MINIPIGS AS LABORATORY ANIMALS - REVIEW

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Abstract

Minipigs are one of the most important laboratory animals because of their similarity to human. The minipigs had several advantages as a laboratory animal. They are small thus much easier for handling. Also, requirements of food, space and even pharmacologic products or anesthetic are significantly reduced. Miniature pigs have a convenient body size for surgical procedures and given their anatomical similarities to humans particularly in terms of skin, skeleton, teeth, gastrointestinal tract, pancreas, liver, kidney, lung, and immune status they are often used as models for humans. In cardiac research, they are often used for studies on cholesterol. They are widely used in pharmacology research and diabetes research. In this review, we summarized several information and guidelines related with minipigs, especially about their history, breeding and using as model laboratory animal.

Key Words: minipig, laboratory animal, breeding

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 (Jang and Lee, 2012).

Pigs were one of the earliest research animals: in ancient Greece Erasistratus (304-250 B.C.) used them to investigate the mechanics of breathing. In Rome, Galen (130-200 A.D.) used them to demonstrate blood circulation. As scientific research became a large and successful endeavor, pigs, like rats, became increasingly popular since most people lack the emotional attachment to swine—they became in many protocols the replacement animal for dogs and cats. And since historically, both rats and pigs are animals devalued by society, their use as tools of science is generally more acceptable by the public. Just as the white rat was genetically modified to suit research needs, the miniature pig has become, like the rat, a research tool par excellence with breeding programs selecting for specific traits that can be utilized in research protocols. And given the state of current pig genome research there are increased studies using mice and swine in tandem for biomedical information that has application to humans. Miniature pigs are purpose bred for research; they are smaller than those bred for production, with a much slower growth curve, but physiologically in all other ways they are similar to agricultural pigs. The exponential growth curve begins at 3 months; for research lasting longer than 3 weeks, miniature swine are preferable both for handling ease and welfare considerations**.** Because pigs have been bred for weight gain, restricting their feed causes them emotional distress. But, since they tend toward obesity, for long term projects a diet low in calories and high in fiber, with plenty of straw and other items for rooting is necessary for their welfare. Miniature pigs have a convenient body size for surgical procedures and given their anatomical similarities to humans particularly in terms of skin, skeleton, teeth, gastrointestinal tract, pancreas, liver, kidney, lung, and immune status they are often used as models for humans. In cardiac research, they are often the model of choice, particularly for studies on cholesterol. They are widely used in pharmacology research and diabetes research. During recent years, pigs have become utilized for their biological parts, e.g. cardiac valve replacement; the area of xenotransplantation, a recent development, has resulted not only in increased scientific research but also new and difficult ethical questions.

Breeds of minipigs

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.

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 Göttingen pigs and each of them show different characters.

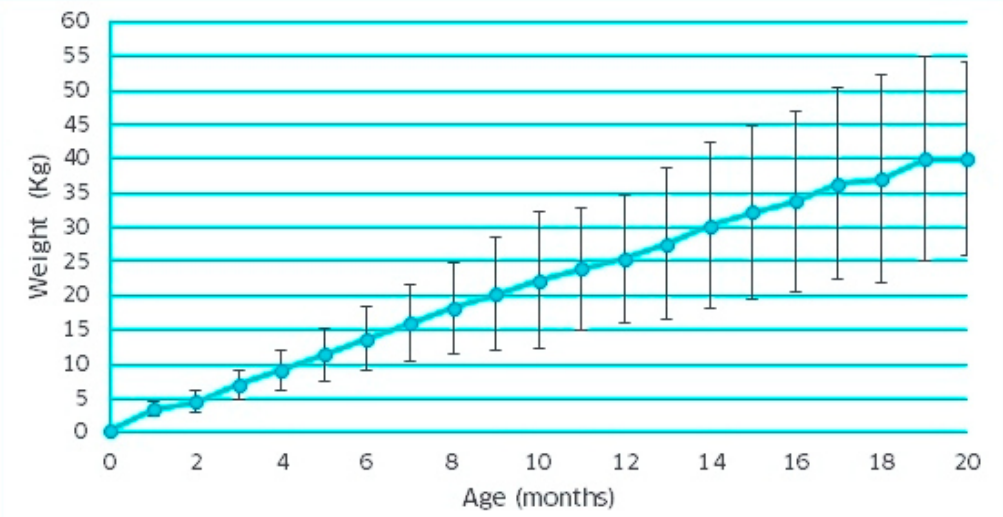
The Yucatan minipigs are one of the native breed pigs in North America. Their body weight at adult stage is around 70-83 kg but recently smaller variant named Micro-Yucatan that weigh approximately 55-70 kg at adult is also developed. They have usually black skin but white colored or dotted pattern also 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, Göttingen 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 Göttingen minipig is small breed in white nonpigmented 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). The Göttingen Minipig was developed at the Georg-August-University in Göttingen, Germany to meet the demand for a non-rodent model with many similarities to humans. In 1960, Minnesota Minipigs were imported from the Hormel Institute, Austin, USA, and Vietnamese potbelly pigs were imported from Wilhelma Zoo, Stuttgart, Germany. A coloured population of pigs resulted from this crossbreeding. In 1965, additional Vietnamese potbelly pigs were imported from Zoo Friedrichsfelde, East Berlin, Germany. This crossbreeding resulted in a smaller and whiter population of pigs. Finally, in 1965–1969, the German Landrace was introduced to develop a white line. Therefore the Göttingen Minipig is the result of crossbreeding the Minnesota Minipig, the Vietnamese potbelly pig and the German Landrace. Genetic management is required in order to minimise inbreeding and drift and to maintain the genetic integrity of the population. The genetics for the entire breeding population are still managed in Göttingen and all breeding data dates back to the 1960s when the Göttingen Minipig was developed. The entire breeding population is found in three physical locations (Dalmose, Denmark, Göttingen, Germany, and North Rose, New York, US). Despite separation into sub-populations, the Göttingen Minipig population is genetically coherent (http://minipigs.dk).

Figure 1. The growth of Gottingen minipigs *(Source: http://www.minipig.dk)*



Breeding management

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).

According Czech legislature, the minimal standards for pig breeding are formulated and minimal floor space for pigs and minipigs are defined (Table1).

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 Göttingen Minipigs A/S, 2010). Appropriate spacing bet-ween 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).

Optimal temperature range suggested by Association for Assessment and Accreditation of Laboratory Animal Care is 16-27℃, optimal humidity will be 50-70% and lightning will be given 12 hours a day at 100 -200 lux. For Göttingen minipigs the temperature given in table 3 is recommended. The correct temperature is measured at floor level. If measured more than 30 cm above floor level, increase the temperature accordingly. When bedding is used, the temperature can be reduced by two degrees. Water should be given ad libitum via automatic watering because shortage of water intake induce health problem in pigs.

Minipigs, especially females, must be fed a restricted diet to control growth. The exact requirements are influenced by a variety of factors that need to be taken into account when designing a dietary regimen. Factors which influence requirements include age, weight, gender, health status, activity, singular/group housing, room temperature and air velocity. To prevent draughts, the air velocity should be less than 0.3 m/sec. Males have a higher maintenance requirement compared to females, and males react to feeding or environmental changes by losing weight faster than females.

Table 1. Minimal floor area for laboratory pigs and minipigs

Live weight (kg)	Minimal area of closed space	Minimal floor area/animal (m^2)	Minimal floor area for laying (m^2/animal) – in thermo neu-tral conditions
< 5	2.0	0.2	0.10
5-10	2.0	0.25	0.11
10-20	2.0	0.35	0.18
20-30	2.0	0.50	0.24
30-50	2.0	0.70	0.33
50-70	3.0	0.80	0.41
70-100	3.0	1.00	0.53
100-150	4.0	1.35	0.70
150 <	5.0	2.50	0.95
adult males	7.5		1.30

Source: http://eagri.cz

Table 2. Space requirement for pig recommended by Association for Assessment and Accreditation of Laboratory Animal Care

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

Source: National Research Council, 2010

Table 3. Optimal temperature for Göttingen minipigs

Age	Temperature (°C)
Less than 1 month	28
1-2 months	26
3-6	22-24
More than 6 months	20-22

Source: http://minipigs.dk

Minipigs in medical research

Pigs are an important animal in biomedical research because of their anatomical and physiological similarities to human. They are widely used in cardiovascular study (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 modelled 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). Pigs and minipigs have been used to model many human dermal diseases and conditions. A non-exhaustive list includes: contact dermatitis/delayed-type sensitivity reactions, dermal melanoma, bullous pemphigoid, radiation exposure, laser exposure, and a genetically psoriasis model may well be under way. Minipigs are intensively used in diabetes researcjh. Rodent models are used extensively in diabetes research and offer valuable insights into the mechanisms of the disease, but many agree that rodents suffer from poor or limited translatability when it comes to predicting the human situation. This warrants models in larger animals. Many structural and functional similarities of the minipig/human gastrointestinal tract and pancreas coupled with similarities in metabolism and glucose tolerance has made the minipig a strong model in diabetes research (Larsen et al., 2003).

Conclusion

Minipigs can provide improvement in biomedical research. But proper management and animal husbandry are very necessary for successful breeding. The welfare conditions according national and European legislature must be kept.

References

Ellegaard Gottingen Minipigs A/S (2010): Taking good care of Ellegaard Gottingen Minipigs. Ellegaard Gottingen Minipigs A/S.
http://minipigs.dk/fileadmin/filer/pdf/Taking_good_care_of_Ellegaard_Goettingen_Minipigs_13.03.13.pdf

Jang, Goo; Lee, Byeong Chun (2012). Minipigs as Laboratory Animals: Facility Management and Husbandry. Reproductive & Developmental biology, 36: 79-85.

Larsen M.O., Gotfredsen C.F., Wilken M., Carr R.D., Porksen N., Rolin B. (2003). Loss of beta-cell mass leads to a reduction of pulse mass with normal periodicity, regularity and entrainment of pulsatile insulin secretion in Gottingen minipigs. Diabetologia, 46(2):195-202.

McAnulty PA, Dayan AD, Ganderup NC, Hastings, KL (2012): The Minipig in Biomedical Research. CRC Press Taylor and Francis Group, 445-468.

National Research Council (2010): Guide for the Care and Use of Laboratory Animals. 8th. National Academy Press, Washington, DC.

Nunoya T, Shibuya K, Saitoh T, Yazawa H, Nakamura K, Baba Y, Hirai T (2007): Use of miniature pig for biomedical research, with reference to toxicologic studies. J Toxicol Pathol 20:125-132.

Piedrahita JA, Olby N (2011): Perspectives on transgenic livestock in agriculture and biomedicine: an update. Reprod Fertil Dev 23:56-63.

Smith AC, Swindle MM (2006): Preparation of swine for the laboratory. ILAR J 47:358-363

Swindle MM (2008): Handling, Husbandry and Injection Techniques in Swine. Sinclair research.

Vodicka P, Smetana K Jr, Dvorankova B, Emerick T, Xu YZ, Ourednik J, Ourednik V, Motlik J (2005): The miniature pig as an animal model in biomedical research. Ann N Y Acad Sci 1049:161-171.

Vyhláška č. 419/2012 Sb., o chovu pokusných zvířat, příl. 7. Available at: http://eagri.cz/public/web/mze/legislativa/pravni-predpisy-mze/tematicky-prehled/100840391.html

Whyte JJ, Prather RS (2011): Genetic modifications of pigs for medicine and agriculture. Mol Reprod Dev 78:879-891.

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