

Fuji Micra's Micro Mini Pigs



← Domestic pig
at 6 month

← Minipig
at 6 month

← Microminipig (MMP)
at 6 month

Global Partnering Opportunity Overview
February 2017



Micro Mini Pigs (MMP)



MMP

Originate from potbellied mini pigs in the United States. Bred and marketed by Fuji Micra since 2009

- Smallest among commercially available minipigs
- White, black and silver
- Outbred strain, 11 SLA types
- Highly homologous to ordinary pigs
- Not genetically modified
- 18th year maintaining closed colony



Fuji Micra Inc.

Located in Shizuoka, Japan (by Mt. Fuji)

President: Noriaki Satake

2006 Microminipig developed by Fuji Nojo Service

2008 Seed stock transferred to Fuji Micra

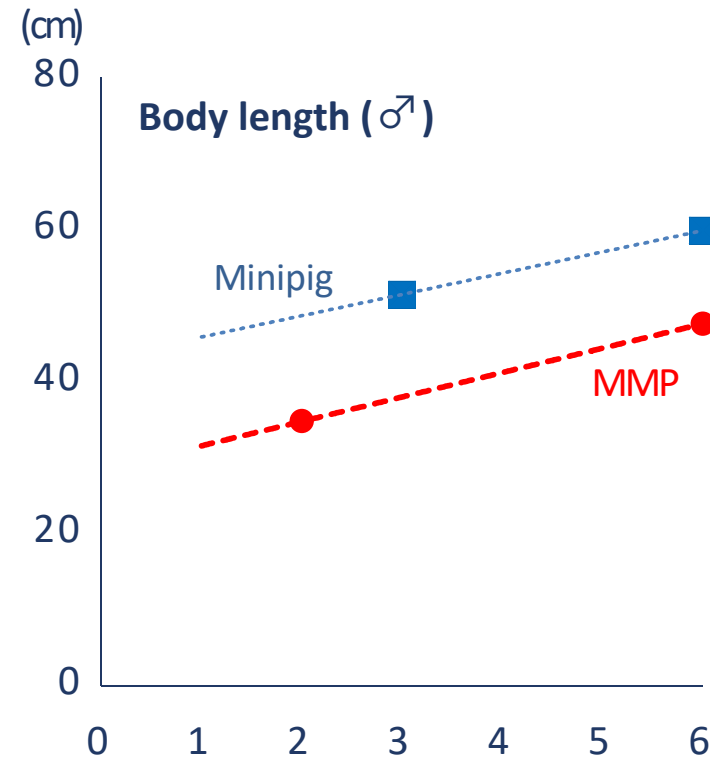
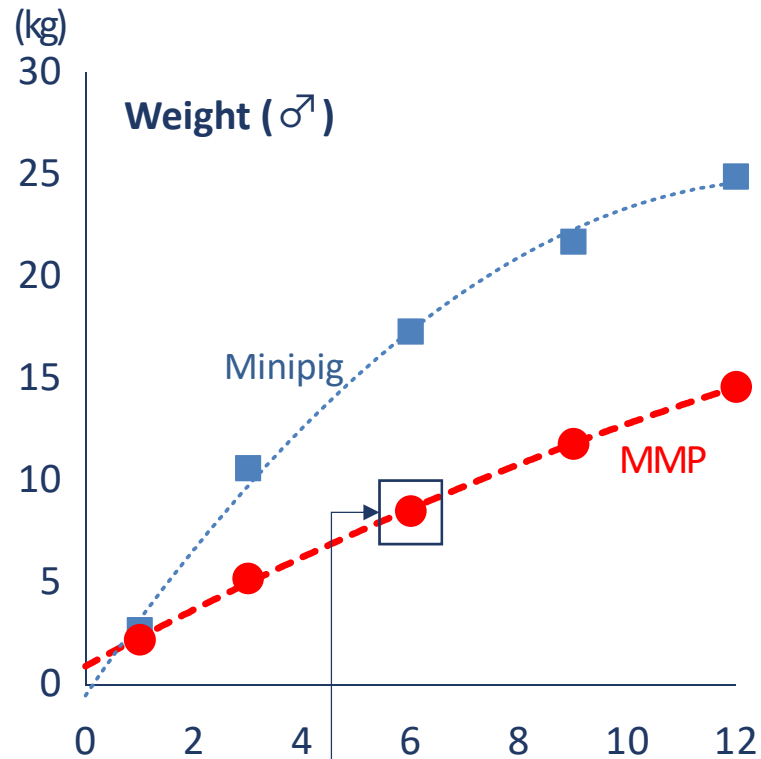
2009 MMP commercially launched

2012 P2A-level facility completed. Production of clean animals started

2016 Custom-order immuno-compromised MMP production started

Customers include: Takeda, Kagoshima U, Kyoto U, Keio U, Jichi Medical U, Toho U, CMIC Bioresearch Center, Shin-Nippon Kagaku, Japan SLC, etc.

MMP: Very small in size



10kg at 6 months

(Organ sizes roughly equivalent to beagle dogs at 10 months / four-year old human child)



- Less investigational compound required
- Lower environmental impact
- Can be bred in dog size cages

MMPs remain small over time



15 kg
White
1 year



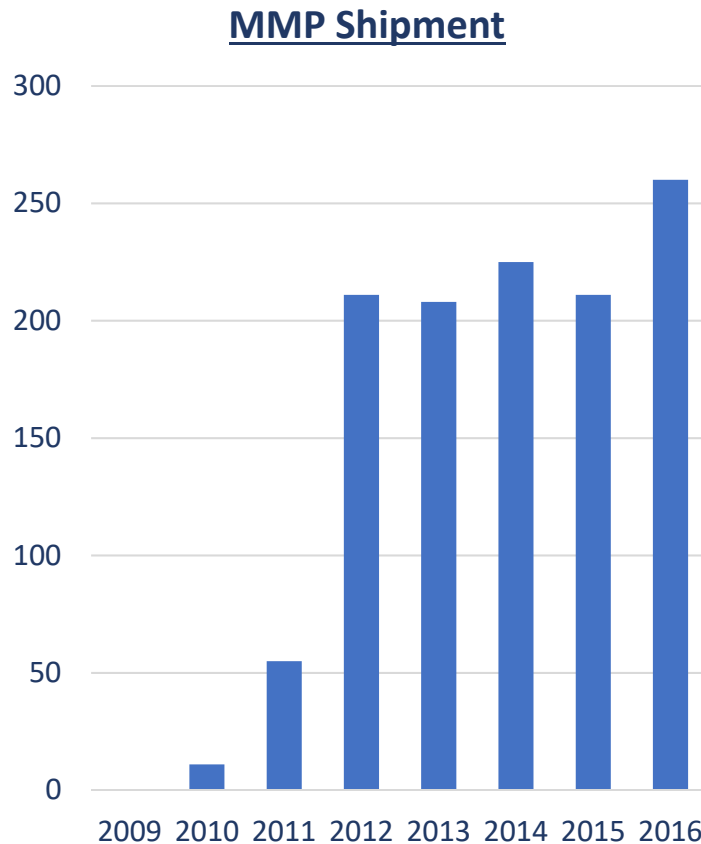
20 kg
Black
1½ years



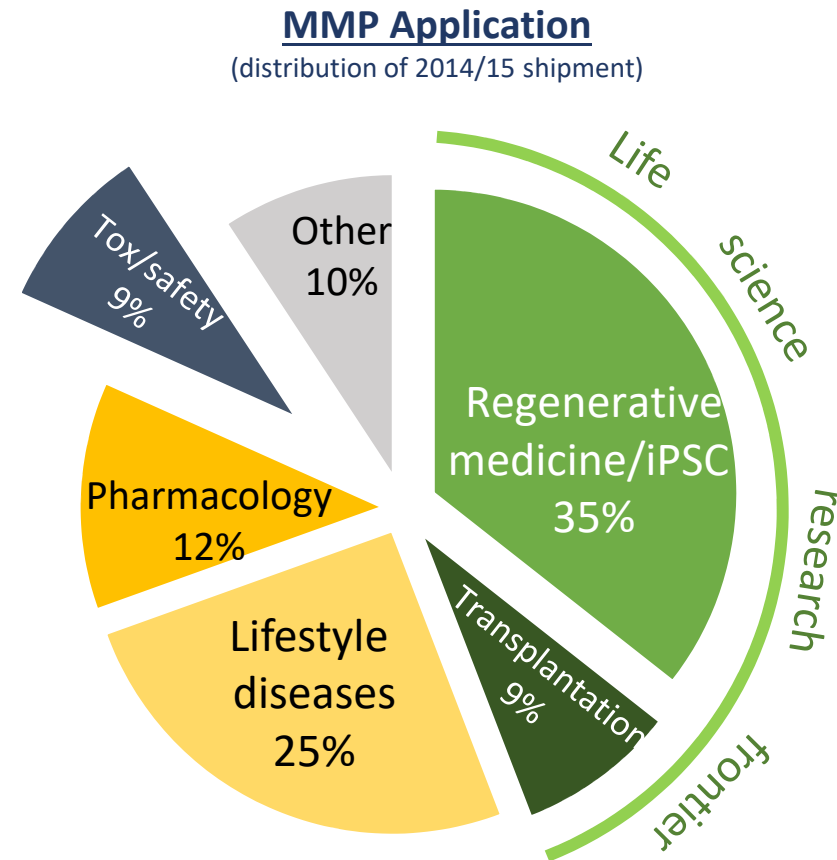
25 kg
Silver
2 years

Hair colour does not equal skin colour. White- and silver-haired animals have white skin. Some black-haired animals have patch of white skin underneath.

MMP leads scientific research



Japan represents one tenth of global research market ...
Further growth potential



Nearly half of MMP demand is for emerging, high value areas of life science research

MMP track record (published studies)



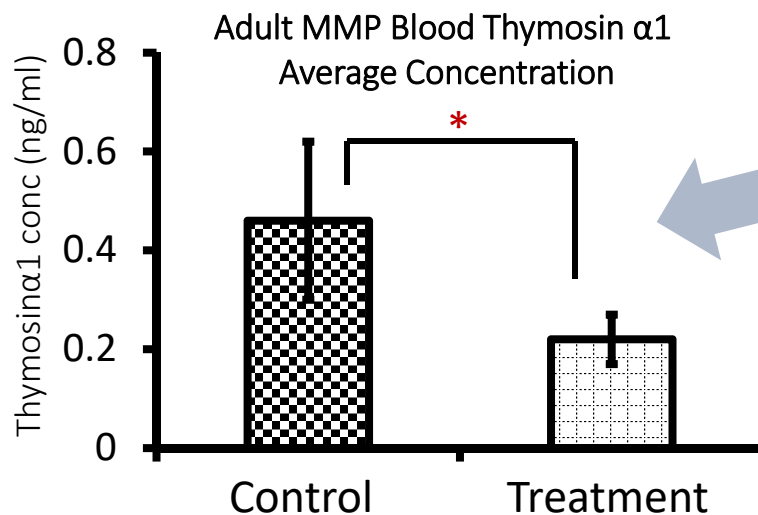
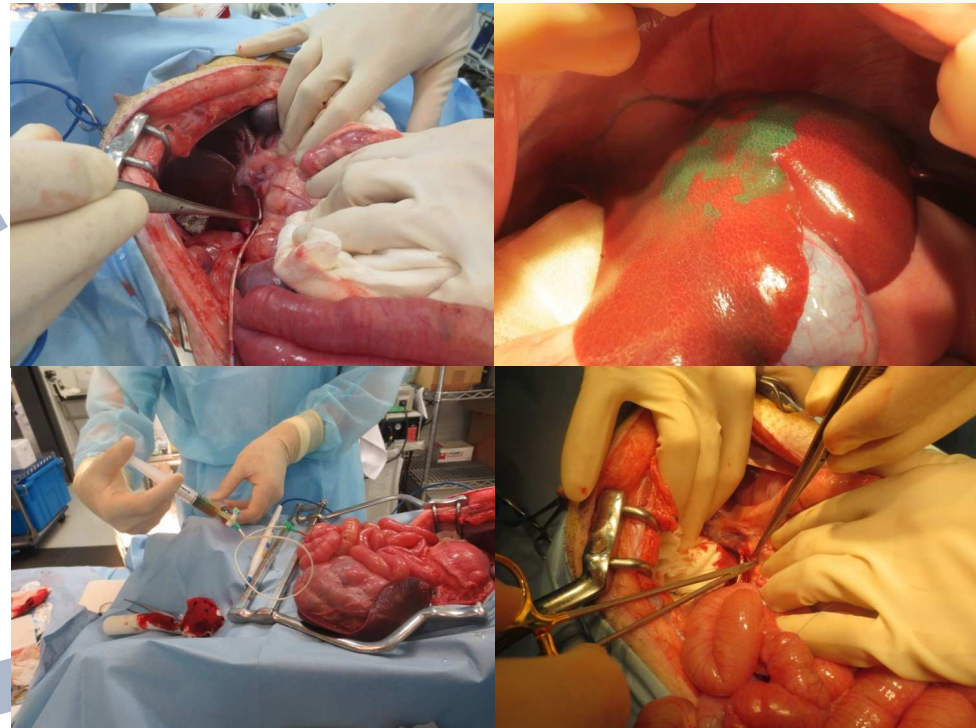
MMP maturity	MMP age (month)	Research focus	Institution(s)
Young	1	Norovirus infection testing	Shizuoka Pref Environmental Health Research Inst, Hamamatsu U
	3	Influenza infection testing	U of Tokyo
	3	Atherosclerosis model	Kagoshima U
	3.5-4.5	Metabolic syndrome model	Osaka City U
	4	Lactoferrin on atherosclerosis	Lion Corp, U of Tokyo, Kagoshima U, Yokohama U, Kyoto Pref U, Ritsumeikan U
Young mature	5-7	Dermal phototoxicity	Shin Nippon Biomedical Laboratories
	6	Proarrhythmia	Toho U
	6-12	Acupuncture on sleep	Kagoshima U
	8	Cytochrome P450-dependent pharmacokinetics	Showa Pharmaceutical U
	9-14	Environmental toxin (perfluoroalkyl acid, PFAA)	National Institute of Animal Health, Agriculture and Food Research Organization
Adult	12	Mesenchymal stem cells for meniscal repair	Tokyo Medical and Dental U
	12	Hydrodynamic gene therapy	Niigata U
	18-24	Partial hepatectomy model	Kyoto U
	23-25	Osteochondral regeneration	Kagoshima U
		Mesenchymal stem cell transplantation	Jichi Medical U

Human cell transplantation model

Thymus gland resection



Human hepatocyte transplantation



*Treated animals remained immuno-compromised showing reduced Thymosin $\alpha 1$.
Tymectomised MMP is a promising human cell transplantation model.*

Transgenic Kusabira Orange(huKO)



huKO pig ♂ MMP ♀

BF

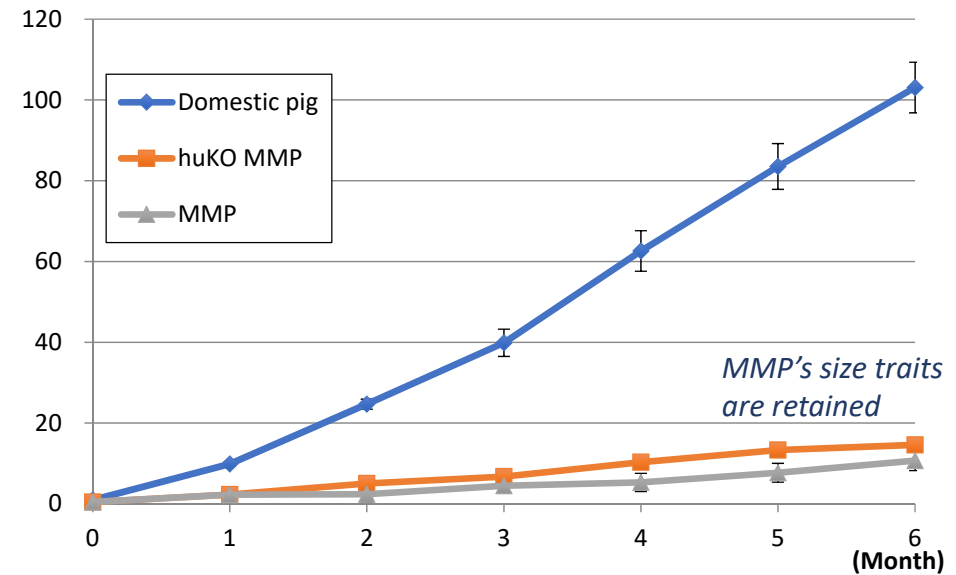


huKO



(Kg)

Body weight



SWINE LEUCOCYTE ANTIGENS (SLA)

SLA1: 1201, 1301

DRA : 020102

SLA2: 1001

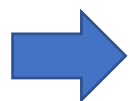
DRB1: 1001

SLA3: 0502

DQA : 0101

DQB1: 0601

The huKO MMP was established from the huKO transgenic pig carrying single copy of huKO gene on 17q23 locus (Matsunari et al., 2008)* by back-crossing with MMP over 3 generations.



Professor Nagashima's team is currently developing new models, e.g., Marfan syndrome, dilated cardiomyopathy, diabetes, immunodeficiency

*H Matsunari et al., Transgenic-cloned pigs systemically expressing red fluorescent protein, kusabira-orange., *Cloning and Stem Cells*, 10: 313-24, 2008.

Interest in safety studies



Dr. Sakai
"Pharmacokinetics"

Prof. Nakamura
"Orchitoxicity"

Prof. Nakamura
"Immune system"

Prof. Nakamura &
Dr. Kunieda
"Hematotoxicity"

Prof. Tanimoto
"MMP toxicopathology"

Dr. Ohtake
"MMP genetic background"

Prof. Sugiyama
"MMP cardio-safety evaluation"



シンポジウム 9

マイクロミニピッグを用いる医薬品の安全性評価

座長：Peter HEINING (Department of Preclinical Safety, Novartis Pharmaceuticals, Switzerland)
杉山 篤 (東邦大学医学部薬理学講座)

S9-1 Global expectations on the microminipig for the safety evaluation of pharmaceuticals

○ Peter HEINING
Department of Preclinical Safety, Novartis Pharmaceuticals, Switzerland

S9-2 マイクロミニピッグの特長と遺伝的背景

○大竹 正剛
静岡県畜産技術研究所中小家畜研究センター

S9-3 マイクロミニピッグの毒性病理

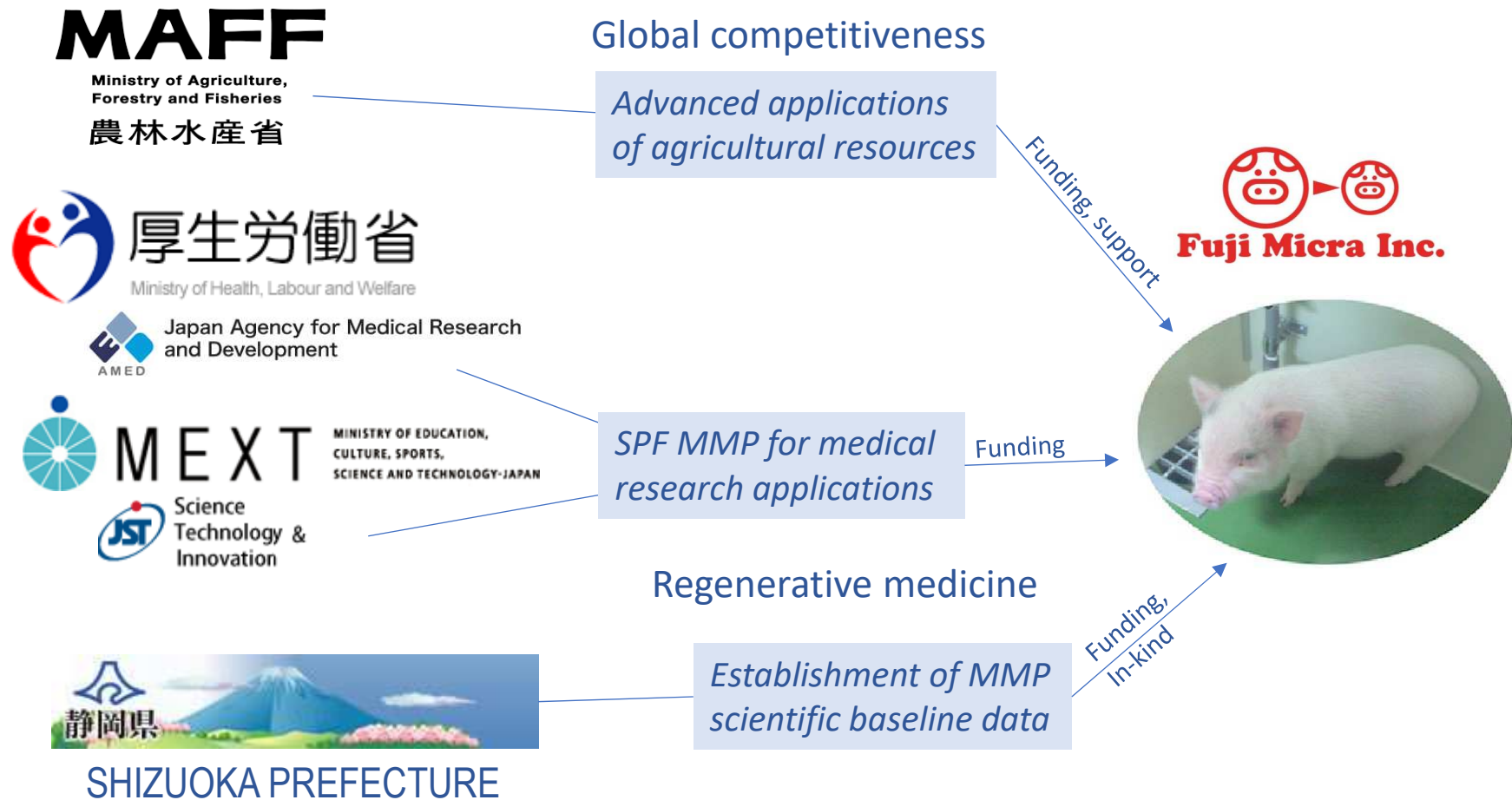
○川口 博明¹, 堀内 正久², 谷本 昭英¹
¹鹿児島大学大学院医歯学総合研究科 病理学,
²鹿児島大学大学院医歯学総合研究科 衛生学・健康増進医学

S9-4 マイクロミニピッグを用いる医薬品の心臓安全性評価

○杉山 篤, 和田 剛, 山本 篤, 東野 浩, 山崎 正, 山崎 隆, 山崎 隆

*MMP gaining interest of toxicology community and pharma companies
Top-tier Japanese global pharma now use MMPs and Novartis and Bayer inquiring global availability*

MMP aligns to key national agenda



Government support for technology development and global reach are expected to continue for the coming years

Research publications

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- Kaneko N, et al. "Microminipig, a non-rodent experimental animal optimized for life science research: preface." *J Pharmacol Sci.* 2011;115(2):112-4.
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- Kawaguchi H, et al. "Reference values of hematological and biochemical parameters for the world smallest microminipigs." *J Vet Med Sci.* 2012 Jul;74(7):933-6.
- Kawaguchi H, et al. "Microminipig, a non-rodent experimental animal optimized for life science research: novel atherosclerosis model induced by high fat and cholesterol diet." *J Pharmacol Sci.* 2011;115(2):115-21.
- Kawaguchi H, et al. "Rapid development of atherosclerosis in the world's smallest Microminipig fed a high-fat/high-cholesterol diet." *J Atheroscler Thromb.* 2014;21(3):186-203
- Morishita S, et al. "Enteric lactoferrin attenuates the development of high-fat and high-cholesterol diet-induced hypercholesterolemia and atherosclerosis in Microminipigs." *Biosci Biotechnol Biochem.* 2016;80(2):295-303.
- Kobayashi E, et al. "The pig as a model for translational research: overview of porcine animal models at Jichi Medical U." *Transplantation Research.* 2012;1:8.
- Miura N, et al. "Coagulation activity and white thrombus formation in the microminipig." *In Vivo.* 2013 May-Jun;27(3):357-61.
- Miyoshi N, et al. "Novel microminipig model of atherosclerosis by high fat and high cholesterol diet, established in Japan." *In Vivo.* 2010 Sep-Oct;24(5):671-80.
- Murata et al. "A preliminary study of osteochondral regeneration using a scaffold-free three-dimensional construct of porcine adipose tissue-derived mesenchymal stem cells" *J. Orthop. Res* (2015) 10:35
- Mogi M, et al. "Simultaneous pharmacokinetics assessment of caffeine, warfarin, omeprazole, metoprolol, and midazolam intravenously or orally administered to Microminipigs." *J Toxicol Sci.* 2012;37(6):11 57-64.
- Murayama N, et al. "Cytochrome P450-dependent Drug Oxidation Activity of Liver Microsomes from Microminipigs, A Possible New Animal Model for Humans in Non-clinical Studies, Drug Metab." *Pharmacokinet.* 24 (4): 404–408 (2009).
- Y Nakagawa et al. "Synovial Mesenchymal Stem Cells Promote Healing After Meniscal Repair in Microminipigs" *Osteoarthritis Cartilage* 23 (6), 1007-1017. 2015 Feb 13.
- Sugiyama A, et al. "Microminipig, a non-rodent experimental animal optimized for life science research: in vivo proarrhythmia models of drug-induced long QT syndrome: development of chronic atrioventricular block model of microminipig." *J Pharmacol Sci.* 2011;115(2):122-6.
- Takeishi K, et al. "Acupuncture Improves Sleep Conditions of Minipigs Representing Diurnal Animals through an Anatomically Similar Point to the Acupoint (GV20) Effective for Humans." *Evidence-based Complementary and Alternative Medicine : eCAM.* 2012;2012:472982. doi:10.1155/2012/472982.
- Yamaguchi T, et al. "Noninvasive metabolic syndrome model using an extremely small minipig, the microminipig." *J Pharmacol Sci.* 2014;126(2):168-71
- Yokoo T, et al. "Site-Specific Impact of a Regional Hydrodynamic Injection: Computed Tomography Study during Hydrodynamic Injection Targeting the Swine Liver" *Pharmaceutics* 2015, 7, 334-343;
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Fuji Micra facility



Temperature:	18 - 28°C
Humidity:	30 - 80%
Lighting time:	12 hours, 8:00-18:00
Water:	Fuji Kawaguchiko City
Air intake:	Forced inspiration
Air discharge:	Forced discharge
Space/animal:	
Under 10 kg	0.20 - 0.40 m ²
10 - 20 kg	0.40 - 0.60 m ²
20 - 30 kg	0.60 - 0.80 m ²
30 - 40 kg	0.80 - 1.00 m ²



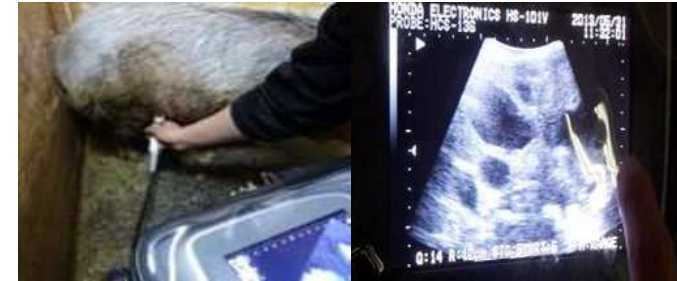
MMP propagation program

Mating



25 days

Pregnancy Testing



21-day estrous cycle

Return to estrous cycle within 7 days of weaning

18 mos cycle

Metric	Average \pm SD
Estrous cycle	20.9 \pm 1.8 (days)
Pregnancy cycle	114.0 \pm 3.7 (days)
Birth weight	400 \pm 80 (g)
Offspring count	4.2 \pm 1.2 (animals)
Suckling period	28.0 \pm 4.0 (days)
Weaning count	3.7 \pm 1.3 (animals)
Weaning weight	2.01 \pm 0.28 (kg)

~114 days

Birth/Lactation



Birth weight ~ 400g

Breast feeding

3 weeks

Vaccination, Feeding

Weaning

To breeding ward

MMP breeding program



Post-weaning (2 - 3kg)



Feed: 80% TDN
Twice daily

2 months (4 - 5kg)



Feed: 83% TDN
Twice daily



Feed: 78% TDN
Twice daily

3 months (5 - 6kg)



Feed: 73% TDN
Once daily

- Selection
- Age
 - Size
 - Sex
 - Colour
 - Character
 - Use

Reorganise colony

Holding ward

Regular testing



Testing		Code	Method
Serum antibody testing	Classical swine fever		ELISA
	Japanese encephalitis	JE	ELISA
	Swine parvovirus	PPV	HI
	Aujeszky's disease	AD	ELISA
	Porcine reproductive and respiratory syndrome	PPRS	ELISA
	Swine influenza (H1N1 subsp)	SIV (H1N1)	HI
	Swine influenza (H3N2 subsp)	SIV (H3N2)	HI
	Swine circovirus type 2	PCV2	PCR
	Swine epidemic gastroenteritis	TGE	SN
	Swine Getah virus	Getah	SN
	Pleuropneumonia virus	APP	LA CF
	Mycoplasma hyopneumoniae	MPS	ELISA
	Swine erysipelas virus	SE	LA
	Toxoplasma	TP	LA
	Hepatitis E virus	HEV	ELISA
Nasal cavity bacterial testing	Bordetella bronchiseptica	AR	Bacteria isolation
	Pasteurella multocida	PAS	Bacteria isolation
	Haemophilus parasuis	HPS	Bacteria isolation
Fecal testing	Rotavirus		Immunochromatography
	E coli		Bacteria isolation
	Clostridium perfringens		Bacteria isolation
	Swine Shigella		Bacteria isolation
	Coccidian		OPG
	Parasite egg		EPG