

Advance Publication

Experimental Animals

Received: 2017.7.19

Accepted: 2017.9.19

J-STAGE Advance Published Date: 2017.10.7

Special field: Management

Type of paper: Review article

Title: Swine used in the medical university-Overview of 20 years of experience

Eiji Kobayashi^{1,2}, Yutaka Hanazono¹ and Satoshi Kunita*¹

¹ Center for Development of Advanced Medical Technology, Jichi Medical University

² Department of Organ Fabrication, Keio University School of Medicine

Running Head: SWINE IN MEDICAL UNIVERSITY

13 text pages

7 tables

1 figure

3 supplementary figures

* Correspondence should be addressed to Satoshi Kunita, DVM, PhD.

Center for Development of Advanced Medical Technology

Jichi Medical University

Yakushiji 3311-1, Shimotsuke, Tochigi, 329-0498

E-mail: sakunita@jichi.ac.jp

Tel: 81-285-58-7078

FAX: 81-285-44-1069

Abstract

Center for Development of Advanced Medical Technology (CDAMTec) in Jichi Medical University was established in FY 2009. It is the first educational research facility specialized for medical research and training using swine in Japan. Preclinical studies on large animals are essential prior to clinical trials to develop regenerative medical products and medical equipment. We have continued comprehensively considering using miniature swine for experiments to develop advanced medical technologies and train physicians with advanced clinical abilities, while paying attention to animal welfare. The center plays a pioneering role in this field by accumulating know-how such as (1) Construction and effective utilization of research facilities, (2) Procurement of quality animal resources, (3) Education and training of technical staff, (4) Establishment of support system for physicians and researchers. We now open up widely these expertise and foundation for medical research and training not only within our university but also outside the university, so as to move faster to practical use of advanced medical technology and contribute to human health and welfare.

Introduction

Given that large animal studies are essential before moving to clinical trials, it is important to look for ways to reduce the number of experimental animals used. We have been proposing 'medical swine to be an ideal non-clinical model systems' [10, 16]. We began using this approach about 20 years ago with a 'total swine system' to model human health and diseases for the purposes of both education of medical skills and development of new devices [19] and therapeutic strategies [3, 14] in the medical university.

Based on our experience in surgical education and research, experimental pigs are valuable models in translational research. In this chapter, we review our activities in this field of 'swine as medical use.'

First, we outline the historical background as to why the swine center which focuses on experimental pigs was established at the medical university. Next, we refer to facilities, etc. for the construction of a swine model system as an integrated approach to non-clinical and clinical research and development. Finally, we state our opinion about a management method as the basis for research and development of

pharmaceuticals, regenerative medical products, and medical equipment based on our experience.

In this topic, we would like to show the importance of pigs as experimental animals that have been developed for purposes other than food and our gratitude for the sacrifice of pigs for medicine, which eventually leads to developing new treatments for humans.

Establishing a swine center based on animal welfare

In Western countries, a large number of training centers have been established to advance simulation-based medical skill training. Medical skill training using human cadavers is also being conducted. In Japan, "a training project for improving practical surgical techniques" was launched in 2012 to further promote surgical training on cadavers, verify the effectiveness of the training, and evaluate the contents of the training and management methods etc. [9]. Meanwhile, medical skill training using living pigs has also gained attention from the viewpoint of safe introduction of new medical devices, more advanced medical safety, and lifelong education. However, other methods that do not depend on the sacrifices of living animals should always be considered ("**R**"**eplacement**, one of 3R's). For many years, one of the authors (Kobayashi) has been involved in surgical training of medical students using live animals at Jichi Medical University [10]. In this section, we would like to explain the past history (Table 1 and Supplementary Figure S1) and discuss the current state of medical education using swine in Japan and how it can comply with the animal welfare standards.

In 2001, as part of Tochigi Prefecture Research and Development Promotion Project in Medical Welfare-related Priority Areas, we started introducing porcine experimental models to medical education (Supplementary Figure S1-A). Initially, we used young livestock pigs. Then, we started to develop experimental miniature pigs (**i.e. purpose-bred animals**) in collaboration with companies.

In 2007, we established "Division of Medical Skill Training" and systematically worked on surgical education and medical skill training of medical students. In addition, we promoted to share sacrificed pigs with the entire university by planning experiments using pigs. That is; for instance, when a researcher wants a porcine liver for an experiment, we attempt the "**R**"**eduction** (one of 3R's) of the number of pigs

used for experiments by sharing the pig with another experimenter who does not need the liver [5]. We also worked on the development of educational materials for medical students and residents using organs and tissues of sacrificed pigs [11].

In 2009, following a MEXT-Supported Program for the Strategic Research Foundation at Private Universities ("Development of Advanced Medical Technology Using Large Animals (Mini Pigs)") (Supplementary Figure S1-C), we established an experimental swine facility in the university and established a system which is not only for our university members but also for country-wide professionals and researchers in companies and other universities (Supplementary Figure S1-D). The facility is currently used by about 700 medical professionals both inside and outside the university for educational purposes such as surgical training. Equipped with C-arms, CT, and MRI, which are imaging devices originally for human clinical use, it conducts non-clinical and clinical integrated regulatory science.

In 2013, the second phase of MEXT-Supported Program for the Strategic Research Foundation at Private Universities ("Mouse to Human: Bridging Studies Using Large Animals") was adopted, and a dedicated cell-processing center (CPC) and a training room equipped with a surgical robot "Da Vinci" were completed. Those who wish to acquire medical skills should always 'refine' their medical skills for patients. However, they should also constantly feel obliged to "**R**"efine (one of 3R's) their skills for the experimental animals they handled. Taken together, the center can meet the expectations of 3R's.

Construction of a center allowing non-clinical and clinical integrated evaluation (of experimental pigs, buildings, and facilities)

Non-clinical and clinical integrated evaluation has been recommended as a strategy for improving product safety more and more strongly (FAD, 2011). In other words, biomarkers and quantitative imaging (e.g. PET, MRI, CT, etc.) are widely used in animal experiments and human trials (Figure 1). In large-scale experimental animals such as pigs, non-invasive tests can be technically easily introduced because of their size similar to that of humans (Table 2). In regenerative medicine, experimental miniature pigs that are 30 to 50 kg are suitable for long-term observation and have been recommended because the number of cells to be administered, their route etc. that greatly affect functional improvement can be reproduced in these pigs.

The authors have developed various models using experimental pigs. Because

the skin properties of Mexican hairless minipigs are similar to those of humans, we tested the tissue permeability of transdermal drugs for humans using the pigs [6]. We reported the characteristics of transgenic pigs with incorporated marker genes which are required for *in vivo* tracking of cells in various cell-therapy researches [8, 13, 18]. We further developed a porcine model as non-clinical and clinical integrated model to verify the function of platelets and T cells [4, 15]. We have established porcine iPS cells [2] and reported their MHC-matched allogeneic transplantation [12]. Recently, we have generated thrombocytopenic pigs [1] and pigs that can be easily engrafted with human cells [7]. The summary records on utilization and achievements of our center are shown in Table 3 and 4.

On the other hand, medical technology-developing and training centers in Japan, where many pigs are used, divert young livestock pigs for the education, training and research purposes. Compared with other countries, edible livestock pigs in Japan are raised very hygienically. It is economical to use livestock pigs for experimental purposes because they were originally produced as a food source. However, even if SPF livestock pigs are used, food safety tests do not include items such as zoonotic hepatitis E virus (HEV) infection, etc. although SPF livestock pigs are often infected with HEV in the infant stages [17]. In terms of food safety, countermeasures against porcine zoonotic diseases are needed. In addition, experimental swine facilities attached to medical institutions should also take adequate measures for the prevention against exposure of immune-incompetent patients to the diseases. As a countermeasure for the above-mentioned risk management, we carefully select the source farms of livestock pigs which are free from HEV infections by checking the periodical microbiological monitoring data including HEV.

Facility Design: What are necessary?

The authors have been interacting with many researchers in other countries, mainly the US and Europe, who promote studies using swine. Not only as a manager of animal experiments but also as a promotor of research using swine, we have studied the facility design. In this section, I overview know-how on the facilities of the swine center which we have acquired through the experience of surgery in humans. Equipment and experimental devices installed in our center are summarized in Table 5.

From the viewpoint of equivalence to human clinical trials, the **laboratory** should be designed just like human **operating rooms** (Supplementary Figure S2). Although HEPA filters are necessary to maintain class 10,000 or better conditions, the

use of electrostatic precipitators or laminar flow devices that reduce exchange of HEPA filters would be a fine choice. From the viewpoint of animal welfare, the **rearing facilities** should be accredited by external authorities such as AAALAC International if considering international tolerability. The management of experimental pigs is different from that of livestock animals. Experimental dogs' cages are mesh, and the feces drops down under cages. In the case of swine, the use of softened concrete (with heating coils) would make it easy to manage sanitation and improve well-being of swine. When building a swine center in an area where buildings are close together, deodorant technology is required before releasing the air from the breeding facilities to the outside. In addition, perioperative management is most important in advanced therapeutic researches. In particular, in the development of implantable medical devices, the same **postoperative management system** as that for humans (e.g. ICU) is necessary.

Management method as the basis for research and development of pharmaceuticals, regenerative medical products, and medical equipment (human resource development, organization management, and corporate involvement)

Although Japan has been touted as a technology superpower, it has had an excess of imports of pharmaceuticals, regenerative medical products, and medical equipments for the past 20 years. In research and development (R & D), experiments on large animals are extremely important before submitting an application for human clinical trials. However, Japan was far behind the global trend regarding the experimental animal species. In other words, although swine have been used as various medical device development models worldwide, Japan had little experience with it. In recent years, catheter-based minimally invasive treatment and robotic surgery have been actively introduced in surgical treatment in Japan. Japan is ten years behind other countries in terms of the experience. We have been conducting translational research on advanced medical technology as an administrator of laboratory animal center and corporate advisor. We have been working on issues in the management of R & D bases in Japan from various perspectives. In the final section we discuss future human resource development and organization management of R & D centers and an ideal state of industry-academia cooperation (Supplementary Figure S3).

Human resource development: In many medical university and research institutions, graduate students in surgery department are conducting experiments on large animals for model development. Since it would be necessary to introduce more advanced human clinical techniques, surgical trainers should be introduced (Supplementary Figure S3-B). Training of veterinarians (e.g. model development and perioperative management, etc.) is also essential (Supplementary Figure S3-C). In Japan, more than 200 facilities for experimental animals are registered at the Japanese Association of Laboratory Animal Facilities of National University Corporations and Japanese Association of Laboratory Animal Facilities of Public and Private Universities. Veterinarians who are familiar with human clinical trials as well as with experimental animals are needed, given that "doctors deal with human lives, and veterinarians deal with animal lives." Without the cooperation of both, it is impossible to justify the lives of the animals sacrificed for experiments.

Organization management: Because laboratory animal facilities have become a part of university organization, their costs are covered by the operating costs of the university. However, because the swine center deals with matters related to the university hospital, such as medical safety and training of residents, hospital accounting should be introduced. Regarding the operation of a swine center, it is recommended that the medical and veterinary university should assign at least one veterinarian and one animal facility manager. The attached hospital should assign at least one radiology technician. Then, the number of staffs (e.g. human resource development, etc.) should be increased, gradually though, depending on the scale of the operation. Staffs currently assigned to operate our center are shown in Table 6.

Corporate involvement: Because research institutes such as universities are operated for the purpose of promoting basic and applied researches, there is not an advantage of good laboratory practice (GLP) management in such institutes. On the other hand, if corporates are involved, there is a great advantage on both sides of research institutes and corporates, because corporates can participate in R & D of new biopharmaceuticals and medical equipment (Supplementary Figure S3-D) from the beginning considering the output (clinical use) and in the development of human resources involved in it. R & D of such advanced medical technology is an unmet need to begin with. Unlike profit-driven medical care, society supports collaborative efforts of university research institutes and companies because they are doing something

helpful for patients suffering from diseases. In Table 7, we propose the future prospects of research and education facilities using swine including cooperation between industry and academia.

Conclusion

Jichi Medical University has established and operated a research center specially designed for swine used in medical research and education of clinical surgeons, while paying attention to animal welfare. The center will continuously contribute to promote advanced medical research and technology as a preclinical and clinical integrated research facility by operating with high transparency and providing the cultivated know-how and resources without gaps between inside and outside the university.

Acknowledgement

The authors would like to take this opportunity to thank Dr. Fumimaro Takaku (Emeritus President of JMU), Dr. Ryozo Nagai (President of JMU) and Dr. Eiju Watanabe (Former Director of CDAMTec) for their guidance and persistent encouragement. We would also like to thank Dr. Naohiro Sata, Dr. Alan Lefor, Dr. Syuji Hishikawa for their cooperation as surgical education faculty and Dr. Hozumi Tanaka as an attending veterinarian. We are particularly grateful for the assistance given by Toru Wakui, Kazusi Miyazawa and many other technical staff. We thank Prof. Ichiro Sekiya for permission to use the photographs. Funding from the MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2008-2012 (S0801045) and 2013-2017 (S13110340) is gratefully acknowledged.

References

1. Abe, T., Kono, S., Ohnuki, T., Hishikawa, S., Kunita, S., and Hanazono, Y. 2016. A swine model of acute thrombocytopenia with prolonged bleeding time produced by busulfan. *Exp. Anim.* 65: 345-351.
2. Fujishiro, S., Nakano, K., Mizukami, Y., Azami, T., Arai, Y., Matsunari, H., Ishino, R., Nishimura, T., Watanabe, M., Abe, T., Furukawa, Y., Umeyama, K., Yamanaka, S., Ema, M., Nagashima, H., and Hanazono, Y. 2013. Generation of naive-like porcine induced pluripotent stem cells capable of contributing to embryonic and fetal development. *Stem Cells* 22: 473-482.
3. Hatsushika, D., Muneta, T., Nakamura, T., Horie, M., Koga, H., Nakagawa, Y., Tsuji, K., Hishikawa, S., Kobayashi, E., and Sekiya, I. 2014. Repetitive allogeneic intraarticular injections of synovial mesenchymal stem cells promote meniscus regeneration in a porcine massive meniscus defect model. *Osteoarthritis Cartilage.* 22: 941-950.
4. Hisakura, K., Murata, S., Fukunaga, K., Myronovych, A., Tadano, S., Kawasaki, T., Kohno, K., Ikeda, O., Pak, S., Ikeda, N., Nakano, Y., Matsuo, R., Konno, K., Kobayashi, E., Saito, T., Yasue, H., and Ohkohchi, N. 2010. Platelets prevent acute liver damage after extended hepatectomy in pigs. *J. Hepatobiliary Pancreat. Sci.* 17: 855-864.
5. Hishikawa, S., Kawano, M., Tanaka, H., Konno, K., Yasuda, Y., Kawano, R., Kobayashi, E., and Lefor, A.T. 2010. Mannequin simulation improves the confidence of medical students performing tube thoracostomy: a prospective, controlled trial. *Am. Surg.* 6: 73-78.
6. Horie, M., Sekiya, I., Nakamura, T., Tanaka, H., Maekawa, K., Nakanishi, M., Muneta, T., and Kobayashi, E. 2009. *In vivo* pharmacokinetics of ketoprofen after patch application in the Mexican hairless pig. *Biopharm. Drug Dispos.* 30: 204-208.
7. Hsu, H.C., Enosawa, S., Yamazaki, T., Tohyama, S., Fujita, J., Fukuda, K., and Kobayashi, E. 2017. Enhancing Survival of Human Hepatocytes by Neonatal Thymectomy and Partial Hepatectomy in Micro-miniature Pigs. *Transplant Proc.* 49: 153-158.

8. Kawarasaki, T., Uchiyama, K., Hirao, A., Azuma, S., Otake, M., Shibata, M., Tsuchiya, S., Enosawa, S., Takeuchi, K., Konno, K., Hakamata, Y., Yoshino, H., Wakai, T., Ookawara, S., Tanaka, H., Kobayashi, E., and Murakami, T. 2009. Profile of new green fluorescent protein transgenic Jinhua pigs as an imaging source. *J. Biomed. Opt.* 14: 054017.
9. Kobayashi, E. 2015. Present and aspects for cadaver surgical training in Japan. *Kyobu Geka.* 68: 204-211. (in Japanese).
10. Kobayashi, E., Hishikawa, S., Teratani, T., and Lefor A.T. 2012. The pig as a model for translational research: overview of porcine animal models at Jichi Medical University. *Transplant Res.* 1: 8.
11. Konno, K., Nakanishi, K., Hishikawa, S., Tanaka, H., Yoshikawa, N., Yasuda, Y., Kobayashi, E., and Lefor, A.T. 2012. Cryo-preserved porcine kidneys are feasible for teaching and training renal biopsy: "the bento kidney". *Transplant Res.* 1: 5.
12. Mizukami, Y., Abe, T., Shibata, H., Makimura, Y., Fujishiro, S.H., Yanase, K., Hishikawa, S., Kobayashi, E., and Hanazono, Y. 2014. MHC-matched induced pluripotent stem cells can attenuate cellular and humoral immune responses but are still susceptible to innate immunity in pigs. *PLoS One.* 9: e98319.
13. Murakami, T., and Kobayashi, E. 2012. GFP-transgenic animals for in vivo imaging: rats, rabbits, and pigs. *Methods Mol. Biol.* 872: 177-189.
14. Nakamura, T., Sekiya, I., Muneta, T., Hatsushika, D., Horie, M., Tsuji, K., Kawarasaki, T., Watanabe, A., Hishikawa, S., Fujimoto, Y., Tanaka, H., and Kobayashi, E. 2012. Arthroscopic, histological and MRI analyses of cartilage repair after a minimally invasive method of transplantation of allogeneic synovial mesenchymal stromal cells into cartilage defects in pigs. *Cytherapy.* 14: 327-338.
15. Satoda, N., Shoji, T., Wu, Y., Fujinaga, T., Chen, F., Aoyama, A., Zhang, J.T., Takahashi, A., Okamoto, T., Matsumoto, I., Sakai, H., Li, Y., Zhao, X., Manabe, T., Kobayashi, E., Sakaguchi, S., Wada, H., Ohe, H., Uemoto, S., Tottori, J., Bando, T., Date, H., and Koshiba, T. 2008. Value of FOXP3 expression in peripheral blood as rejection marker after miniature swine lung transplantation. *J. Heart Lung Transplant.* 27: 1293-1301.
16. Tanaka, H., and Kobayashi, E. 2006. Education and research using experimental pigs in a medical school. *J. Artif. Organs.* 9: 136-143.
17. Tanaka, H., Yoshino, H., Kobayashi, E., Takahashi, M., and Okamoto, H. 2004.

Molecular investigation of hepatitis E virus infection in domestic and miniature pigs used for medical experiments. *Xenotransplantation*. 11: 503-510.

18. Teratani, T., Matsunari, H., Kasahara, N., Nagashima, H., Kawarasaki, T., and Kobayashi, E. 2012. Islets from rats and pigs transgenic for photogenic proteins. *Curr. Diabetes Rev.* 8: 382-389. (Review).
19. Yano, T., Yamamoto, H., Sunada, K., Miura, Y., Taguchi, H., Arashiro, M., Yoshizawa, M., Hayashi, Y., Miyata, T., Tanaka, H., Kobayashi, E., and Sugano, K. 2011. New technique for direct percutaneous endoscopic jejunostomy using double-balloon endoscopy and magnetic anchors in a porcine model. *Dig. Endosc.* 23: 206.

Figure Legends

Figure 1. Examples of a non-clinical and clinical integrated research and development method

Articular cartilage regeneration treatment by MSC: arthroscopy image (A) and intra-articular image (B) in a pig. An arthroscopy image (C) and an intra-articular image (D) in a human clinical trial. Photographs were provided by courtesy of Prof. Ichiro Sekiya.

Supplementary Figure S1. Process of the development of the porcine system at Jichi Medical University

A. Started with purchasing livestock pigs from farmers (Establishment of a system in which pigs raised with the budget of Ministry of Economy, Trade and Industry can be used for drug development / medical device development (2001))

B. Sir Roy Calen, Professor Emeritus of Cambridge University (England), who gave an invited lecture at the commemorative ceremony for the establishment of Division of Medical Skill Training (2007)

C. Construction of the experimental swine facility following the adoption of a fund MEXT-Supported Program for the Strategic Research Foundation at Private Universities (2008)

D. Opening ceremony of Jichi Medical University Center for Development of Advanced Medical Technology (commonly known as Pig Center) (2009)

Supplementary Figure S2. Inside of the completed swine rearing facility

A. The ceiling is covered with sound-proof plates as shown by the arrows).

B. A weight scale is placed in the passageway for pigs.

C. Animal rearing room with solid floor cages.

D. Adequate rearing space was given in compliance with the Institute for Laboratory Animal Research (ILAR) Guide recommendations.

Supplementary Figure S3. Facilities and equipments

- A.** The appearance of the present center.
- B.** Simulation training of brain-dead transplant for the medical team.
- C.** Training of making a pig pancreatectomy model for veterinary physicians.
- D.** Testing of an imaging equipment usable in human surgery.

Table 1. History of the Center for Development of Advanced Medical Technology

2001	Started introducing porcine experimental models to medical education after abolishing all experiments using dogs donated from public health centers as part of Tochigi Prefecture Research and Development Promotion Project in Medical Welfare-related Priority Areas (5 million yen for starting budget). Initially, we used young livestock pigs and then introduce miniature pigs (Clawn and Mexican hairless strains) co-operating with the companies.
2008-2012	Financial support stage I from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) for Supported Program for the Strategic Research Foundation at Private Universities (total 324 million yen including 168 million yen from MEXT)
2009	Completion of the main building (543.35 m²)
2012	1st extension of the main building (763.91m²) to increase area for rearing pigs
2013	2nd extension of the main building (900.04m²) including a cell-processing room (CPC) and a room with "da Vinci" surgical system
2013-2017	Financial support stage II from MEXT for Supported Program for the Strategic Research Foundation at Private Universities (total 256 million yen including 136 million yen from MEXT)
2017	Certified as the Joint Usage/Research Center by MEXT

Table 2. Points that pigs are suitable for experiments compared with other animal species and necessity as an experimental animal

➤ Pigs have similar size, anatomical features, and physiological parameters such as blood pressure, heart rates, etc. as those of humans.
➤ Pigs can take any kinds of food and drink including alcohol as humans do.
➤ Pigs suffer from obesity, diabetes, etc. that humans do from.
➤ Medical devices such and endoscopy, catheter, etc. can be used in pigs.

Table 3. Projects and trainings conducted in 2015 budget year

➤ Intra-institutional research projects:	used by 393 persons* from 24 sections
➤ Joint research projects with outside institutions:	used by total 488 persons from 7 institutions
➤ Company projects:	used by total 7 persons from 6 companies
➤ Intra-institutional trainings:	used by total 356 persons from 9 sections
➤ Trainings by outside institutions:	used by 246 persons with 6 institutions
Total number of projects and trainings:	used by 1,490 persons from 52 sections trainings: /institutions/companies

*Persons = cumulative total number of people

Table 4. Achievements

➤ 91 papers published for recent 3 years (between 2013-2015)
➤ 140 papers presented in meetings for recent 3 year (between 2013-2015)
➤ Several patents filed
➤ Some of medical devices developed in the center have been commercialized.

Table 5. Equipment and experimental devices

A main building equipped with the followings:

➤ Conference room
➤ Animal room equipped with cages for 27 pigs (1.35-1.7m ² /cage)
➤ Surgical room (class 10,000 HEPA-filtered) equipped with 4 surgical beds
➤ Intensive care unit (ICU, class 10,000 HEPA-filtered) with telemetry system
➤ Cell-processing room (CPC)
➤ "da Vinci" surgical system
➤ Non-invasive imaging devices: CT, MRI, C-arm, Ultrasound
➤ Isolators for germ-free rearing

Table 6. Staffs

- 4 concurrently-served professors
 - 2 visiting professors
 - 2 full-time associate professors (including 1 medical doctor)
 - 2 full-time lecturers (including 1 veterinarian)
 - 6 full-time technical experts (including 2 veterinaries)
 - 3 full-time secretaries
-

Table 7. Current problems to be solved and future prospects

- Development of human resources
 - Raising funds
 - Increasing cooperation with other facilities using pigs
 - Promotion of globalized activities
-

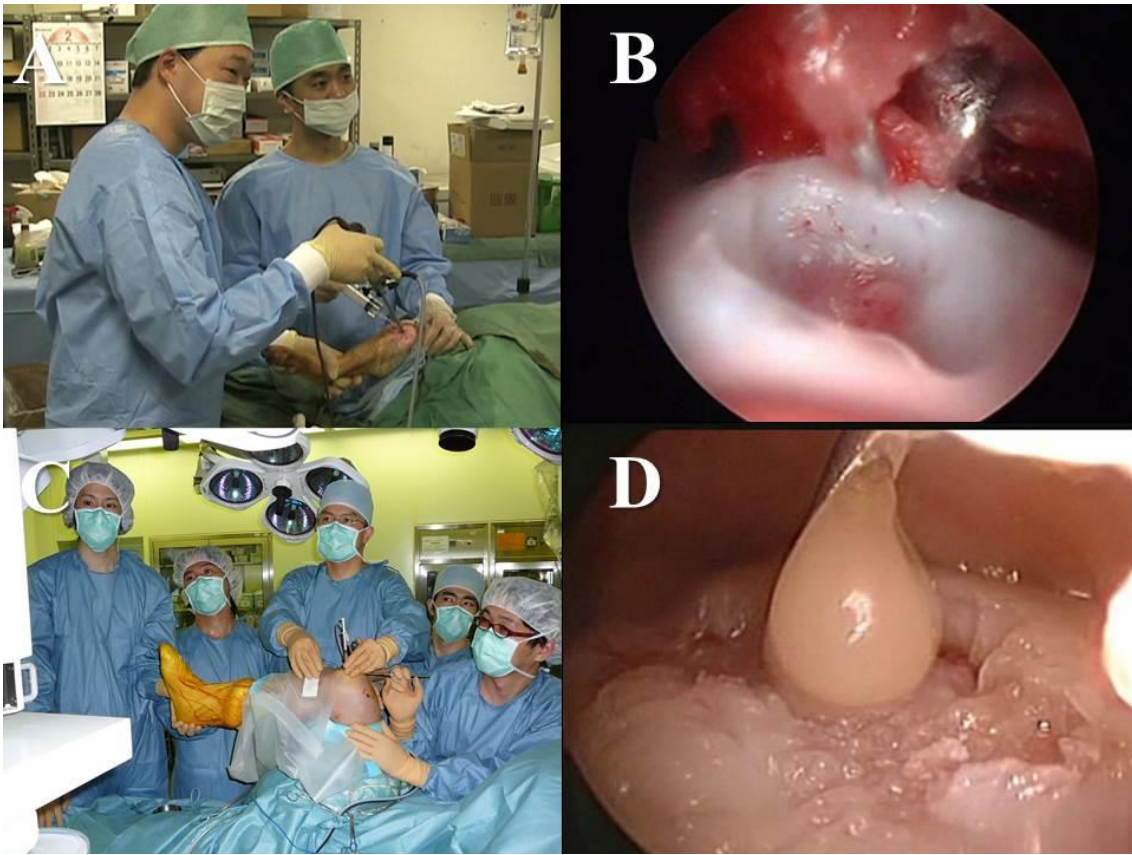
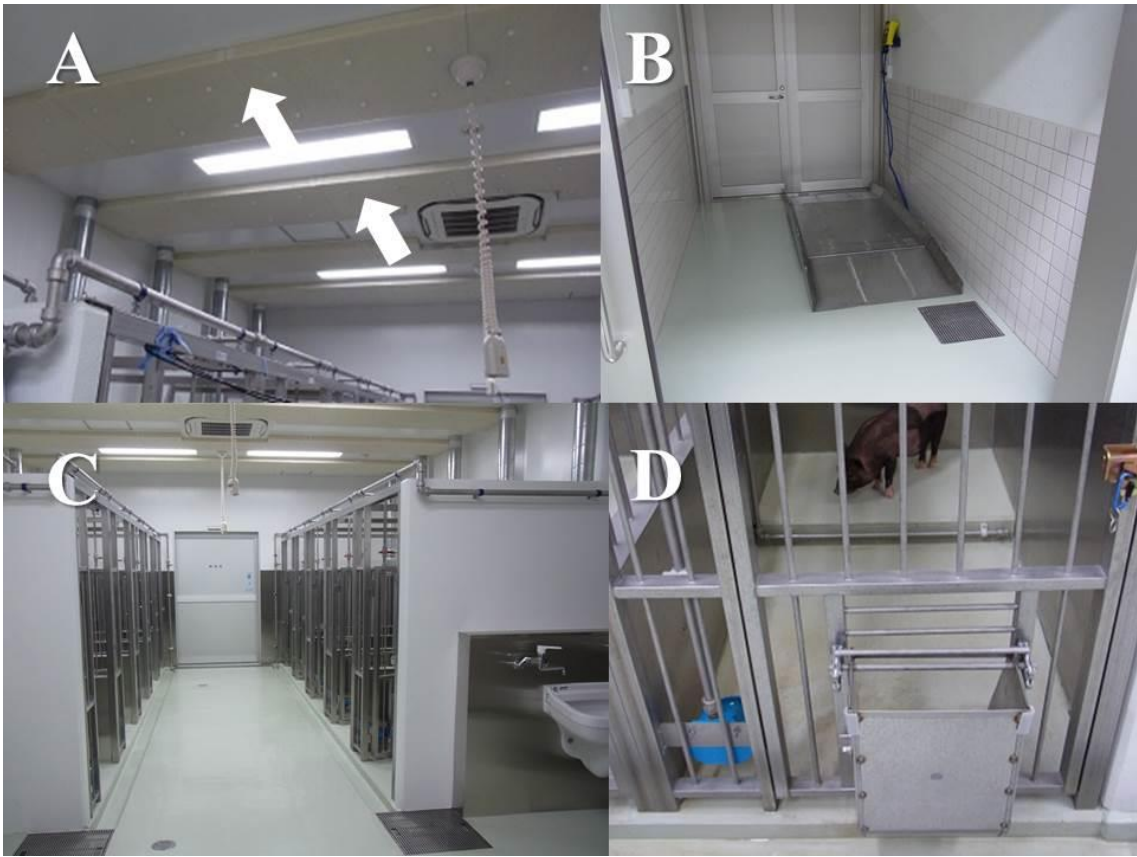


Figure 1.



Supplementary Figure S1.



Supplementary Figure S2.



Supplementary Figure S3.