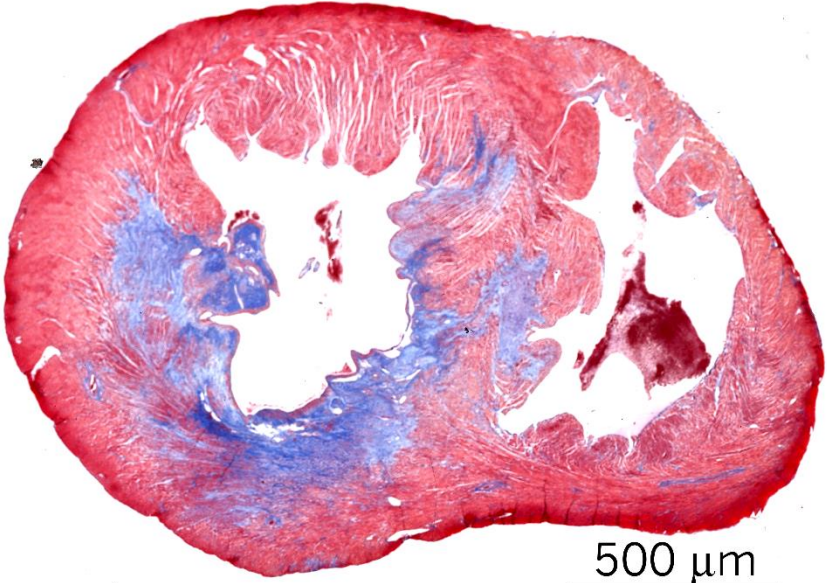




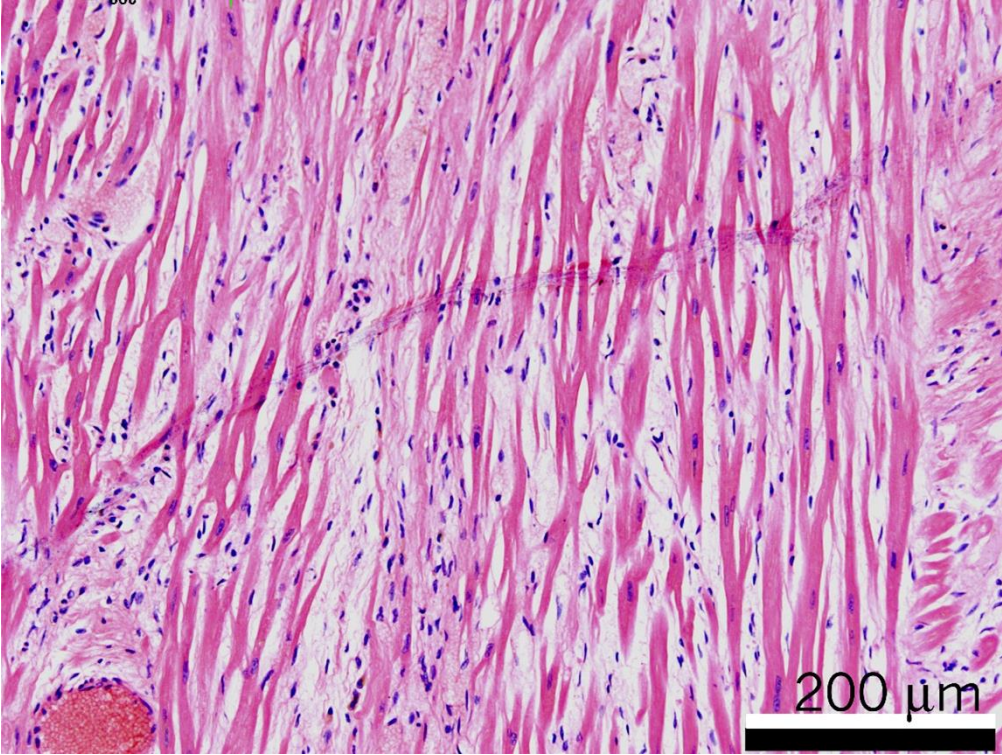
To keep the characteristics of WHHLMi rabbits, we have to analyze and record followings.

- The **serum lipid levels** periodically
- The degree of **coronary atherosclerosis**
- Development of **thin-capped fibroatheroma** in the coronary plaques
- Development of **myocardial infarction**

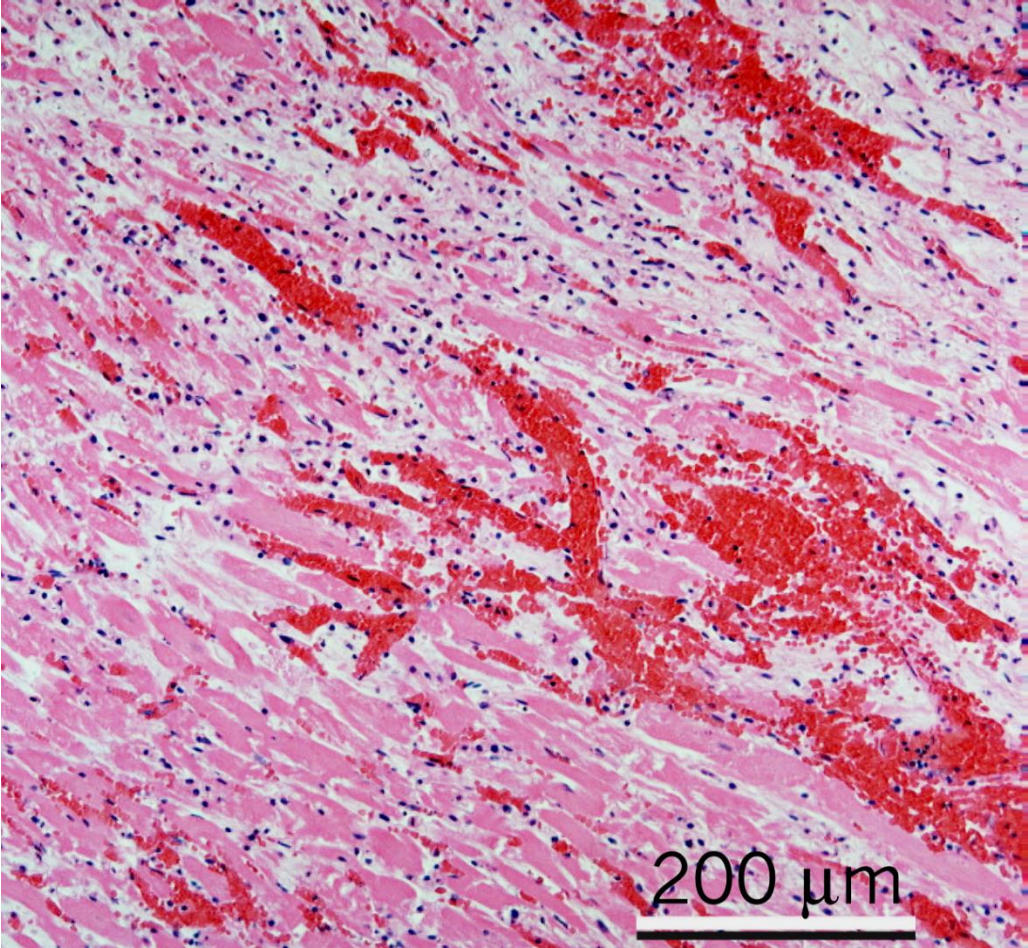
Myocardial lesions



500 μm



200 μm



200 μm

To keep the characteristics of WHHLMi rabbits, we have to analyze and record followings.

- The **serum lipid levels** periodically
- The degree of **coronary atherosclerosis**
- Development of **thin-capped fibroatheroma** in the coronary plaques
- Development of **myocardial infarction**
- Health condition

Selection of rabbits for mating

1. **Age** of rabbits: 6-24 months-old
2. Serum **cholesterol** levels more than
1,000 mg/dL at 6 months age,
800 mg/dL at 12 months age,
700 mg/dL at 18 months age.
3. Serum triglyceride levels : less than 400 mg/dL
4. Genetic background: **Parents** suffered from
myocardial infarction,
serial severe coronary plaques, and
thin-capped fibroatheroma in coronary plaques
5. Others: good healthy conditions (outward
appearance, body weight, feeding)

		Age	Breeding Record	Dam	Shire	Body weight				
26-123-65f	female	2012/3/20	16	1/11(1/3)	25-116-25f	?OKxOK	27-114-29m	?OK*?OK	12	3.27
26-123-69f	摂餌不良3/15~20	20	16	10/26(0/1)	25-116-34f	MIxMI	25-113-24m	?MI*MIOK	12	3.29
26-123-72m	male	2012/3/20	16	(X)(X)5/17(2/2)5/31()	25-116-34f	MIxMI	25-113-24m	?MI*MIOK	12	3.29
24-121-1f	female	2012/1/17	19	10/5(1/3)3/26(X)5/17(0/3)	23-101-2	MI	24-09Y-38	MI	18	3.55
24-121-2f	female	2012/1/17	19	10/5(3/6)3/26(X)5/17(0/6)	23-101-2	MI	24-09Y-38	MI	18	3.43
24-121-3m	male	2012/1/17	19	低Ch 4)(0/1)1/11(5/5)	23-101-2	MI	24-09Y-38	MI	18	3.23
24-121-4m	male	摂餌不良 11/28~		10/26(3/3)	23-101-2	MI	24-09Y-38	MI	18	3.03
25-121-5f	female	2012/1/17	19	10/5(5/7)5/17(5/7)	24-107-16	?xMI	26-107-29	OKOK	18	3.07
25-121-6m	male	2012/1/17	19		24-107-16	?xMI	26-107-29	OKOK	18	3.03
25-121-7m	BN(+/-)	2012/1/17	19	4/26(6/9)	24-107-16	?xMI	26-107-29	OKOK	18	2.88
25-121-8m	male	2012/1/17	19		24-107-16	?xMI	26-107-29	OKOK	18	2.83

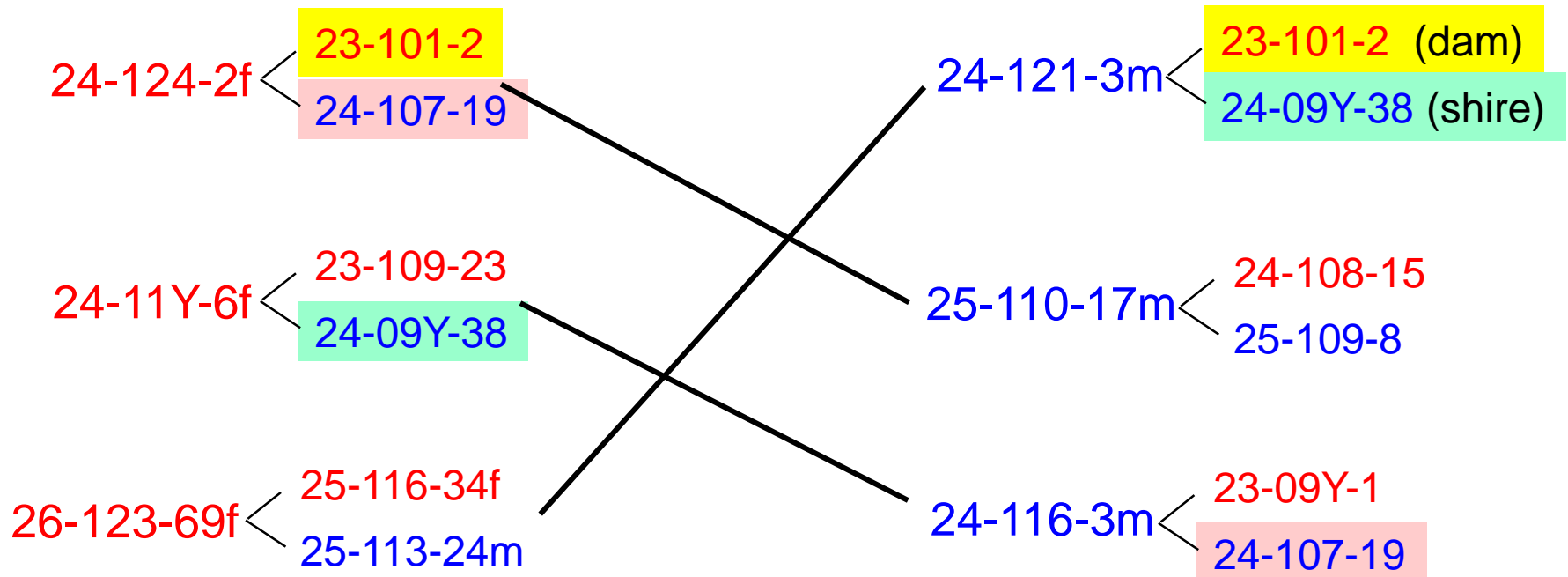
		Month-age	Cholesterol	Triglyceride								
			2	6	12	18	2	6	12	18		
26-123-65f	female	2012/3/20	16	1/11(1/3)	911	1098	980		256	137	233	
26-123-69f	摂餌不良3/15~20	20	16	10/26(0/1)	835	1026	996		252	143	305	
26-123-72m	male	2012/3/20	16	(X)(X)5/17(2/2)5/31()	803	1186	914		195	114	311	
24-121-1f	female	2012/1/17	19	10/5(1/3)3/26(X)5/17(0/3)	888	1055	875	629	225	159	221	258
24-121-2f	female	2012/1/17	19	10/5(3/6)3/26(X)5/17(0/6)	819	1042	954	872	261	221	345	505
24-121-3m	male	2012/1/17	19	低Ch 4)(0/1)1/11(5/5)	835	1055	629	580	342	120	235	354
24-121-4m	male	摂餌不良 11/28~		10/26(3/3)	944	1045	691	462	318	167	446	466
25-121-5f	female	2012/1/17	19	10/5(5/7)5/17(5/7)	881	967	832	612	253	139	165	188
25-121-6m	male	2012/1/17	19		858	1134	875	714	269	82	150	158
25-121-7m	BN(+/-)	2012/1/17	19	4/26(6/9)	1022	1281	803	626	297	122	161	153
25-121-8m	male	2012/1/17	19		855	1111	845	701	236	92	143	273

Record of WHHLMi rabbits

- ID number, gender, birth day, removal day, removal cause, dam, shire
- % area of myocardial lesions in the left ventricle
- Maximum stenosis of left circumflex artery, plaque feature
- Degree of aortic lesion
- Accumulation of mesenteric fat
- Serum cholesterol and triglyceride levels at 2, 6, 12, 18, 24, 30, 36 months old

Matching of mating pairs

1. Check the ID number of parents of candidate rabbits using in mating.
2. Avoid rabbits with the same dam or shire in matching.



Reproductive ability of WHHLM rabbits in 2012

Mating pairs: 77

Delivery dams: 66 Gestation rate: **0.857**

Total of pups: 406

Litter size: 5.68 ± 2.60 (mean \pm SD)

Total weanlings: 189 Weaning rate: **0.512** \pm 0.349

Weanling pups: 2.95 ± 2.26

Reproductive ability: **2.455** (weanlings/mating pairs)

Induction of ovulation

1. Maturation of follicles

Prior to mating, **FSH** is administered intramuscularly to rabbits at a dose of **1 AU twice a day for 3 days**.

FSH: Gonapure Injection 75 (Aska Pharmaceutical Co.Ltd, Tokyo)

2. Induction of ovulation

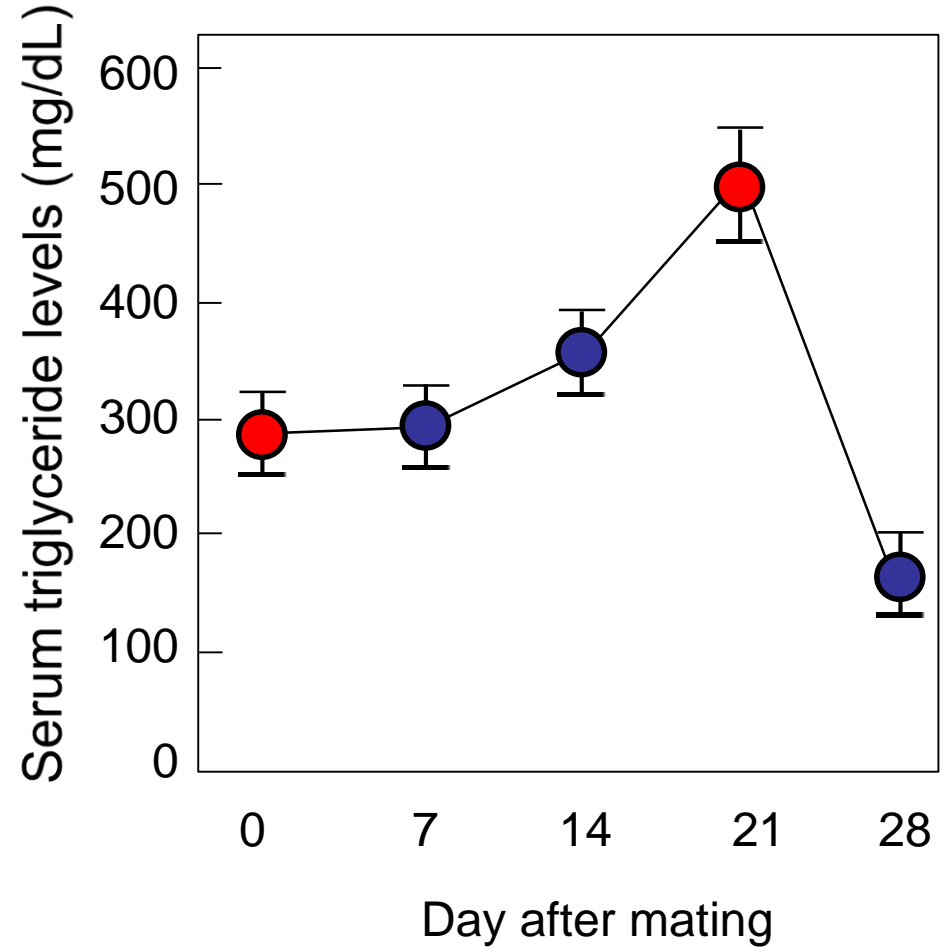
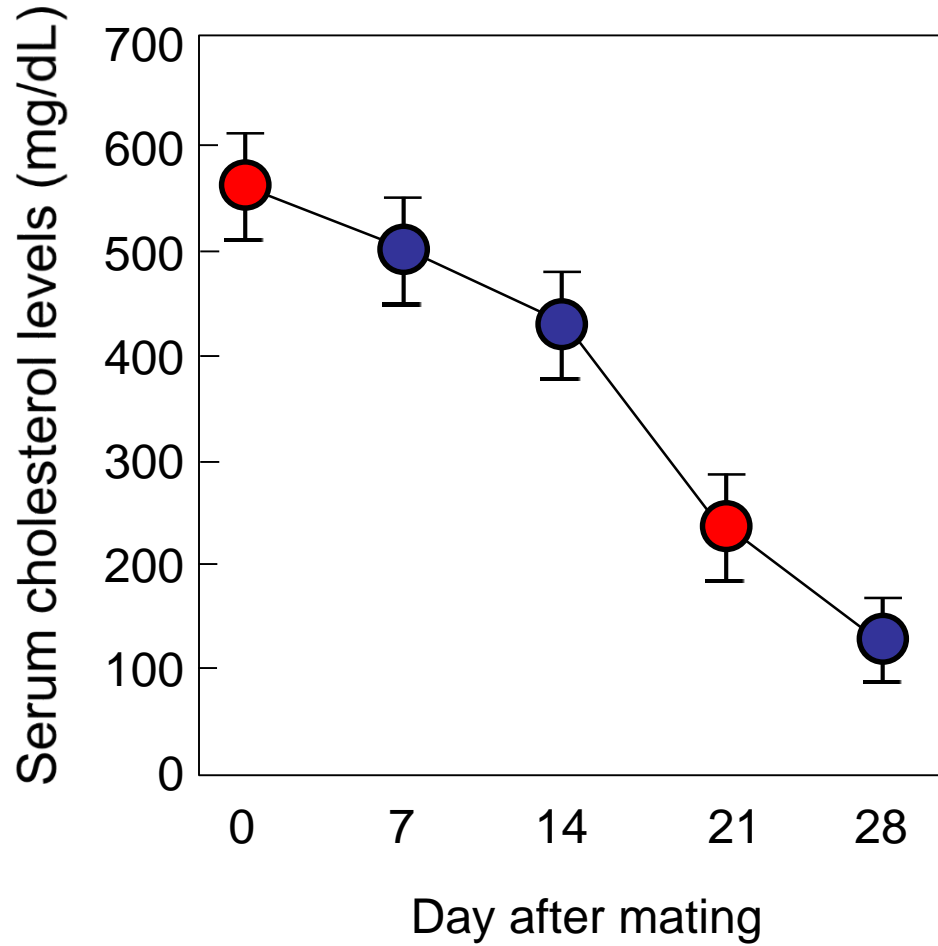
30 min before mating, **hCG** is administered intravenously to rabbits at a dose of **100 IU**.

hCG: Gonatropin 1000u (Aska Pharmaceutical Co.Ltd, Tokyo)

Mating of rabbits

1. Prepare 4 mating boxes, which are disinfected with alcohol and weak-acidic hypochlorite solution.
Two males and two females are put into each mating box.
2. Put a female into a mating box in which a male rabbit is already put.
3. Measure the time starting mating.
4. Observe behavior of the female and male.
Whether does the female raise the tail and/or buttock?
Whether does the male try to mounting?
5. Restrain the female gently, to induce the male, if the female does not accept the male.
6. If the male does not show any interest in the female after 10 min progress, put the female into another mating box in which another male is already put.
7. Generally, mating is performed twice with the same pair after 5 min rest.

Diagnosis of pregnancy



Changes in serum lipid levels during pregnancy

Before mating

21 days after mating

ID	B.W	T-CH	TG	B.W	T-CH	% change	TG	% change	Judgment
23-093-10	3.61	1064	364	3.62	312	29.32	316	86.8	○
23-096-6	3.60	960	296	3.51	976	101.7	273	92.2	×
23-096-16	3.32	1116	172	3.4	276	24.73	380	221	○
24-097-3	3.39	1108	135	3.41	724	65.34	99	73.3	○
25-09Y-13	3.37	1096	187	3.35	412	37.6	279	149	○
22-099-14	3.38	1124	210	3.39	432	38.4	162	77.1	○
22-099-16	3.13	1108	172	3.19	1104	99.6	190	110	×
22-099-20	3.08	1084	262	3.13	520	48.0	189	72.1	○
23-101-25	3.00	1076	201	3.03	164	15.2	235	117	○

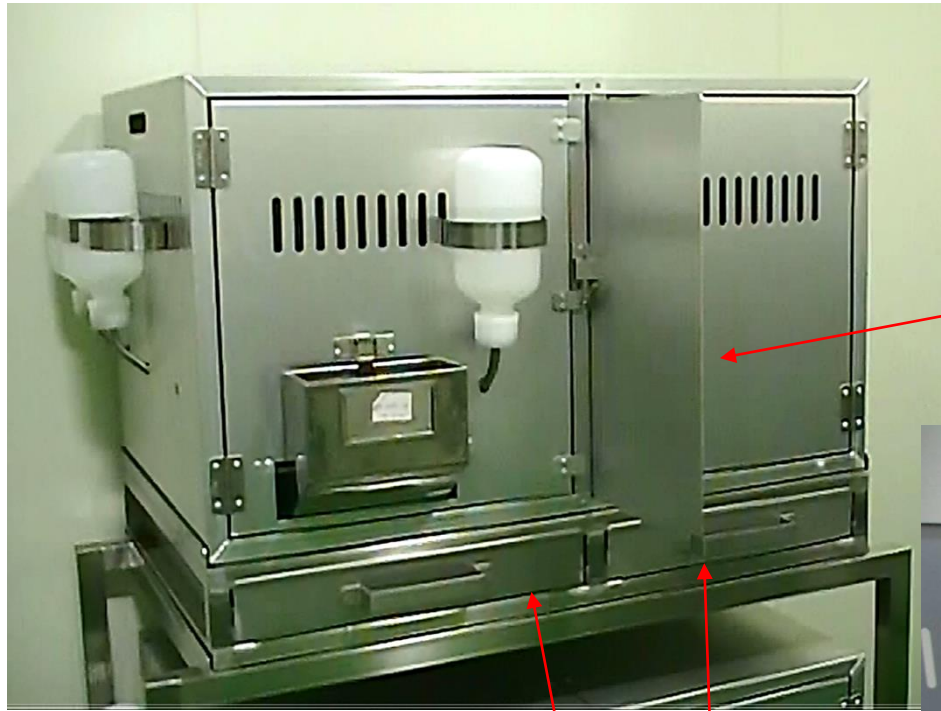
Preparation of delivery cages

27th day of pregnancy:

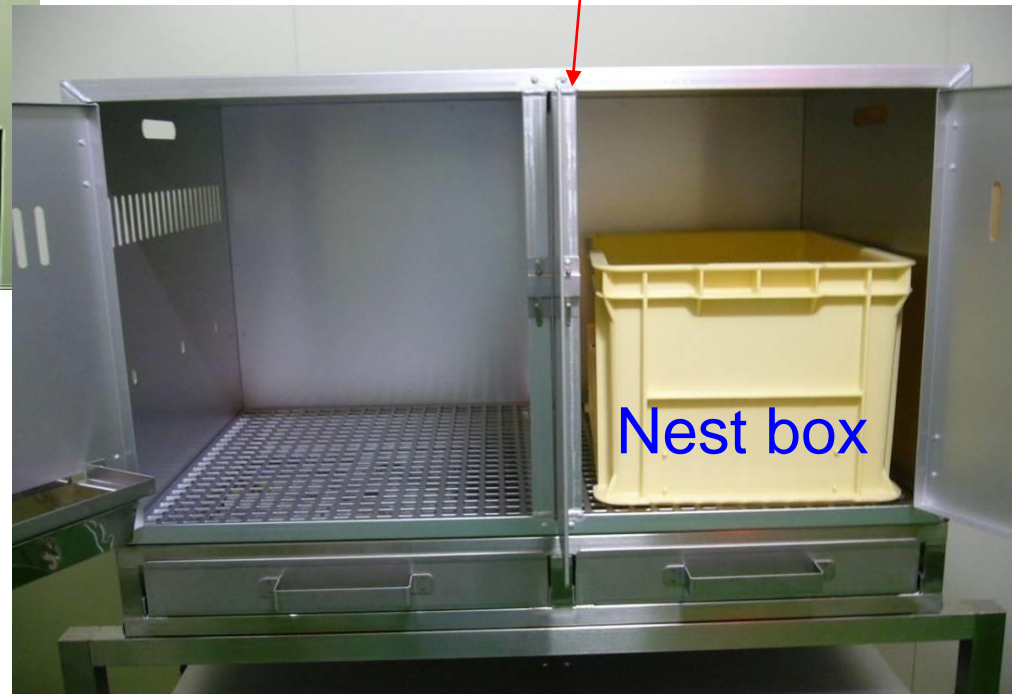
- Sterilize delivery cages, beddings (wood shavings for mice and rats, shredder cutting papers), and paper sheets for feces tray.
- Disinfect nest boxes and drinking bottles.

28th day of pregnancy: accommodate pregnant rabbits in the delivery cages set in the delivery room

Preparation of delivery cages and delivery room



Feces tray



Partition board

Nest box

Nesting box with new born pups



We used sterilized wood shavings and thinly sliced papers by a shredder as bedding in a nest box

Nest box with pups



We used sterilized wood shavings and thinly sliced papers by a shredder as bedding in a nest box

Preparation of delivery room

1. Lighting: half-light during suckling period (for 21 days)
2. Lighting cycle: 12 hours light and 12 hours dark
3. Room temperature: 22-25°C
4. Relative humidity: 55-70%
5. Ventilation rate: 15/hour

Care of rabbits around delivery period

28th day of pregnancy: accommodate rabbits to delivery cages set in the delivery room and provide chow (360 g) and water (3 bottles, 1,500 ml) for 3 days.

31st day of pregnancy: provide chow (800 g) and water (4 bottles, 2,000 ml) for 4 days

32nd day of pregnancy: Almost rabbits give birth.

Since delivered rabbits are very nervous, nobody enters delivery room until 3 days after delivery, and everyone must keep quite around the delivery room by weaning, to avoid eating new born pups or abandoning nursing.

Care of rabbits after delivery

4 days after delivery: confirm newborn pups and provide chow (600 g) and water (3 bottles, 1,500 ml) for **3 days**

Confirm newborn pups

- 1) Provide chow.
- 2) Close partition board quietly after mother rabbits start to eating.
- 3) Provide water bottles for 3 days.
- 4) Open the door of delivery area and count newborn pups, but must not touch them.
- 5) If pups were died, remove them after wearing globe.
- 6) If pups would be small due to avoiding nursing, put them out to other foster mother to nurse.

Care of rabbits after delivery

6 days after delivery: provide chow (about 300 g/day) and water (2 bottles, 1,000 ml/day)

After 6 days, chow and water are provided everyday.

10 days after delivery: dispose feces for the first time after close partition board.

After 13 days, dispose feces and change floor plate every Mon, Wed, Fri, and provide 360 g chow and 2 water bottles (1,000 ml) every day.

2 weeks after delivery: remove nest box

Care of rabbits after delivery

3 weeks after delivery: Move rabbits from delivery room to general animal room and make adapt pups to a water supply nozzle. Thereafter, delivery cages are washed and sterilized.

Group housing of a mother and the weanlings



Care of rabbits after delivery

3 weeks after delivery: Move rabbits from delivery room to general animal room and make adapt pups to a water supply nozzle. Thereafter, delivery cages are washed and sterilized..

4 weeks after delivery: Pups are weaned from their mother rabbits and are measured body weight.

3-4 pups are accommodated together in the same cage.

Group housing of weanlings



Group housing of weanlings



Care of rabbits after delivery

- 3 weeks after delivery: Move rabbits from delivery room to general animal room and make adapt pups to a water supply nozzle. Thereafter, delivery cages are washed and sterilized..
- 4 weeks after delivery: Pups are weaned from their mother rabbits and are measured body weight. 3–4 pups are accommodated together in the same cage.
- 7 weeks of age: Measured body weight, determine the gender of pups, and give ID number to each pups.

Naming of weanlings

Dam
Shire

M. 生		Mating: Oct 26, 3023 Birth: Nov 27, 2012				
♀ × ♂						
24-114-6f	25-124-22f	-23m	-24m			
24-116-3m						
25-123-7f	26-124-25f	-26f	-27f	-28f	-29f	
25-116-44m						
25-123-8f	26-124-30f	-31f	-32m			
24-113-13m						
24-123-18f	25-124-33f	-34f	-35f	-36m	-37m	
25-116-44m						
26-123-21f	27-124-38f	-39m	-40m			
24-121-4m						
25-123-37f	26-124-41f					
25-110-17m						
25-123-38f	26-124-42f	-43m	-44m			
23-113-7m						
24-124-2f	25-124-45f	-46f				
25-110-17m						

ID number of weanlings

ID number of WHHLM I rabbits

28-097-1f 29-120-5m 27-13Y-10f 30-13Z-21m

Female or male

Serial number of rabbits born in the same month

Birth year and month: born at JUL, 2009; OCT, 2012;
NOV, 2013; and DEC, 2013, respectively

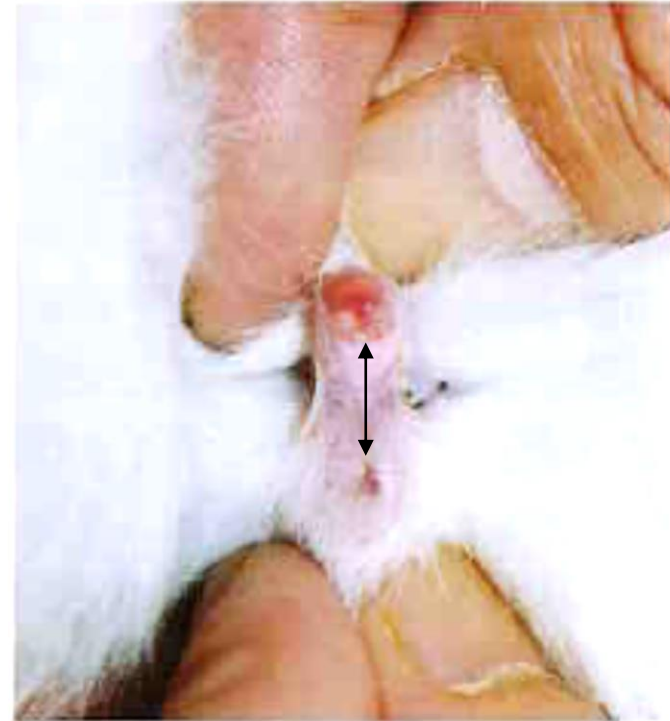
Generation: add one to generation number of the mother rabbit.

Determination of pups gender

Determined from shape of external genitalis and length between genitalis and annulus



female



male

- Weaning (group housing) : 4 weeks of age
- Determine the gender: 7 weeks of age
- Individual housing: 7 or 8 weeks of age

Care of WHLMI rabbits

Room temperature: 20-24°C

Relative humidity: 55-70%

Lightening circle: 12 hours light and 12 hours dark
(lighting: 6:00-18:00)

Chow feeding: 120 g/day (LRC-4, Oriental Yeast company)

Supplement: hey cub for prevention of hair ball
cabbage for pups and rabbits showing anorexia
(Chow and supplements are sterilized with an autoclave.)

Water: ad libitum (tap water, automatic water supply)

Health observation: everyday

weak-acidic hypochlorite solution



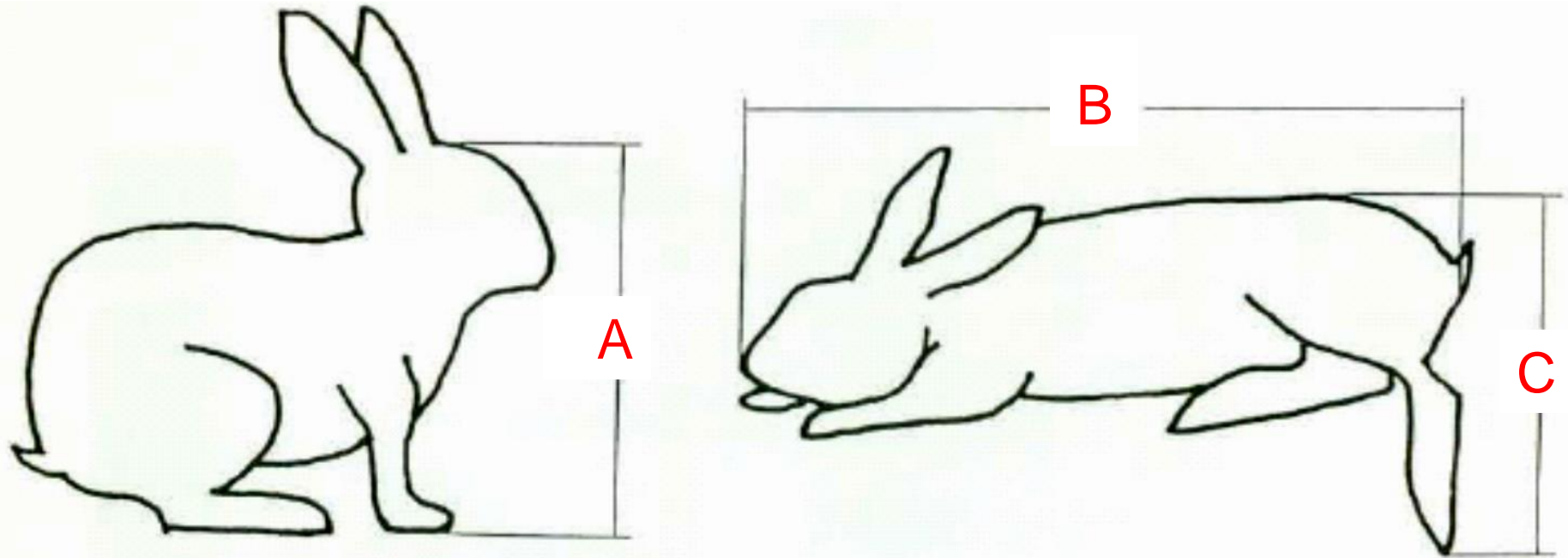
weak-acidic hypochlorite solution



Disinfecting for
breeding apparatus,
floor of animal rooms
and operational room,
benches, and others

Cages for rabbits

Body size of WHHLMI rabbits



Body weight	Withers height (A)	Body length (B)	Leg length (C)
3.5 kg	32 cm (45 cm) (from top of ear)	54 cm	28 cm

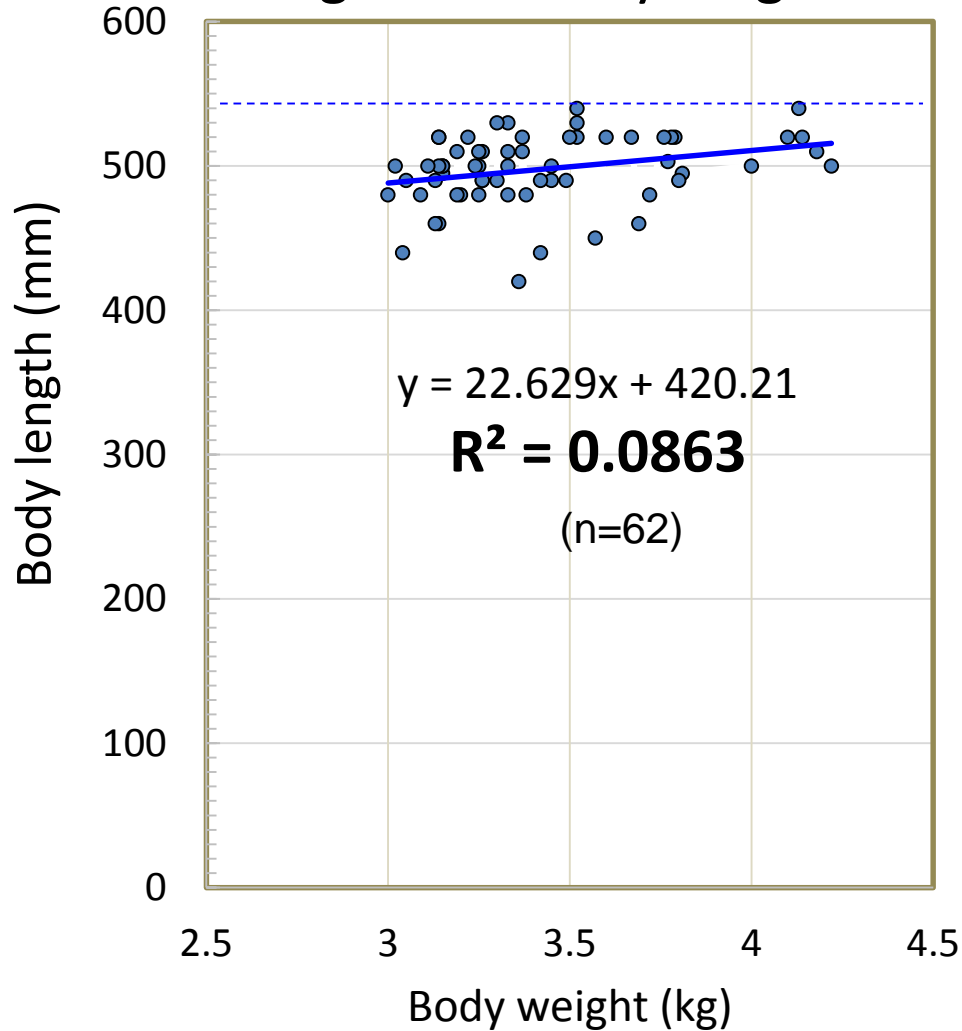
Data indicate maximum value

Size of cages for WHHLM rabbits

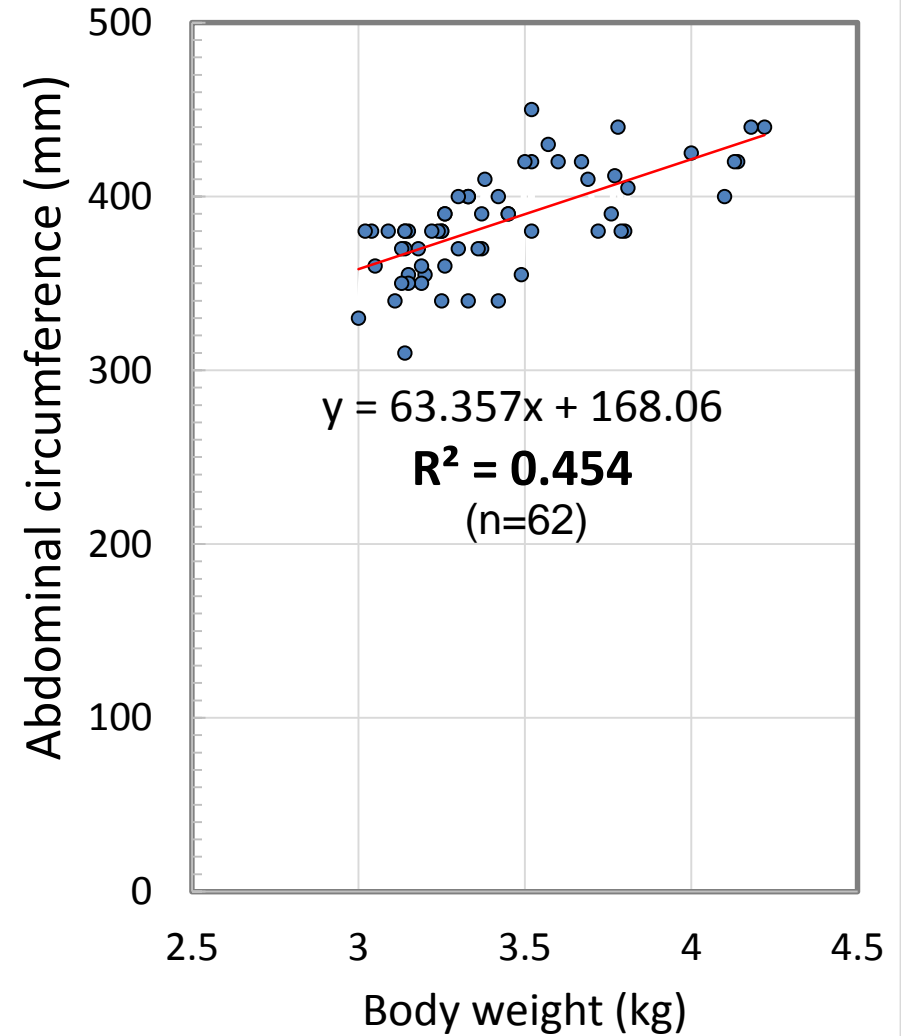
	Body weight	floor area (cm ²)	Height (cm)
Kobe Univ	< 3.5 kg	3250 (50x65)	45
<hr/>			
ILAR2011	<2.0kg	1400	40.5
	2.0—4.0kg	2800	40.5
	4.0—5.4kg	3700	40.5
ETS 2013	<3.0 kg	3500	45
	3.0—5.0 kg	4200	45

Changes in body size of WHHLM1 rabbits by increasing body weight

Relation between body weight and body length

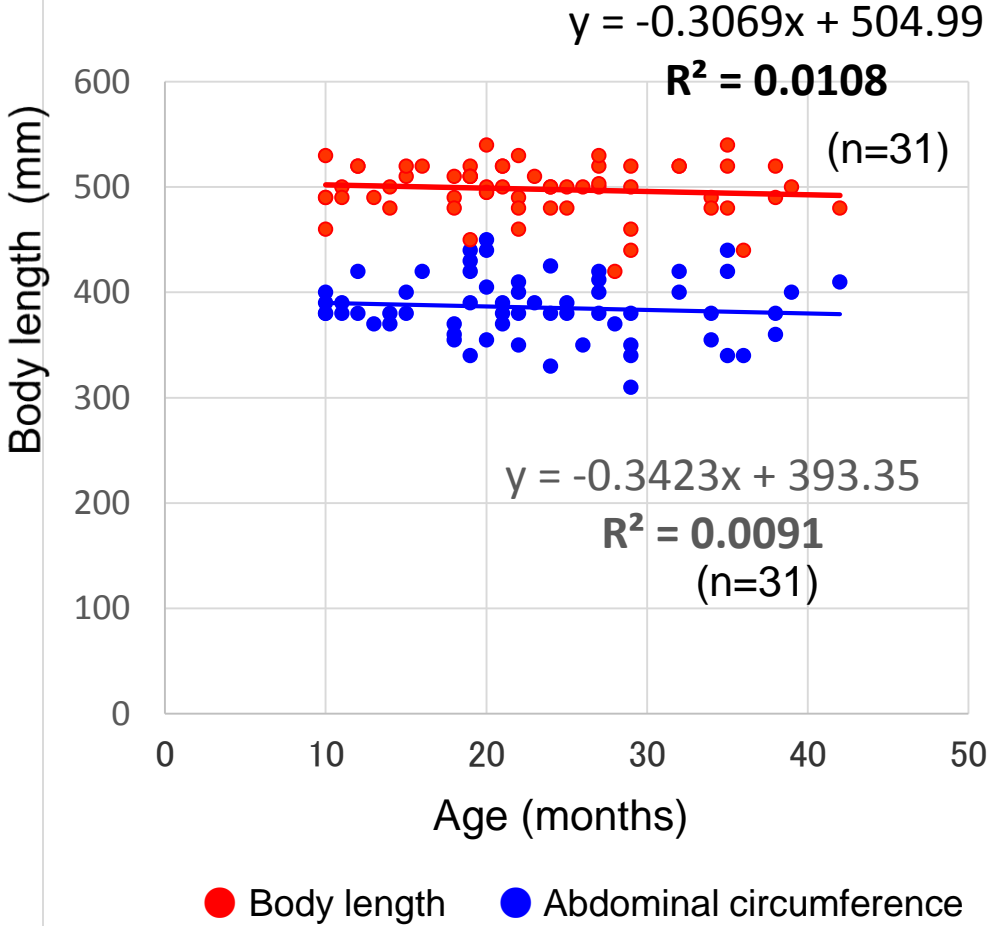


Relation between body weight and abdominal circumference

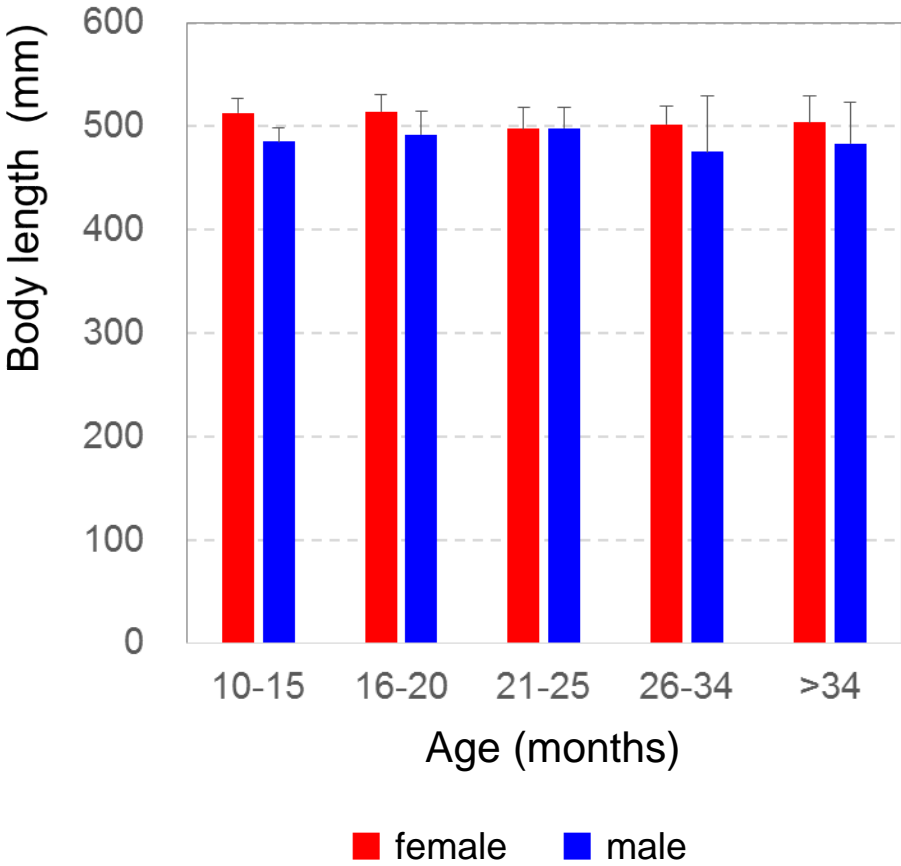


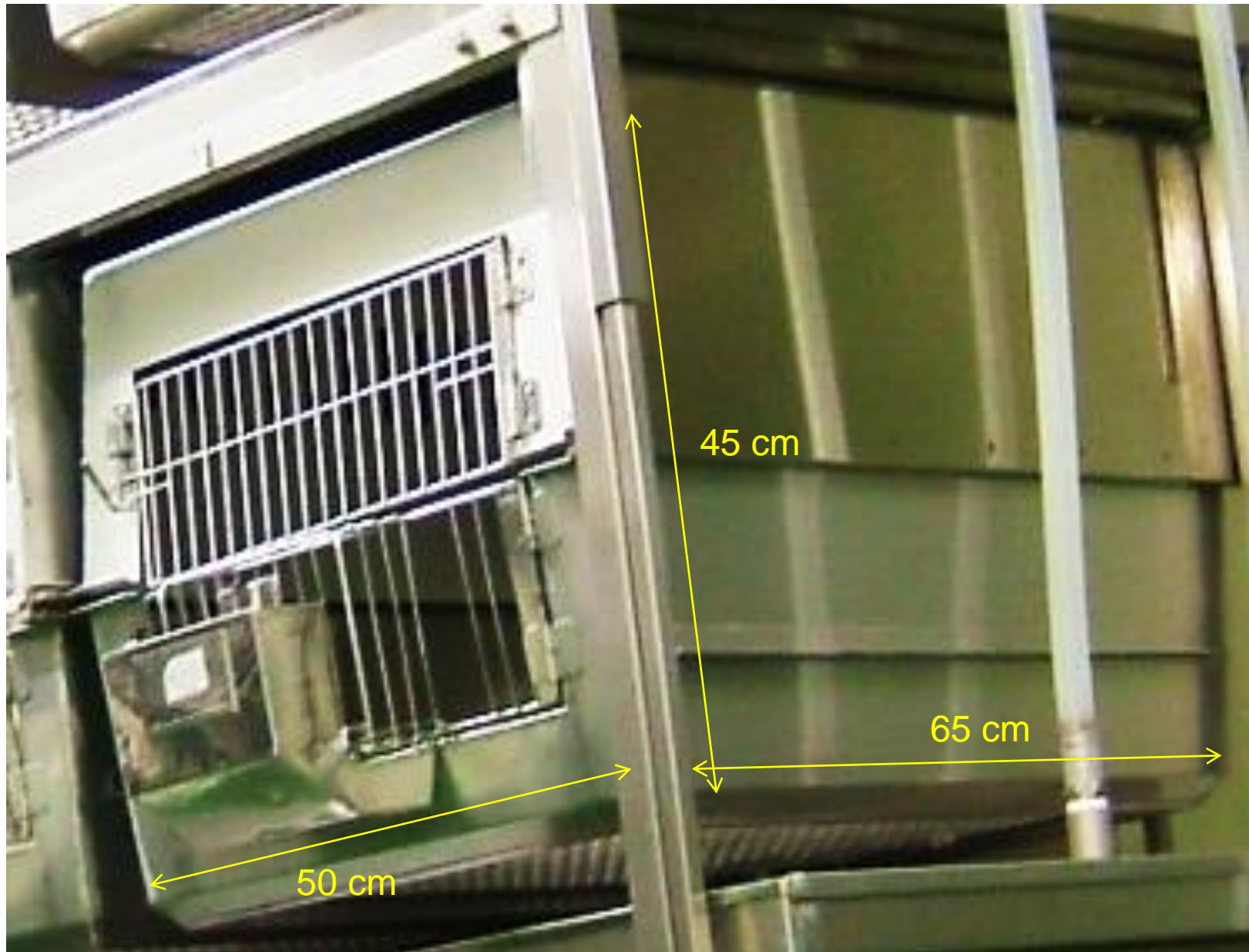
Changes in body size of WHHLM rabbits born after 2009

Relation of age and body size



Changing in body length with aging





45 cm

65 cm

50 cm



50 cm



Health management and examination of pathogenic microbe

Consideration of **welfare** of rabbits on breeding



A large haycube for an environmental enrichment

Health observation

部屋番号 号室 うさぎ

ケージ 番号	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	備考	
A -																																	レ:異常なし a:入室 b:退室 c:飲水 d:糞汁 e:残餌 f:死亡 g:給食 h:下痢 i:排便 j:脱毛
B -																																	
C -																																	

- レ: no particular
- a: accomodate
- b: exit
- c: soft stool
- d: rhinorrhea
- e: leftovers
- f: death
- g: fasten
- h: diarrhea
- i: bended neck
- j: 脱毛

Animal Health Report

Species : Rabbit Strain : WHHLMJ Sex : Male(8) , Female(7) No. of tested : 15 heads

Materials : Stamped Tapes on Ear
Nasal swab , Fecal contentSampled Date : July 28, 29, 2010, August 2, 2010
Tested Date : July 28, 2010 ~ August 4, 2010

Examination of pathogenic microbe (once in a year)

Items
<i>Pasteurella multocida</i>
<i>Bordetella bronchiseptica</i>
<i>Salmonella</i> spp.
<i>Mycoplasma pulmonis</i>
Sendai virus
<i>Clostridium piliforme</i>
Eimeria spp.
Ear mange
Body mange

Items	Bacteriology	Serology	Parasitology	No. of positive /tested
<i>Pasteurella multocida</i>	○			0/15
<i>Bordetella bronchiseptica</i>	○			0/15
<i>Salmonella</i> spp.	○			0/15
<i>Mycoplasma pulmonis</i>		○		0/15
Sendai virus		○		0/15
<i>Clostridium piliforme</i>		○		0/15
Eimeria spp.			○	0/15
Ear mange			○	0/15
Body mange			○	0/15

ID No	Sex	age (months)	B.W. (kg)	Room and rack No.	Cage No.
22-077-10	♂	36	3.39	427-1	3
24-087-14	♂	24	3.49	427-2	15
22-080-17	♂	21	3.08	427-3	33
24-082-6	♂	19	3.58	427-4	40
23-093-1	♀	16	3.48	427-5	54
23-096-10	♂	12	3.13	427-6	63
23-097-16	♀	12	3.24	427-7	81
22-098-6	♀	11	2.81	427-8	87

ID No	Sex	age (months)	B.W. (kg)	Room and rack No.	Cage No.
23-099-4	♂	10	2.94	427-9	105
22-099-15	♀	10	3.22	427-10	116
24-099-24	♂	10	2.58	427-11	125
23-09Y-7	♂	8	2.62	427-12	132
23-101-17	♀	6	2.53	424-3	27
23-101-23	♀	6	2.59	424-4	33
24-101-31	♀	6	2.79	427-14	162

Inspected by :

Nobue Hirayama, D.V.M.

Manager :

Masashi Shiomi, Ph.D.

Institute for Experimental Animals, Kobe University Graduate School of Medicine
Kusunoki-cho 7chome, chuo-ku Kobe 650-0017 Japan

Probable cause of anorexia in rabbits

1. no drinking of water

Check water supply nozzles.

2. malocclusion of incisor tooth

Cut incisor tooth



3. Formation of a hair ball in stomach.

Administer metoclopramide hydrochloride solution orally.

Feces become small and are linked together like beads of a rosary, in addition to anorexia



To prevent formation of hair ball in stomach

Remove hairs
in feeder.



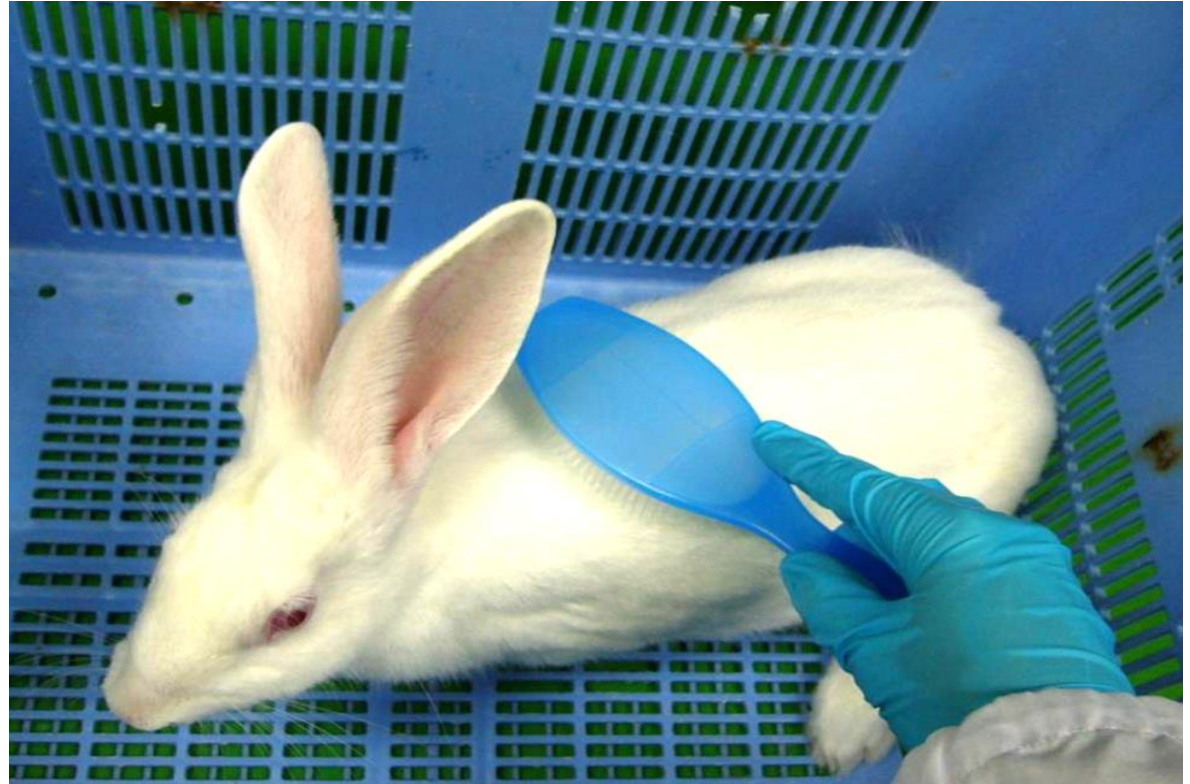
To prevent formation of hair ball in stomach

Give sterilized hey cub 5 g/ day.



To prevent formation of hair ball in stomach

Brushing is effective for removal of hair fell off and for acclimating rabbits to humans.



Treatment of rabbits showing anorexia in case of Hair Ball

Preparation of metoclopramide hydrochloride solution

- Grind tablets of metoclopramide hydrochloride solution
- Add 2 ml of glucose solution and suspend
- Administered the suspension at a dose of **1 mg/kg** once or twice a day



Treatment with **metoclopramide hydrochloride** solution

- Administered orally for **3-5** days.
- Give sterilized hey cub and cabbage during anorexia.



Probable cause of anorexia in rabbits

1. no drinking of water

Check water supply nozzles.

2. malocclusion of incisor tooth

Cut incisor tooth

3. Formation of a hair ball in stomach.

Administer metoclopramide hydrochloride solution orally.

4. Derangement of Intestinal flora or intestinal inflammation

Administer chlortetracycline hydrochloride solution orally.

Treatment of rabbits showing anorexia with derangement of Intestinal flora or intestinal inflammation or diarrhea

Administer **chlortetracycline hydrochloride** solution (0.1 g/100 ml) orally for **5 days** using drinking bottles.

It is important to confirm whether rabbits drink the drug solution.



To prevent buck injury of rabbits

Not to frighten rabbits,

- Knock door of animal rooms before enter.
- Talk to rabbits gently while waking in the animal rooms.

Rabbits are very nervous and timid.

To prevent nail injury, clipping nails is important .

Nail clipper





Floor plate of cage with square punching (15 mm x 15 mm)



Effective for prevention of plantar ulcer.

Anesthesia, pain relief, and euthanasia

Anesthesia for surgical experiment

Kylazine (3 mg/kg) + Midazoram (1 mg/kg) +
Ketamin (15 mg/kg)

Local anesthesia

Xylocaine 2%

Continuous anesthesia

Ketamin 60 mg/kg/h, 12 ml/kg/h

Euthanasia and evaluation of coronary lesions

- Exsanguination from carotid artery or femoral artery after anesthesia with **pentobarbital sodium (50 mg/kg) with xylocaine**.
- Observation of **xanthoma** and **mesenteric fat** accumulation, and findings of each organ.
- Extirpation of organs: **heart**, brain, **aorta** from proximal to bifurcation of femoral artery with renal artery, carotid arteries, and other tissues showing abnormal findings.
- Preparation of histopathological sections about **coronary lesions** and **myocardial lesions**, and evaluate the degree and findings.

