Preparation of Swine for the Laboratory

Alison C. Smith and M. Michael Swindle

Abstract

Swine are an important model in many areas of biomedical research. These animals have been used predominantly as preclinical models involving surgical and interventional protocols. The systems most commonly studied include cardiovascular, integumentary, digestive, and urological. Swine are intelligent social animals and require species-specific socialization and handling techniques. It is important to acclimate the animals to the facility and to personnel before they are placed on chronic protocols. Gentle handling techniques instead of forceful procedures are essential to their socialization. They require sturdy caging with specific construction standards, and toys for environmental enrichment. Because the species is covered by both the Animal Welfare Act and the US Department of Agriculture, interstate transport requires a health certificate with destination state-specific disease screening standards. This manuscript provides an overview of best practices that have been utilized in the authors' facility.

Key Words: animal models; animal welfare; husbandry; miniature pig; swine

Introduction

The use of swine in research, specifically as a preclinical model, has increased dramatically since the early 1980s. The species' unique anatomical and physiological characteristics make it a suitable model for many organs and systems. However, swine also have unique behavioral and husbandry considerations that must be addressed when they are used as laboratory animals. Resources that address these issues include a series of technical books (Bollen et al. 2000; Stanton and Mersmann 1986; Swindle 1983, 1998b), proceedings books (Tumbleson 1986; Tumbleson and Schook 1996), and review articles (Swindle and Smith 1998; Swindle et al. 1988, 1996, 1998a, 2005). In this manuscript, the most common considerations are described.

Common Models

The most common biomedical models in swine involve the cardiovascular, integumentary, urinary, and digestive systems. Emerging models are being developed for the study of stroke, diabetes, toxicology, and endotoxic shock. Swine are also considered to be one of the primary models for training in surgery, organ transplantation, and endoscopic and laparoscopic techniques, as well as being a preclinical model for the testing of pharmaceuticals and biomechanical devices (Jensen et al. 1996; Lindberg and Ogle 2001; Pond and Mersmann 2001; Stanton and Mersmann 1986; Svendsen 1998; Swindle 1998b; Tumbleson 1986; Tumbleson and Schook 1996).

The cardiovascular systems of swine are similar to humans' in terms of morphology and physiological function. The swine heart is similar morphologically to the human heart except for the presence of the left azygous (hemiazygous) vein, which contains systemic blood from the intercostal vessels and which enters the coronary sinus. Consequently, blood from the coronary sinus is mixed myocardial and systemic blood. Swine have prominent Purkinje fibers, a conduction system that is more neurogenic than myogenic, and nerve cells within the atrioventricular node that are also different from humans. However, the blood supply to the myocardium and the conduction system is similar to 90% of the human population, and swine have little collateral circulation in place. Consequently, the animals are used as models of myocardial infarction and arrhythmia treatment. Their blood vessel size and morphology are similar to humans' and the aorta has a true vaso vasorum. Swine also develop atherosclerosis in a very similar manner to humans when fed an atherogenic diet. The primary models for the cardiovascular system include the study of myocardial infarction, heart failure, atherosclerosis, vascular grafts (neointimal hyperplasia), aneurysm, interventional devices, pacemakers, and biomechanical heart valves (Pond and Mersmann 2001; Stanton and Mersmann 1986; Swindle 1998b; Tumbleson and Schook 1996).

The skin of the pig is relatively hairless, with a fixed rather than loosely attached subcutaneous component. The blood supply, glands, and structures of the integument are similar to humans' except for the relative absence of sweat glands. Swine have been a primary model for plastic and

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reconstructive surgery, transdermal toxicology, and wound healing (Pond and Mersmann 2001; Swindle 1998b; Tumbleson and Schook 1996).

The internal renal anatomy of the kidney is that of a true multirenculate, multipapillate organ with a true calyceal system. This characteristic makes the internal anatomy of the kidney more similar to humans' than other commonly used animal models. Swine have been used primarily for intrarenal surgery, intrarenal reflux, hydronephrosis, and renal hypertension (Pond and Mersmann 2001; Swindle 1998b; Tumbleson and Schook 1996).

Secondary only to the cardiovascular system, various models of the digestive system have been studied. The gross anatomy of the intestinal tract has substantial differences from other species. The stomach has a muscular outpouching, the torus pyloricus, in the pyloric region. The mesenteric vessels form vascular arcades in the subserosa rather than in the mesentery. The cecum and ascending and transverse colonic segments are coiled into a series of centrifugal and centripetal coils, known as the spiral colon, in the left upper quadrant of the abdominal cavity. The pancreatic and biliary ducts enter the duodenum separately. The liver has a similar anatomy to humans' except for the presence of fibrous septae, which are apparent histologically. The pancreas consists of two lobes and encircles the cranial mesenteric vessels, making surgical removal more difficult than in other species. In spite of the gross anatomical differences, the function of the digestive tract is that of a true omnivore, and consequently, the physiological function is similar to humans (Jensen et al. 1996; Lindberg and Ogle 2001; Pond and Mersmann 2001; Swindle 1998b; Tumbleson and Schook 1996).

Many other models have been developed in swine, and new models are continually being developed. Genetic models of malignant melanoma (Sinclair) (Hook et al. 1982), ventricular septal defect (Yucatan) (Swindle et al. 1990), and metabolic syndrome (Ossabaw) (Dyson 2006) have also been developed. The production of transgenic, knockout, and cloned pigs is further developing the number of biomedical models that will be used in the future (Lai et al. 2002; Nagashima et al. 2003; Walker et al. 2002).

Breeds and Characteristics

The breeds of swine that are predominantly used in research in the United States are domestic farm breeds and the following miniature breeds: Yucatan mini and micro, Hanford, Sinclair, and Göttingen. Other miniature pigs in use in the United States include the Ossabaw and the NIH, which are characterized and used for diabetes and transplant immunology, respectively. Worldwide there are hundreds of breeds of farm pigs and at least 45 miniature breeds. However, the scope of this manuscript is such that only the breeds commonly in use in the United States are discussed.

Domestic farm breeds are numerous in the United States. Commonly used breeds in research include Duroc, Yorkshire, Landrace, and crossbreeds. Domestic swine are bred for meat production and have a growth rate that increases from 1 kg at birth to 100 to 110 kg at 4 mo of age. Both farm and miniature breeds reach sexual maturity between 4 and 6 mo of age. Some breeds of swine, such as the Landrace and Pietrain, are susceptible to malignant hyperthermia, which is genetically transmitted. This condition can be a complication of anesthetic protocols, therefore it is important to avoid using susceptible animals. The rapid growth rate of domestic swine limits their usefulness in chronic studies. As a general rule, domestic swine should not be used in protocols longer than 4 to 6 wk unless growth is part of the study. Larger animals are difficult to handle and are an occupational safety hazard for personnel (Swindle 1998b).

The Yucatan breed has been developed both as a miniature and a micro variety. The difference between the two results from selecting for animals that are smaller at sexual maturity. At sexual maturity, the miniatures weigh approximately 20 to 30 kg and the micros, approximately 14 to 20 kg. The Yucatans are usually black and have a short snout and wattles. However, white and spotted animals are available. With sexual maturity, the skin over the neck and shoulders thickens greatly, and in older breeding animals, it may reach a thickness of 2 cm and become very firm. This characteristic makes surgical procedures in this area more difficult. Yucatans have been bred to develop a genetic model of ventricular septal defect.

The Hanford pig is the largest of the miniature breeds. At sexual maturity, they weigh approximately 25 to 40 kg. These pigs are generally white and have elongated snouts. The Hanford pig has the largest heart and blood vessels, and sexually mature animals are used in the testing of implanted devices sized for humans.

The Sinclair breed reaches a weight of approximately 16 to 22 kg at sexual maturity. A genetic model of malignant melanoma has been developed in this breed. Varieties of Sinclair include dark, white, and spotted, and the animals have a shortened snout like the Yucatan. The Sinclair is a general-purpose miniature pig that is selected when the size and the reduced growth rate are important to the study.

The Göttingen pig is the smallest of the common miniature breeds. It weighs approximately 10 to 14 kg at sexual maturity. These pigs are white and have a rounded appearance with a shortened snout. This breed is available in a barrier-raised pathogen-free status and is mainly used in pharmaceutical and toxicology studies.

The health status of the selected pig is important. Purchasing pigs from small farms or livestock auctions is similar to purchasing unconditioned random-sourced dogs and cats. Major producers tend to raise healthy animals in barrier systems and to have a comprehensive preventative medicine program. The institutional veterinarian should review the supplier's program to ensure that the pigs will be of sufficient quality to meet the needs of the research program. The term specific pathogen free (SPF[1]) has an exact commercial definition in swine. It is a good starting point, but the purpose of the SPF program is to eliminate diseases and parasites responsible for poor growth rates for meat production. SPF pigs are monitored at slaughterhouses by gross examination and not by culture and histology (Swindle 1996, 1998a).

Behavioral Attributes

Good husbandry in the laboratory environment requires knowledge of the behavioral traits of swine. Swine are naturally social creatures. In the wild, they live in large, close-knit groups called sounders, which comprise females and their young. A social dominance order is established among groups of females. Aggressive interactions diminish in stable social groups. When males reach maturity, they leave the group and may form bachelor herds until they are old enough to mate, generally at 4 yr of age. Adult boars are generally solitary, but females stay with the sounder unless they are rearing young. Pigs are omnivorous, and they use their strong snouts for rooting in the soil. Their feeding and exploratory behaviors are closely linked. Wild pigs are generally active at dusk, at dawn, and at night (Bollen et al. 2000).

Within a few days of birth, newborn piglets establish a social order called the teat order. When the teat order is established, each piglet will consistently suckle at the same teat, with the more dominant piglets generally suckling from the more productive anterior teats. If piglets from different litters are mixed after weaning, a new social order is established based on aggressive interaction, which will be maintained as long as the group is together. If subordinate individuals are separated from the group, they will be attacked upon reintroduction, whereas a dominant animal can be separated and reintroduced without incident (Bollen et al. 2000).

Transportation Issues

Transportation of swine is stressful to the animals. They may lose 10% of their weight even on a relatively short truck ride. They are susceptible to stress-induced respiratory disease and diarrhea after shipping. Consequently, pigs should be conditioned at the research facility for approximately 1 wk before being placed on chronic protocols such as survival surgery. Upon arrival, animals should receive a clinical examination and be bathed and treated for disease conditions. Depending on the source of the animals and the goals of the research institution, animals may require fecal examinations and vaccination (Swindle 1998b).

There are specific standards for transportation of pigs in interstate commerce, which are regulated by the US Department of Agriculture (USDA[1]). Out of state suppliers are responsible for meeting the standards, which vary slightly from state to state. Herds must be certified as free from diseases such as pseudorabies and Brucellosis, and must be accompanied by a health certificate. Institutions that ship animals to other research institutions must also meet these shipping standards. A licensed USDA-certified veterinarian must complete the health certificate and ensure that the shipment is in compliance with shipping standards.

The requirements of the Animal Welfare Act for transportation and handling are found in Subpart F, 3.125-3.142 (OFR 2002). These standards are not specific to pigs, but state that provision must be given to transporting animals in safe, structurally sound cages in compatible groups. Cages must have adequate ventilation and have either wire floors or adequate bedding to keep the animals clean from waste. Animals must be offered water at least every 12 hr and food at least every 24 hr during transport. The animals must be observed at least every 4 hr during surface transportation to ensure their welfare. Temperature should not be allowed to be colder than 7.2°C (45°F) or to exceed 29.5°C (75°F) during transport. Animals must be handled and held in facilities and enclosures that do not allow the animals to commingle with inanimate cargo and that prevent the animals from being exposed to hazardous environmental conditions.

Husbandry

Pigs housed in the laboratory may be housed individually or in small groups in pens. Individual housing is common in a research setting. Individually housed swine should have visual, olfactory, and auditory contact with other swine to prevent social deprivation.

When group housing is utilized, the behavioral characteristic of forming social hierarchies should be considered. Both the size of the pens and the size of the pigs will dictate how many animals can be housed per pen; however, group sizes should be limited to between 10 and 15 animals, otherwise a stable social hierarchy will not be maintained. Ideally, partitions should be provided so that subordinate animals can avoid aggressive animals. Groups of animals should remain together as much as possible. If regrouping is necessary, aggression can be minimized by bringing unfamiliar pigs together in a newly cleaned cage just before feeding or sleeping times. In a research setting, groupings should be established when a new shipment of pigs is received. Adequate feeding space should enable the animals to eat simultaneously; however, differences in body weights may occur due to competition when trough feeding is used for group-housed swine.

Because adult boars are solitary, individual housing is appropriate. Barrows (castrated males) may be group housed using the same guidelines as for females.

Space guidelines for swine have been established by the

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[1] Abbreviations used in this article: IACUC, institutional animal care and use committee; SPF, specific pathogen free; USDA, US Department of Agriculture.

National Research Council in the *Guide for the Care and Use of Laboratory Animals* (NRC 1996). In general, more space is recommended for research swine than for swine in agriculture. The *Guide* states that "animals should be housed with a goal of maximizing species-specific behaviors and minimizing stress-induced behaviors" (p. 22).

Pens should be sturdy because swine are strong and forceful animals. They rub their sides along the sides of their pens with considerable force and use their snouts to manipulate loose objects, therefore feeders and waterers should be securely fastened to cages (Figure 1). Automatic waterers are preferable to bowls because they provide constant access to clean water. Flooring may be solid concrete or raised grids. Concrete floors should be textured to provide secure footing, and bedding should be provided for rooting and nesting activity. Grid floors increase ease of sanitation because bedding is generally not provided. Spacing of the floor grids should be appropriate to prevent hoof damage. Room temperature is more critical with grid flooring than with concrete flooring because animals do not have the opportunity to nest on grid flooring. In general, regular hoof trimming is necessary with grid flooring because hooves do not wear down as they do on concrete floors. Swine develop a dunging pattern and will defecate at the opposite end of the cage from where they are fed. For this reason, cage design should include consideration of the location of feeders and watering devices to accommodate this behavior (Swindle 1998b).

Wild swine spend most of their active time foraging for food, and they eat small amounts continuously. If fed ad libitum, laboratory swine become obese, therefore restricted feeding is necessary. Restricted feeding of a low-energy ration will provide a satisfying larger volume of food intake compared with a higher energy diet (Bollen et al. 2000).

When swine are not sleeping, they are exploring and rooting. Laboratory swine require environmental enrichment to satisfy their intense need to chew and root, especially when no bedding is provided. Straw or wood shavings are excellent enrichment items that allow swine to engage in normal rooting activity and that provide insulation while allowing animals to control their microenvironment. However, these materials increase the effort required for adequate cage sanitation, particularly with slatted flooring, and care must be exercised to prevent the materials from clogging drains during routine cleaning.

Figure 1 This stainless steel pen has fiberglass slatted flooring, automatic watering, an attached food bowl, and a Teflon ball as an enrichment toy.

A variety of items may be considered elements of environmental enrichment, including balls, large plastic dog toys, mats, items that hang from chains, and items such as a nylon brush or broom head attached to fencing. Hanging items satisfy the need to chew and rub, and items provided on the floor may be used for rooting. Effective sanitation is essential for all enrichment items because swine will avoid them if they become soiled. Frequently rotating the enrichment items and providing them for a limited time each day will help maintain their novelty.

Training of Species and Staff

Although wild swine are crepuscular and nocturnal, the activity of laboratory swine is tied to the activity and presence of humans, rather than to the light cycle. Laboratory swine spend 70 to 80% of their time lying or sleeping unless it is feeding time or people enter the room. The influence of human caretakers and handlers on swine behavior in a laboratory setting is significant, and the importance of this influence cannot be overemphasized. Swine are extremely intelligent animals with excellent memories. They remember both good and bad experiences, and they easily become accustomed to daily routines, including special handling practices unique to research settings, such as restraint in a sling or running on a treadmill. They are relatively insensitive to noise and are themselves very noisy; however, sudden, extremely loud noises frighten them. Their responses to acute stressors are vocalization and attempts to escape.

Personnel working with swine should interact with them so that fearful responses are minimized (Figure 2). Pigs that are gently handled will allow themselves to be petted and will bond with their handlers. Pigs are readily trained using positive reinforcement and gentle handling. Much more effort is necessary to overcome the effects of negative handling experiences compared with the effort expended in using positive reinforcement for training (McGlone et al. 2001).

Gentle handling techniques should also be used to adapt laboratory swine to restraint. The restraint technique used will be dictated by the size of the animal and the experimental procedures required. Restraint techniques used for swine include manual, mechanical, and chemical restraint. Pigs should be approached quietly. They will be less threatened if approached by a person in a crouched position than

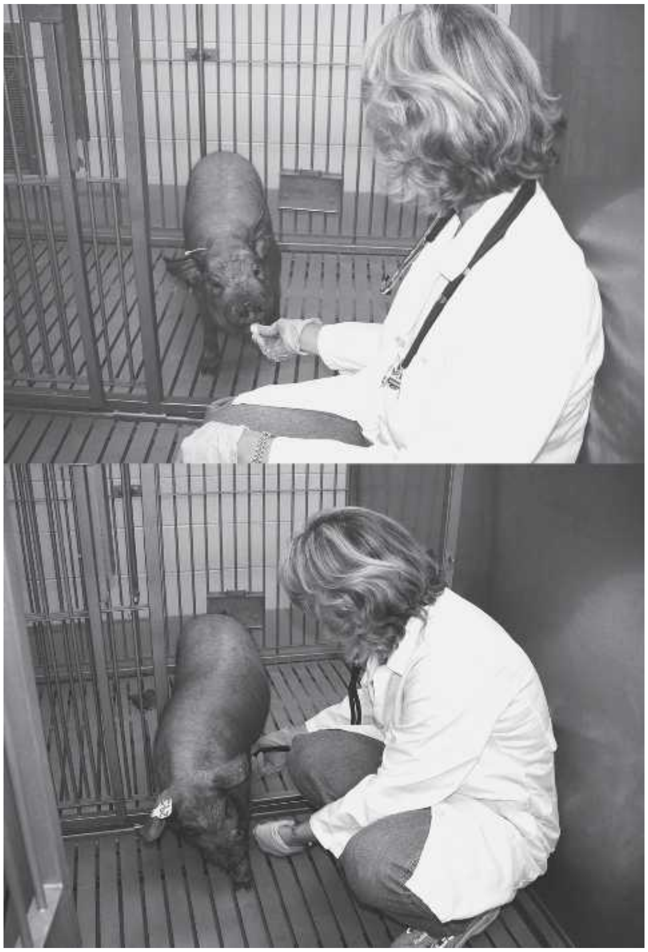

Figure 2 This Yucatan pig is being enticed with a food treat (top) to induce the animal to perform auscultation (bottom). Notice that the pen has a central dividing panel that allows two pigs to be housed in the unit for social contact. This separation is preferable to housing pigs together in the same pen during the postsurgical period because of the possibility of wound damage.

Figure 3 This farm pig has been acclimated to the sling and is sleeping during restraint.

by one who is standing and bending over them. Rubbing them on the abdomen has a calming effect and is a petting technique that can be utilized for brief examinations. For brief restraint, piglets and animals that weigh less than ~20 kg can be restrained manually.

Mechanical restraint using a sling apparatus can be utilized for all sizes of swine for brief procedures such as dosing or obtaining blood samples, or for relatively noninvasive procedures that require prolonged restraint. Swine that have been properly acclimated to the sling will relax and frequently fall asleep during prolonged restraint (Figure 3).

Large animals can be trained to walk out of their cages and be guided, or they will require chemical restraint. Chemical restraint, which can be achieved with minimal stress in the animal's home cage, should be used routinely for invasive procedures to prevent negative handling experiences.

Considerations of the Institutional Animal Care and Use Committee (IACUC[1])

In general, most of the same considerations applied to other species during IACUC review of experimental protocols pertain to protocols utilizing swine. However, there are several issues with respect to swine that deserve special consideration. Swine that will have prolonged restraint in a sling should be acclimated to the sling. Generally, 1 wk of short exposures involving food treats for positive reinforcement is sufficient training for longer durations. Intermittent use of food treats during prolonged restraint is also good reinforcement if it is not contraindicated by the protocol.

Swine that will be utilized in chronic procedures of any kind should be acclimated to the facility for a minimum of 1 wk before they are enrolled in a study. This will allow the animals to recover from the physiological stress of shipping and to accommodate to different husbandry conditions.

Individual medical records should be maintained on all swine, regardless of whether their use is for acute or chronic procedures. Records should include vendor and USDA information, results of incoming physical examination, and a chronological medical history. For animals utilized in surgical protocols, medical records should include the anesthetic regimen, monitoring during anesthesia, as well as a complete operative and perioperative account of all procedures, measurements, and observations.

Swine that have undergone surgery should be individually housed postoperatively to prevent injuries inflicted by cage mates. Group housing of freshly operated swine is strongly discouraged due to the tendency for increased aggression during this period. This practice is well tolerated as long as pigs can see, hear, and smell other pigs in the same room. Once animals are completely recovered from all sequelae of surgery, it is appropriate to re-establish group housing.

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