26 Swine in Biomedical Research

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ABSTRACT

This chapter provides an overview of the use of domestic and miniature breeds of swine in biomedical research. Differences between breeds of pigs and the most significant anatomic and physiological characteristics of the species related to their selection as biomedical research models are discussed. Husbandry and handling techniques that have proven to be beneficial for using these animals in research are detailed. Also included are the key references for developing and using swine and resources for additional training and refinement are listed.

Key Words: Swine, Miniature pig, Surgery, Animal models, Animal husbandry.

INTRODUCTION

Swine (*Sus scrofa domestica*) have been used extensively in biomedical research and have become a standard general surgical model for preclinical studies. Their usage continues to grow while other large animal models are decreasing in number. Since the 1980s there have been a number of conferences held to specifically identify uses of swine in research. There are detailed publications on the technical issues[1–6] involved in the use of this species and reference publications on biology[7–10] and models.[1,2,11–16] Detailed publications on anesthesia, analgesia, and perioperative care are available.[1–3,17–19]

In this chapter their common uses are described as well as some of the technical issues involved in their selection and use as biomedical models. Because of the large number of porcine models utilized, the anatomic and physiological parameters of the various systems that justify their use in biomedical research are detailed.

BREEDS AND SOURCES OF SWINE

Most breeds of swine are animals raised commercially for meat production. These animals are commonly referred to as domestic or farm breeds in the literature. There are hundreds of these breeds and common examples are Yorkshire, Landrace, Duroc, Hampshire, Pietrain, and Poland China. There are over 50 breeds of miniature pigs worldwide but only a few of them are important in biomedical research. Smaller miniature breeds are sometimes referred to as microswine. Some of the most common miniature swine used in research are the Yucatan, Hanford, Sinclair, Göttingen, Ossabaw, Banna, Vietnamese Potbellied, and Meishan.[1,2,4,8]

The most significant difference between miniature and domestic swine is the growth rate. Farm breeds grow from a birth weight of 0.5–1 kg to a weight of >100 kg in 4 months. This is an exponential growth rate that is an economic necessity when raising swine for food consumption. However, in a biomedical research facility this growth rate is a complication for research as well as a potential occupational health hazard. Large swine can be very difficult to handle and lifting anesthetized animals can lead to ergonomic injuries. In the same 4-month period of time miniature pigs grow from 0.25 kg to 7–20 kg depending upon the breed. Of the most commonly used minipigs the size ranges from smallest to largest are Göttingen, Sinclair, Yucatan, and Hanford. The Hanford achieves the body weight of an adult human in a year, while the smaller breeds weigh <30 kg at the same age.[1,2,4,8]

Consequently, using domestic breeds for long-term chronic experiments is discouraged. Unless growth is part of the study, projects using farm pigs for chronic experiments should be limited to 3–6 weeks depending on the size of the animal at the start of the experiment and the housing capabilities of the institution.

Selection of miniature pigs for an experiment is based upon the size and physiological characteristics of the particular breed. Piglets may be weaned from the sow between 3 and 6 weeks of age. Sexual maturity in swine generally is reached between 4 and 6 months of age. Consequently studying animals <4 months of age would be considered to be a study conducted in neonatal or juvenile animals. Depending upon the goals of the study this may be a complication.

In general all pigs of the same weight have similar sized organs and structures. The difference would be the physiological maturity of the organ of interest because the size of the pig is breed dependent. Most organs and systems are physiologically mature by sexual maturity. However, the range of weights between miniature and domestic breeds at sexual maturity may be as much as a 100 kg difference. Consequently, the size of an organ such as the heart may be substantially different at the same age between breeds.

The health status of the source herd is also important.[2,20,21] Pigs purchased from auctions or conventional farms may have complicating diseases and parasites. In fact, their use may be similar to using unconditioned pound source dogs. Large commercial suppliers and operations that specialize in supplying swine for

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research will generally have a program for prevention of diseases. In swine there is a designation of specific pathogen free (SPF), which has a proprietary connotation in the United States. These are animals that have been raised to prevent diseases that interfere with weight gain. SPF swine are not necessarily free of all diseases and it should be used as a starting point when evaluating a source of animals. Some pigs are available as Cesarian-derived and barrier-reared animals. Housing of these animals with conventional swine is not appropriate because they will become infected with organisms that may be subclinical in the other animals. The institutional veterinarian should be consulted to preapprove the source of animals after evaluation of the herd health program.

Shipping of swine between facilities may also be a substantial problem that must be overcome.[2,22] There are intense and specific regulations regarding the shipping of swine in interstate commerce within the United States, as regulated by the U.S. Department of Agriculture (USDA), and even more intense regulations need to be followed when shipping between countries. No exceptions are made for animals used in research. The institutional veterinarian should be knowledgeable about the regulations and shipping requirements for animals.

It is important to adequately describe the type of pig used in an experimental study in the materials and methods section of journal articles. This should include the breed, sex, weight, age, and health status of the animal. Hemodynamics should be indexed to the body surface area when they are reported. When making comparisons between studies these factors need to be taken into consideration.

GENETIC MODELS

There are only a handful of spontaneous mutations in swine that have been developed as genetically reproducible models. A colony of swine with ventricular septal defect (VSD) had a high incidence of perimembranous VSD that is similar to the most common form of VSD in humans. Affected animals also had a high incidence of patent foramen ovale. Pedigree studies indicated that the defect was inherited as a polygenic trait.[23] Pigs develop other congenital heart anomalies that occur in humans, but these have generally been described as single case reports and have not been genetically reproducible. Swine with mutations of the predominant bloodborne cholesterol transporter, low-density lipoprotein (LDL), have elevated plasma cholesterol levels and have been used to model familial hypercholesterolemia in humans.[24] Sinclair miniature swine spontaneously develop cutaneous melanoma lesions that are classified histologically as malignant melanomas. Features of the swine model shared with human melanomas are the genetic predisposition, spontaneous appearance, similar histological appearance, capability for malignant transformation, and pattern of metastasis. Unlike human melanomas, tumor development in swine is not related to exposure to ultraviolet radiation, spontaneous tumor regression occurs that is immunologically mediated in most pigs, and the melanomas develop only in darkly pigmented animals.[25] Currently, female Ossabaw swine fed a high fat/cholesterol chow are being developed as a model of the human metabolic syndrome, characterized by central obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and hypertension.[26]

Current emphasis is on developing transgenic and knockout swine for applications in the areas of xenotransplantation, improving agricultural production, pharmaceutical production, and the development of new animal models. The four primary methods for producing transgenic pigs include pronuclear injection, oocyte transduction, sperm-mediated gene transfer, and nuclear transfer (cloning). The various methods and the pigs that have been produced using these techniques have been summarized by Prather.[27] A transgenic swine model of retinitis pigmentosa has been produced that is characterized by early and severe loss of rod photoreceptors and a slower degeneration of cone photoreceptors, similar to the condition in humans.[28] A major goal in producing genetically modified swine has been focused on overcoming the immunological barriers to xenotransplantation. Cloning technology has been used to produce α-Gal-deficient pigs; however additional immunomodulating strategies will be necessary for xenografting to become clinically feasible.[29]

INDUCED MODELS

Most of the models using swine are induced, many by surgical means, rather than being spontaneous or genetic in origin. The most common use of swine is in cardiovascular research, including atherosclerosis.[2,11,13–15] Investigational surgical procedures of various types are also a very common area of usage. Swine have become the standard general surgical model, having replaced the dog and primates over the past two decades. Included in these invasive procedures would be endoscopy, laparoscopic surgery, interventional catheterization, transplantation, and xenographic technologies.[1–3,5,6,30] In addition to the cardiovascular system, swine have also been used extensively to study diseases of the digestive, urogenital, and integumentary systems.[1,2,13–16,31] Swine have been used to a lesser degree for other systems, but are emerging in a variety of new areas, such as safety pharmacology and toxicology.[2,12,32]

CARDIOVASCULAR Cardiovascular models are based upon shared anatomic and physiological characteristics with humans.[1,2,7,11,13–15] Sexually mature Hanford pigs have a similar sized heart and blood vessels, however, many of the smaller pigs can be used for cardiovascular experimentation if adult sized structures are not important. Anatomically the most significant difference from humans is the presence of a left azygous vein (hemiazygous) that drains the intercostal vessels into the coronary sinus of the heart. Therefore, coronary blood flow return in the pig is mixed systemic and myocardial blood. This vessel may be ligated if total coronary blood flow is required.

The blood supply to the conduction system is right side dominant as in 90% of the human population and significant collateral circulation in the coronary system is not present. If a coronary vessel is obstructed to create a myocardial infarction, a complete infarct is created. Infarcts may be created acutely by ligation of a coronary vessel or by injection or blocking agents such as microspheres. Chronic or gradual infarction may be created by surgically applying osmotic ameroid constrictors around the blood vessel or by creating atherosclerotic lesions in the vessel lumen.[1,2,7,11,13–15,33–35] Myocardial infarction is an intensive area of research in swine because of these characteristics.

None of the cardiac conduction systems in animals exactly matches that of the human. In the case of the pig the conduction tends to be neurogenic rather than myogenic; there are nerve fibers in the atrioventricular node and the bundle branches. The pig has large well-differentiated subendocardial Purkinje cells. However, the anatomy and parameters of the intracardial electro-

gram overlap the values in humans and the pig has been used in studies of cardiac system ablation and pacemaker testing.[36–38]

Pigs are susceptible to development of atherosclerosis in a manner similar to humans. Over time they develop complex lesions. In general, pigs can be fed a diet with 2–4% cholesterol and 40% fat to develop generalized lesions over a period of approximately 6 months. The process can be accelerated by damaging the endothelium with a balloon catheter. This offers the advantage of being able to localize the lesion and decreasing the time to production of a significant lesion to 3 months.[2,39,40]

Almost any model that can be created surgically in other large animal species can be developed in swine.[2] A major category in this area is the production of heart failure and cardiac hypertrophy. Concentric hypertrophy is produced by pressure overload of the ventricle. Pressure overload is created by placement of a Silastic band around one of the great vessels of the heart. The band can be placed without constriction in young animals and the band will produce constriction and overload as the animal grows. Alternatively, it can be placed tightly to produce a constriction and pressure gradient. In either case hypertrophy of the affected ventricle will develop over approximately 3 months.[2,41]

Volume overload can be produced by severance of the tendinous chordae producing mitral regurgitation. It can also be produced by creating an arteriovenous fistula or by creating a patent foramen ovale. The closer to the heart that the shunt is created, the faster is the production of volume overload heart failure and eccentric hypertrophy. Fistulas created in the leg will lead to hypertrophy and heart failure in approximately 3 months. Fistulas between the aorta and pulmonary artery may lead to heart failure within a week.[2]

Rapid epicardial pacing >180 bpm leads to the development of dilative cardiomyopathy and congestive heart failure in approximately 3 weeks. These animals tend to become compromised very quickly and frequently have to be maintained on diuretics because of the rapid development of disease.[42,43]

PULMONARY The pulmonary system consists of three lobes on either side plus an accessory lobe on the right lung. The pulmonary system of the pig is very friable and easily injured. The mediastinum is thin and friable and for practical purposes should be considered incomplete. The lungs are the shock organ for the pig, as in humans. For this reason, pigs have been used as endotoxic shock models. Pigs undergo the same phases of hypodynamic and hyperdynamic shock phases after being exposed to organisms, toxins, or lipopolysaccharides.[2]

Pigs have also been used in studies of adult respiratory distress, asthma, and oxidative stress. Predominately, they have been used in studies of surgical techniques and repair.[2]

INTERVENTIONAL CATHETER AND MINIMALLY INVASIVE SURGERY TECHNIQUES Swine have become the standard model for interventional catheterization, endoscopy, and robotic and laparoscopic surgical procedures.[1,2,34,35,37,38,44–53] At first they were utilized as models for training physicians in the techniques. This evolved into the replacement of dogs and primates as models for testing of these devices and techniques.

Anatomic and physiological characteristics of the vascular system contribute to the selection of this model for testing of intravascular devices.[39,44,46,50,51] The size of the blood vessels in larger minipigs is similar to that of humans. In vascular anastomosis procedures or when intravascular stents are implanted pigs can develop neointimal hyperplasia more readily than other animals at the procedural margin. Neonatal shunts, such as atrial septal defects (ASD) and patent ductus arteriosus (PDA), can be reopened using balloon catheter procedures. These shunts will remain open and can be used for the testing of closure devices. Other models such as aneurysms of various types can be created surgically. Growth of the cardiovascular system from birth until sexual maturity can be used as a model of growth of the human heart into the early third decade of life.[2]

Abdominal, thoracic, and central nervous center minimally invasive techniques are routinely performed in porcine models, mainly as feasibility and training procedures. Long-term testing of devices for preclinical trials are growing in number and the model has gained acceptance by the Food and Drug Administration (FDA).[2]

DIGESTIVE SYSTEM Physiologically the digestive system of the pig is similar to humans probably because pigs are true omnivores. However, the anatomy of the gastrointestinal tract has some significant differences from humans. The stomach has a muscular outpouching called the torus pyloricus that can cause delayed gastric emptying with some substances. The mesenteric vessels form vascular arcades in the subserosa rather than in the mesentery. The cecum and large intestine form a series of centrifugal and centripetal coils that adhere together to form the spiral colon in the left upper quadrant of the abdomen. The bile duct and the pancreatic duct enter the duodenum separately, rather than as a common duct as is found in humans.[2,16,54]

Swine are used in models involving the physiology of digestion and metabolism. They have special importance in the study of diabetes and metabolic syndrome. Pancreatitis and various aspects of islet cell transplantation have also been studied in porcine models. Endoscopic techniques, especially of the biliary system, are routinely studied in porcine models.[2,16,54–57]

UROGENITAL SYSTEM Swine have true multirenculate, multipapillate kidneys making the internal renal anatomy of the calyceal system analogous to humans. Swine have been used extensively in the study of renal function, renal hypertension, hydroureteronephrosis, intrarenal reflux, and the study of interventional devices for the correction of defects.[2,58–62]

The reproductive system of the female is a typical bicornuate type of uterus with epitheliochorial placentation. The fallopian tubes are long and torturous compared to other mammals. The male has the same types of accessory sex glands as the human, except that the dominant glands are the bulbourethral glands rather than the prostate, which is rudimentary. The male also has a corkscrew-shaped tip to the penis, a large preputial diverticulum, and a sigmoid flexure. All of these characteristics of the penis make it impossible to routinely catheterize the male bladder.[2,58–62]

INTEGUMENTARY SYSTEM/WOUND HEALING Swine have been standard models for wound healing and dermal toxicology because of the anatomic and physiological characteristics of the skin. Swine are relatively hairless with a skin that is tightly attached to the subcutaneous tissues as in humans. They have a comparable histology except that they have a paucity of eccrine sweat glands, a fatty subcutis, and a relatively higher pH value. There are similarities in cellular turnover time, permeability, transdermal absorption, and dermal metabolism. The microvascular anatomy is consistent with that of humans and the sizes of flaps, grafts, and dermal wounds have been standardized for comparison of therapeutic agents.[2,63–67]

OTHER SYSTEMS The systems described above represent the majority of the ones in which swine are used as a large animal model. Other organs and systems are utilized to a lesser degree and usually for a specific focus.[2]

For example, the musculoskeletal system is rarely utilized for studies because of the massive structure of the bones and muscle, as would be expected for an animal primarily used for food production. Also the epiphyseal closure may take as long as 4 years in domestic breeds, while the majority of the species are not maintained for that many years. However, some specialized efforts related to studies of bone and cartilage metabolism have been undertaken.[2]

The central nervous system (CNS) and sensory organs are emerging areas of using porcine models.[68–74] Pigs have been used in ophthalmic research, mainly because of characteristics of the retina and vitreous humor. They are being developed as stroke and CNS ischemia models.

The use of swine, miniature breeds in particular, for pharmacokinetic and toxicological studies has been increasing.[12,75,76] Like other species, swine have both similarities and differences from humans and multiple species are used for preclinical studies. In terms of the cytochrome P450 system pigs have CYP1A, CYP2E, and CYP3A activity similar to humans. There is either no information or low activity for CYP2B, CYP2C, and CYP2D. Pigs have high glucuronidation and acetylation activity but low sulfation activity. Pigs have 16 blood types (A–P) with weak blood group antigens. Factors V, VIII, and IX are higher compared to humans and pigs have faster clotting times. As a general rule in our laboratory when we are trying to use a new agent in swine, we use the human pediatric dosage.

Xenotransplantation studies are performed for both tissue and solid organ transplants. The research is mainly related to the transgenic technology issues discussed above. None of the various forms of rejection has been completely conquered. There also exists the issue of poor physiological function posttransplant, even if the rejection issues have been resolved. There is a major interest in protecting against xenozoonoses resulting from either mutation or a combination of viruses in human recipients. The reasons for using swine for this model are their availability as compared to nonhuman primates and because of the anatomic and physiological similarities.[2,77]

HUSBANDRY AND HANDLING

HUSBANDRY Swine are social animals and prefer to have contact with other swine. Laboratory swine can be housed individually or in small groups in pens. Individual housing is common in a research setting. If housed individually pigs should have visual, olfactory, and auditory contact with conspecifics to prevent stress associated with social deprivation.

Proper management practices and knowledge of normal swine behavior are required to successfully group house swine. Group sizes will be based on the size of the pens and the size of the pigs; however, group sizes should be limited to 10–15 animals to maintain a stable social hierarchy. Cage partitions should be provided to allow subordinant animals to avoid more dominant animals. Groups of animals should remain together as long as possible as dominance fighting occurs when new animals are added to the group. In a research setting, new groups should be established when a new shipment of pigs is received. Providing additional troughs or greater trough feeding space will allow all animals to eat simultaneously; however, differences in body weights may occur because of competition during feeding.[3]

In the United States, space recommendations are specified for swine used in biomedical research in the *Guide for the Care and Use of Laboratory Animals*.[69] The Council of Europe has recently revised their housing standards for research swine.[70]

Caging for swine should be sturdy since pigs are strong animals. Although pigs do not climb, they rub their sides along the sides of their cages with considerable force. As an expression of their rooting behavior, they will find and manipulate any loose items in their environment. Consequently, caging should be of solid construction and free of sharp edges and protrusions to prevent injury. Chain link fencing may be used for small swine. Other suitable materials include aluminum and stainless steel caging, which can be configured into vertical bars or solid panels. Regardless of cage material, the fencing should interface with the flooring securely and without gaps to prevent pigs from getting their hooves caught.

Swine require secure footing and can develop stress ulcers if flooring is slippery. Cage flooring can be either solid or on raised grids. If solid concrete or epoxy floors are used they should be textured to provide secure footing, and bedding of wood shavings or straw should be provided for rooting and nesting behavior. Grid floors increase the ease of sanitation since bedding is not usually provided. Spacing of the floor grids should be appropriate to prevent hoof damage. Plastic-coated diamond grids with five-eighths inch openings are suitable for housing swine of multiple sizes; however, swine will chew the plastic off the metal as soon as a tear in the plastic occurs. Fiberglass slatted floors with a slat width of 4.4 cm and a space of 0.64 cm between slats work well in the authors' experience. The slats can be coated with a medium grit to promote hoof wear. Regular hoof trimming at 3–6 month intervals is required for chronically housed animals if flooring does not maintain hoof wear.[2]

Cages that do not use bedding should be hosed daily to remove waste and minimize odors. Daily removal of soiled bedding is recommended when deep bedding is used. A complete change of bedding should be done one or two times a week. Cages should be sanitized no less than every 2 weeks by pressure washing for cleaning units in place or by cage washing modular units after they have been broken down. Pigs should be removed from their cages during routine cleaning to prevent contact with detergents and chilling from being wet. However, they readily accept baths and can be kept clean using a mild soap and warm water.

Swine readily use automatic watering systems, which are preferred to buckets. Swine require approximate 2.5 liters of water/kg of feed and can spill or soil water that is supplied in buckets. Feeders should be securely attached to cage sides to be able to withstand the excitement associated with feeding and the tendency of the pigs to root cage objects. Stainless steel or Teflon feed troughs are easily sanitized and are preferable to bucket feeders used in agricultural settings. Consideration should be given during cage design to the placement of the location of feeders and watering devices since swine will develop a dunging pattern and will defecate at the opposite end of the cage from where they are fed.

Laboratory swine should be provided with environmental enrichment to satisfy their intense need to chew and root, especially if bedding is not provided. We have successfully used large plastic balls, plastic dog toys, items that hang from chains, and

nylon brushes attached to fencing. Hanging items satisfy the need to chew and rub while items provided on the floor are used for rooting. Enrichment items need to be included in routine cage sanitation since pigs will avoid them if they become soiled. Rotation of enrichment items and limiting access to them for several hours a day will help maintain their novelty.

HANDLING Laboratory swine are easily trained using gentle handling techniques and positive reinforcement. Food items such as canned dog food, cookies, apples, and carrots can be used for training. Agricultural methods of handling and restraint, such as snout snares and suspension by the rear legs, are aversive and stressful to swine and are inappropriate in a research setting. Swine adapt readily to daily routines, including special handling practices common to research settings. Small pigs may be carried in the handler's arms, similar to handling a large dog. Larger pigs can be gently restrained against the side of a cage using a swine board. If prolonged restraint is necessary animals can be trained to use a humane restraint sling following a period of acclimation.

Pigs should be approached quietly in a crouched position since bending toward them is perceived as threatening. Rubbing pigs gently on the abdomen has a calming effect and can be used to aid physical examination. Swine can be trained to move out of their cages and guided with the use of a pig board or they can be trained to walk using a harness and leash.[71]

Laboratory swine that are cared for and handled gently interact readily with people. In fact, human–pig interaction is an important part of their environmental enrichment and contributes to their well-being in a research setting.

CONCLUSIONS

Swine will continue to be developed as translational research models and it is expected that their use will continue to outpace the growth of other large animal species in this arena. We receive frequent inquiries to troubleshoot protocols from investigators outside our institution. In the authors' experiences most of the complications that occur in porcine protocols are related to anesthetic, perioperative care and surgical issues. Anesthetic and perioperative care protocols must be designed with specific attention to the physiological effects of the agents and their potential for interference with the experimental goals. Surgical complications are usually related to inadequate aseptic technique or improper wound closure techniques.

There are current courses, CD Rom training programs, and technical publications[2,3,5,6] to help ameliorate these issues. Many of the helpful techniques are located online in various websites, which are listed below:

1. Contains a swine literature database from the Animal Welfare Information Center:
   http://www.nal.usda.gov/awic/pubs/swine/swine.htm
2. Contains reviews of models and Sinclair, Hanford, and Yucatan information:
   http://www.sinclairresearch.com/
3. Tutorial on swine procedures in research: Laboratory Animal Training Association:
   http://www.latanet.com/online/onlinetr.htm
4. Biology and diseases of swine:
   http://www.ivis.org/advances/Reuter/swindle/chapter_frm.asp?LA=1
5. Basic information on swine:
   http://www.aphis.usda.gov/vs/ceah/cahm/Swine/swine.htm
   http://www.nal.usda.gov/awic/pubs/swinehousing/swinehousing2.htm
6. Göttingen minipig background information:
   http://minipigs.dk
7. CD Rom training series on husbandry, handling, injection techniques, anesthesia, analgesia, and perioperative care:
   http://www.latanet.com/desktop/drs.html
   http://www.latanet.com
8. National Swine Research Resource Center–Transgenic and Cryopreservation Technology:
   http://www.nsrrc.missouri.edu/
9. Medical University of SC-Swine Training Courses:
   http://research.musc.edu/dlar/Swine%20Training%20Courses.htm

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