# Minipigs as Laboratory Animals: Facility Management and Husbandry

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## ABSTRACT

**Minipigs are regarded as one of the most important laboratory animal in that anatomical and physiological properties are similar to human and their reproduction efficiency is relatively higher compared to other large animal species. Particularly, several diseases that cannot be mimicked in rodent models are successfully occurred or induced in pig models therefore it has been interested in a valuable model for human diseases. Pigs are also 'standard' species in xenotransplantation research. To maximize experimental outcome using minipigs, establishment and management of proper animal facility, right animal husbandry and control of pathogens are very important. In this review, we summarized several international guidelines related with minipigs published by several companies or governments and discuss optimal conditions for providing informative ideas to the researchers who want to use minipigs in their future studies.**

**(Key words : Minipig, Facility, Husbandry, Laboratory animal)**

## INTRODUCTION

Pigs are an important animal in biomedical research because of their anatomical and physiological similarities to human. They are widely used in cardiovascular study because heart of the pig is very similar to human except the presence of the left azygous vein which enter the coronary sinus (Smith & Swindle, 2006). The pigs are also very useful for dermatologic research because they have almost hairless skin and it is tightly attach to the subcutaneous tissue like that of human (Nunoya *et al*., 2007). The gastrointestinal and urinary systems are also similar to human therefore they are one of important animal model for nutritional studies. More importantly several diseases cannot be mimicked in rodent animal model are successfully modeled using pigs therefore it will be almost essential for the advanced biomedical research to use pigs as laboratory animals (Whyte & Prather, 2011). Their size is ideal for practice or development of procedures for human clinic and multiple collections of samples including blood or other body fluid. In addition, they can reach at puberty relatively faster (4 to 6 months) and litter size is also much bigger compared to other large animal species such as dog or sheep thus its production efficiency is relatively high. For these many reasons it is very reasonable to regard the pig as best candidate for large experimental animal (McAnulty *et al*., 2012).

While conventional farm pig breeds such as Landrace, Yorkshire, Duroc, or Hampshire are extensively used in pork industry, smaller pigs, named minipigs or miniature pigs, are produced by cross-breeding of various purebred or wild species for special needs. The minipigs had several advantages as a laboratory animal. They are small thus much easier for handling (Vodicka *et al*., 2005). Also, requirements of food, space and even pharmacologic products or anesthetic are significantly reduced (McAnulty *et al*., 2012; Piedrahita & Olby, 2011). Unlike conventional farm pigs, the minipigs are commonly maintained in intensively controlled facilities. To design a study using minipigs as laboratory animals it is important to know their characters. In the present review, we discuss about management and husbandry of minipigs for research purposes to improve understanding of minipigs for researchers who want to use minipigs in their future study.

## BREEDS OF MINIPIGS

There are at least 45 breeds of minipigs available world-widely (Smith & Swindle, 2006). Most widely used breeds for biomedical research are Yucatan, Hanford, Sinclair and Gottingen pigs and each of them show different characters (Table 1).

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**Table 1. Overview of minipig breeds widely used in biomedical research (McAnulty *et al.*, 2012)**

| Breed | Average birth weight (g) | Average adult weight (kg) | Average litter size | Color |
|---|---|---|---|---|
| Gottingen | 450 | 45 | 6.5 | White |
| Hanford | 730 | 80～95 | 6.7 | White |
| Sinclair | 590 | 55～70 | 7.2 | Black, Red, White, Roan |
| Yucatan | 500～900 | 70～83 | 6 | Black, Slate grey, White |
| Micro-Yucatan | 600～700 | 55～70 | 6 | Black, Slate grey, White |

The Yucatan minipigs are one of the native breed pigs in North America. Their body weight at adult stage is around 70～83 kg but recently smaller variant named Micro-Yucatan that weigh approximately 55～70 kg at adult is also developed. They have usually black skin but white colored or dotted pattern also available. Yucatan is very good as laboratory animal because they have very good temperature and are easy to handle. They are used in many types of research including cardiovascular study. Especially there is a genetic model for ventricular septal defect (McAnulty *et al.*, 2012).

The Sinclair minipig is also known as Minnesota minipig or Hormel minipig because it was firstly developed by Hormel Institute in Minnesota, United States. Body weight of Sinclair minipig is about 55～70 kg at adult. They show various colors and patterns including black, red white and roan. They have complex genetic background thus also used for establishing other minipig line including NIH Minipig, Nebraska, Gottingen and Minipig of Czech Republic. The Sinclair minipig is a general-purpose breed and well known as model animal for melanoma study because they has significant incidence (McAnulty *et al.*, 2012).

The Hanford minipigs are one of biggest minipig breed. Their body weight is about 80～95 kg at adult even though they have less subcutaneous fat compared to other breeds. They have white haircoat and skin thus can be very good for dermal studies. Also their heart size is very similar to human therefore it's also widely used for cardiovascular study (Nunoya *et al.*, 2007).

The Gottingen minipig is small breed in white non-pigmented color. At adult their body weight is about 30～45 kg. They are mainly used for toxicologic test but also widely used for diabetes, orthopedic, dental and surgical practice purposes (McAnulty *et al.*, 2012).

**Production of Transgenic Pig for Biomedical Research**

Transgenesis technologies make animal models more valuable. Since the first transgenic pig was produced in the middle of eighties, various transgenic pigs are produce for many purposes. In eighties and nineties many scientists tried to produce transgenic pigs for agricultural purpose, however, recently most of transgenic pigs are produced for xenotransplantation or disease model. Recently sort of transgenic pigs are well categorized and reviewed by Whyte and Prather elsewhere (Whyte & Prather, 2011).

Pronuclear injection technique is one of 'standard' protocol to produce transgenic mice. In this technique, high amount of DNA is microinjected into pronuclear of zygotes recovered from oocytes donors and transplanted into surrogate. In pig it was used since mid-eighties and several groups successfully produced transgenic pigs using the technique. However, only 1% of injected eggs are developed into transgenic pigs.

Alternatively, viral transduction protocols were also tried to produce transgenic pigs. In this technique, oocytes or sperm is infected with viral vectors and used for production. This method slightly improves efficiency of transgenic pig production, however, it still too low for widely used. Moreover, both pronuclear injection and viral transduction protocol cannot provide technical background for producing gene targeted animals.

Since the first cloned animal derived from the somatic cells was produced in 1997, somatic cell nuclear transfer technique became a standard protocol for producing transgenic animals in large animal species. Especially this technique opens up the way to make gene targeted pigs. Until now many transgenic pigs including several knock-out pigs are produced using this technique. Efficiency of this technique is improved compared to the pronuclear injection or gene transduction, however, still very low (about 3%) and need to be improved.

Theoretically, every single somatic cell can be used for somatic cell nuclear transfer technique. Therefore various transgenic techniques were tried at in vitro cell culture level for designing more complicated transgenic pigs. Most of all, zinc finger nuclease technique for gene knock-out and targeting is one of most interested by many researchers in this field. (Yang *et al.*, 2011; Hauschild *et al.*, 2011; Whyte *et al.*, 2011). Recently sperm mediated gene transfer technique is combined with ectopic germ line tissue xenograft technique (Honara-

mooz *et al*., 2008) or intracytoplasmic sperm injection technique (Umeyama *et al*., 2012) and opens up new direction of further transgenic pig production studies. Conditional expression system (Moon *et al*., 2012), RNA interference (Dieckhoff *et al*., 2007) or transposon system (Kim *et al*., 2011) also provide good tools for advanced transgenic studies.

Stem cell techniques are also very important in pig transgenesis study. Embryonic stem cells injection into blastocyst is one of most important technique to produce gene targeted mouse. However, except mouse embryonic stem cells were not established in any other mammalian animals and it was one of major hurdle to produce transgenic large animals. Promisingly, recent report showed that chimeric pig can be produced using porcine induced pluripotent stem (iPS) cells (West *et al*., 2011). Even though germ-line transmission is still not clear in the report it clearly provides new direction for transgenic pig research. On the other hand spermatogonial stem cells also have big potential for producing transgenic pig. Though transgenic pigs was not produced using this technique yet, Kim *et al* showed that possibility of this technique in pig transgenesis (Kim *et al*., 2010). Recent advance about transgenesis techniques in farm animals were well reviewed elsewhere by Kues and Niemann (Kues & Niemann, 2011).

One thing should considered to produce transgenic minipig is use of conventional farm pig as surrogate for minipig embryo. In the previous study we observed that minipig embryos are not grown well in the uterus of conventional farm pig due to gene expression profile is different from normal farm pigs (Koo *et al*., 2009).

### Facility Management and Animal Husbandry

Animal husbandry is very important for laboratory animals. Stable and consistent physiological state is critical for success of research thus animal facility should be designed and maintained to reduce stress as much as possible. Pigs are socialized animal thus they can be housed in small group in pen. However, sometimes they tease with each other thus it is best to house them individually in pens. In this case, each pigs should have visual, olfactory, and auditory contact with each other to prevent social deprivation (Smith & Swindle, 2006).

Space requirement for pigs have been suggested by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care). It is shown in Table 2 (National Research Council, 2010). Flooring design is also considerable. If solid materials are used it is recommended to make texture on the surface for secure footing and bedding should be provided for rooting and nesting (Smith & Swindle, 2006). Grid floor is good for sanitation but provide poor insulation and will require a slightly higher room temperature (Ellegaard Gottingen Minipigs A/S, 2010). Appropriate spacing between each bar is about 6～12 mm. If floor did not wear hoof, it should be trimmed regularly (every 60～90 days). For this reason Sinclair research recommended to use fiberglass slatted floors contain medium grit (Swindle, 2008).

**Table 2. Space requirement for pig recommended by AAALAC**

| Animal/enclosure | Weight (kg) | Floor area/animal ($m^2$) |
|---|---|---|
| 1 | <15 | 0.72 |
| | Up to 25 | 1.08 |
| | Up to 50 | 1.35 |
| | Up to 100 | 2.16 |
| 2～5 | <25 | 0.54 |
| | Up to 50 | 0.9 |
| | Up to 100 | 1.8 |
| >5 | <25 | 0.54 |
| | Up to 50 | 0.81 |
| | Up to 100 | 1.62 |

(National Research Council, 2010)

Optimal temperature range suggested by AAALAC is 16～27℃, however, Ellegaard *et al* suggested more detailed reference ranges following the age of pigs (Ellegaard *et al*., 2010). Optimal humidity will be 50～70%

**Table 3. Reference ranges for environmental factors of minipig facility**

| Environmental factor | Age | Reference range |
|---|---|---|
| Temperature | Newborn pig | 32℃ |
| | Up to 8 weeks | 29℃ |
| | Up to 16 weeks | 24℃ |
| | Up to 36 weeks | 17.4℃ |
| Humidity | | 50～70% |
| Light | | 12 hours daily, 100～200 lux |

(Ellegaard Gottingen Minipigs A/S, 2010; Ellegaard *et al*., 2010)

**Table 4. Total daily amount of food**

| Weight (kg) | Food for males (g) | Food for females (g) |
|---|---|---|
| 5～9 | 240 | 220 |
| 9～13 | 240～300 | 220～280 |
| 13～17 | 300～340 | 280～320 |
| 17～21 | 340～380 | 320～360 |
| 21～25 | 380～420 | 360～400 |
| 25～35 | 420～600 | 400～600 |

(Ellegaard Gottingen Minipigs A/S, 2010)

**Table 5. List of bacteria and fungi for designated pathogen free condition**

| Name of pathogen | Suggested for DPF condition | FELASA* | Ellegaard Gottingen Minipigs | Sinclair Bio Resources | Optifarm Solution Medipig |
|---|---|---|---|---|---|
| *Actinobacillus equuli* | ○ | | | | |
| *Actinobacillus pleuropneumoniae* | ○ | ○ | ○ | Vaccination | ○ |
| *Actinobacillus suis* | ○ | | | | |
| *Actinobaculum (Eubacterium) suis* | ○ | ○ | ○ | | |
| *Arcobacter* spp. | ○ | | | | |
| *Aspergillus* spp. | ○ | | | | |
| *Bacillus anthracis* | ○ | | | | |
| *Bordetella bronchiseptica* | ○ | ○ | ○ | Vaccination | |
| *Brachyspira* spp. | | | ○ | | ○ |
| *Brucella suis* | ○ | | | ○ | ○ |
| *Campylobacter* spp. | ○ | | ○ | | |
| *Candida* spp. | ○ | | ○ | | |
| *Chlamydia* spp. | ○ | | | | |
| *Clostridium* spp. | ○ | | ○ | | |
| *Coxiella burnetii* | ○ | | | | |
| *Cryptococcus neoformans* | ○ | | | | |
| *Eperythrozoon suis* | ○ | | | | |
| *Erysipelothrix* spp. | ○ | ○ | ○ | Vaccination | ○ |
| *Escherichia coli* (verotoxigenic) | ○ | | | | |
| *Haemophilus parasuis* | ○ | ○ | ○ | Vaccination | ○ |
| *Histoplasma capsulatum* | ○ | | | | |
| *Lawsonia intracellularis* | ○ | | ○ | | ○ |
| *Leptospira* spp. | ○ | ○ | ○ | ○ | |
| *Listeria* spp. | ○ | | ○ | | |
| *Microsporum* spp. | ○ | | ○ | | |
| *Mycobacterium* spp. | ○ | | | | |
| *Mycoplasma* spp. | ○ | ○ | ○ | | ○ |
| *Pasteurella* spp. | ○ | | ○ | Vaccination | ○ |
| *Pseudomonas pseudomallei* | ○ | | | | |
| *Rhodococcus equi* | ○ | | | | |
| *Salmonella* spp. | ○ | ○ | ○ | ○ | ○ |
| *Serpulina hyodysenteriae* | ○ | | | | ○ |
| *Serpulina pilosicoli* | ○ | | | | |
| *Shigella* | ○ | | | | |
| *Staphylococcus hyicus* | ○ | ○ | ○ | | |
| *Streptococcus* spp. | ○ | ○ | ○ | ○ | |
| *Trichophyton* spp. | ○ | | ○ | | |
| *Yersinia* spp. | ○ | ○ | ○ | | |

* Federation of European Laboratory Animal Science Associations.
(Bollen *et al.*, 2010; McAnulty *et al.*, 2012)

and lightning will be given 12 hours a day at 100~200 lux (Table 3).

Water should be given *ad libitum* via automatic watering because shortage of water intake induce health problem in pigs. For food high-fibre/low-energy diet is ideal for minipig due to it provide a satisfying larger volume of food intake. Ellegaard Gottingen minipig A/S provides guideline for daily food for feeding their Gottingen minipig products (Ellegaard Gottingen Minipigs A/S, 2010), however, it also can be adopted to other minipig breeds (Table 4).

On the other hand, it seems that transgenic pigs need special care. In most case transgenic pigs are produced using somatic cell nuclear transfer or other assisted reproduction techniques. These intensive procedures seem to be induced high mortality in early stage of the animal. In our personal observation, transgenic cloned piglets are easily died within a week of the birth. One reason for this early death is infection of neonatal pigs. In our case, intensive neonatal care of the transgenic piglets in specific pathogen free (SPF) condition resolve this problem.

**Biosafety Issues and Barrier Facility for Xenotransplantation**

One of the most actively and intensively researched area using the pigs, especially transgenic pigs, is xenotransplantation study. Though this technology already tried in human at least one case in New Zealand (Elliott, 2011), however, lots more consideration should be-needed for using this technique widely. Most important consideration to use pig for xenotransplantation is potential transfer of infectious pathogens from the organ-source pig to the patient. To remove this potential risk, the pigs should be maintained free from specified bacteria, fungi, protozoa and viruses that can be arise zoonotic diseases. The SPF state are initially designed for protect weak or immune-defected animals from the pathogens to minimize effects on experimental results. Thus, for the human use of the animal, another pathogen regulated status for free of pathogens that might cause zoonotic in human is required. This status is frequently called designated pathogen free (DPF) condition. Though there is no 'gold standard' for DPF condition yet, several organizations, including U.S. Food and Drug Administration, World Health Organization (through Changsha Communique) and United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA) suggest guidelines for DPF status and other issues related with xenotransplantation. List of bacteria, fungi, and virus for developing DPF status compared to several SPF/DPF programs performed in companies is suggested in Table 5 and 6. Full list of pathogens for establishing SPF or DPF condition can be found elsewhere (Bollen *et al.*, 2010; Schuurman, 2009).

Biosecure barrier facility is the only way to establish DPF status. It is not necessary to maintain totally germ-free status, however, several basic things should be considered to maintain DPF status. First animal facility sh-

**Table 6. Selected list of viruses for designated pathogen free condition (Bollen *et al.*, 2010)**

| Viral infection | Ellegaard Gottingen Minipigs | Sinclair Bio Resources | Optifarm Solution Medipig |
|---|---|---|---|
| Aujeszky's disease | ○ | ○ | ○ |
| Encephalomyocarditis virus | ○ | | ○ |
| Hemagglutinating encephalomyelitis | ○ | | |
| Transmissible gastroenteritis | ○ | ○ | ○ |
| Porcine circovirus | ○ | | ○ |
| Porcine cytomegalovirus | | | ○ |
| Prcine epidemic diarrhea | ○ | | ○ |
| Porcine influenza | ○ | ○ | ○ |
| Porcine parvovirus | ○ | Vaccination | ○ |
| Porcine reproductive & respiratory syndrome | ○ | ○ | ○ |
| Porcine rotavirus | ○ | | ○ |
| Swine fever | ○ | | ○ |
| Vesicular stomatitis | | ○ | |
| Swine hepatitis-E | | | ○ |
| Japanese B encephalitis | | | ○ |

ould be located far from other conventional pig facility and also the facility is totally closed from environment of outside. Air should be supplied after flow thorough at least 0.3 um size of filter. Water and food should be given after disinfection or irradiation. All the materials should be autoclaved first before entering the facility. More importantly, all people who entering the facility should take shower first and wearing special clothe. In most case contamination is occur by human.

When new animals are entering the facility, they should be disinfected first. Ideally for introducing new pigs into facility it is recommended to produce new piglet by Cesarean section and transferred the animal to the facility through the disinfectant cabinet rather. This procedure efficiently removes potential risk of most contamination pathogens except several viruses can be infected through placenta.

At last but not least, most of DPF status did not control endogenous viruses such as porcine endogenous retro virus (PERV). The pathogenicity of PERV in human is still controversial. However, it surely infect human cells thus all the efforts to eliminate PERV from DPF pigs and to establish monitoring procedure for clinic should be continued (Denner *et al*., 2009).

In Korea, guideline for xenotransplantation is still not officially available. However, recently Korean Food and Drug Administration (KFDA) statement kick-off of meetings for establishing new guideline for xenotransplantation and related studies in Korea.

## CONCLUSION

Minipigs can provide tremendous improvement in biomedical research. However, proper facility management and animal husbandry is very critical requirement for success of animal studies. In addition, consideration for the animal welfare is another important issue in this research field. Researcher should approve their procedure by Institutional Animal Care and Use Committee (IACUC) before conducting their study. For these reasons, use of appropriate facility and coordinating of researching scientist, facility staffs and veterinarian is very important. Development of good animal facility and cultivation of professional manpower is continuously required.

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