Issues to Consider for Preparing Ferrets as Research Subjects in the Laboratory

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Abstract

The domestic or European ferret (*Mustela putorius furo*) has been domesticated for thousands of years. Ferrets have been used for hunting and fur production, as pets, and as models in biomedical research. Despite the relatively small numbers used in the laboratory, ferrets have some unique applications including study of human influenza and severe acute respiratory syndrome (SARS)-associated corona virus. They have served as models for peptic ulcer disease, carotenoid metabolism, cystic fibrosis, and drug emesis screening, among others. Most research ferrets are males, due to estrus-related health problems in females. They may be housed conventionally and are easy to care for when their biology and behavior are understood. Due to the small number of ferret suppliers, animals are often shipped long distances, requiring air transport and intermediate handlers. It is important to minimize shipment stress, especially with weanling and pregnant animals. Additional expertise is required for success with pregnant and whelping ferrets and for rearing of neonates. The animals have specific dietary requirements, and proper nutrition is key. Successful housing requires knowledge of ferret behaviors including social behavior, eating habits, a general inquisitive nature, and a species-typical need to burrow and hide. Regular handling is necessary to maintain well-being. A ferret health care program consists of physical examination, immunization, clinical pathology, and a working knowledge of common ferret diseases. Various research methodologies have been described, from basic procedures such as blood collection to major invasive survival surgery. Ferrets have a distinct niche in biomedical research and are hardy animals that thrive well in the laboratory.

Key Words: behavior; biology; ferret; husbandry; methodology; models; *Mustela*; reproduction

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Background Information

History, Taxonomy, and Uses of the Domestic Ferret

The domestic or European ferret (*Mustela putorius furo*) has been domesticated for thousands of years (Boyce et al. 2001). Thought to be a descendant of either the European or steppe polecat, the domestic ferret should not be confused with the indigenous black-footed ferret, *Mustela nigripes*, which is an endangered species (Cohn 1991). The ferret belongs to the order Carnivora, family Mustelidae, and is related to mink, otters, and weasels.

Uses of the ferret have varied. For hundreds of years the animals were used to hunt rabbits and other small mammals. "Fitch" ferrets, which are polecat-ferret crosses, were used for fur production (Bell 1999). The use of ferrets in biomedical research dates back to the early 1900s. More recently, beginning in the late 1970s, ferrets became popular as household pets, and it is estimated that there are currently several million pet ferrets in the United States (Boyce et al. 2001).

Ferrets as Animal Models in Biomedical Research

It is difficult to know exactly how many ferrets are used in biomedical research. According to the 2004 US Department of Agriculture (USDA[1]) Animal Care Report, a total of 1.1 million animals were used in biomedical research (APHIS 2004). Of these, 171,000 were reported in the "All Other Covered Species" category. It is likely that <10% of this number represents ferrets. Despite these relatively small numbers, ferrets have a distinct niche and some unique applications in research.

One of the earlier ferret models involved the study of human influenza, a disease to which ferrets are highly susceptible. To date, this model continues to be an important use of the ferret and includes work on pathogenesis, treatment, vaccine development, and investigation of Reye's syndrome (Maher and DeStefano 2004). The ferret is also

[1]Abbreviations used in this article: AWA, Animal Welfare Act; IATA, International Air Transport Association; SARS, severe acute respiratory syndrome; USDA, US Department of Agriculture.

used to study infection of the highly pathogenic avian H5N1 viruses (Zitzow et al. 2002).

It has been determined that ferrets are susceptible to another human respiratory pathogen, the severe acute respiratory syndrome (SARS[1])-associated corona virus (Martina et al. 2003). The first eruption of this disease affected thousands of people in Asia, causing SARS to be recognized as a serious new pathogen (Stadler et al. 2003). Ferrets have been utilized to study this disease and to investigate development of a vaccine (Weingartl et al. 2004).

Investigation of airway physiology and pathophysiology is another application of the ferret in respiratory disease research. There is remarkable similarity in lung physiology and morphology between ferrets and humans. As a result, ferrets are used as a model for the study of cystic fibrosis, including a ferret airway xenograft model to study gene therapy (Li et al. 2002).

In addition to the study of respiratory diseases, ferrets have been used as a model for human gastrointestinal disease. Most notable is the ferret model for peptic ulcer disease, which has resulted from the anatomical and physiological similarity of the ferret stomach to the human stomach, and the fact that both species possess a *Helicobacter* gastritis-causing organism (Fox et al. 1990; Whary and Fox 2004). Ferrets have been used to study the epidemiology of this disease as well as its pathogenesis and treatment (Patterson et al. 2003).

Ferrets have also been useful as a model for nutrition research due to their similarity to humans in carotenoid metabolism (Wang et al. 1992). Furthermore, ferrets have been used to study the influence of carotenoids on the development of certain cancers and cardiovascular disease (Lee et al. 1999; Raila et al. 2002).

In addition to many basic research applications, ferrets have also been investigated as a nonrodent test system in pharmaceutical drug development, especially as a surrogate for dogs when test material is in limited supply (Gad 2000). A ferret model for evaluating the emetic potential of drugs is another use of the ferret in the field of drug development (Crawford et al. 2002).

Finally, ferrets are used in cardiovascular research, including myocardial infarct models, in neural development and visual system studies, in skeletal research, for pediatric endotracheal intubation training, and for the investigation of renal disease secondary to toxin producing intestinal infection with *Escherichia coli* (Crawford et al. 2002). These uses of the ferret comprise a variety of applications that dictate necessary knowledge about selection, husbandry, health care, research methodologies, and possibly reproduction.

Research-related Ferret Selection Criteria and Biology

Ferret selection criteria may vary depending on the model system or research application (e.g., the purchase of timed pregnant females for visual system studies in neonates). Often, however, the only requirement is a healthy ferret within a particular weight range. It is advantageous to be familiar with ferret biology to facilitate decision making.

Sexually intact female ferrets are not often used in research. They breed seasonally, ovulation is induced, and females experience persistent estrus if induction of ovulation does not occur (Quesenberry and Carpenter 2004, Ch. 4). After several weeks of estrus, clinical signs of estrogen-induced bone marrow suppression may result. Ovariohysterectomy before sexual maturity is preventive; however, we at Marshall BioResources find that it is both common and expedient to simply choose males.

Whether or not age is a study-specific requirement, it is important for the researcher to be familiar with normal ferret growth in order to plan properly for housing needs. As depicted in Figure 1, growth is rapid, with sexually intact males weighing twice that of females by 24 wk of age.

Investigators sometimes request preshipment castration and/or "descenting" of male ferrets. Surgical removal of the scent glands, or anal gland sacculectomy, is invariably performed for the purpose of odor reduction (Mullen and Beeber 2000). Researchers should be aware that neither procedure, alone or in combination, will entirely eliminate the mustelid scent. Even neutered, descented ferrets produce a slight musky odor due to normal sebaceous secretions that can be attenuated through bathing.

The natural or "wild-type" color of the ferret is sable, sometimes also called fitch. It is the color pattern requested by most researchers, in contrast with the many coat color variations that abound in the pet industry. One research study, however, reported the use of albino ferrets as pseudopregnant recipients of embryos from cinnamon-colored parents (Li et al. 2003).

Individual identification of ferrets is not mandated by the USDA Animal Welfare Act (AWA[1]) regulations, but may be required by other oversight bodies or by the scientific protocol. As a small fur-bearing mammal, ferrets have a thick hair coat and small head and ears. These factors complicate not only identification but also procedures such as blood collection, as further described below in Methodology. Tattoos and neck collars are largely unsuitable compared with larger laboratory species such as the dog, cat, and rabbit. However, ear tags and microchips are reliable methods of identification, which can be applied or implanted before shipment.

Unless restricted by the research, it is recommended that ferrets be fully immunized against rabies and canine distemper virus. Breeding facilities routinely vaccinate, but if ferrets are purchased before the animals have reached the designated vaccination age, immunizations become the responsibility of the research institution.

It is appropriate to expect the breeding facility to provide a health history for each ferret. Because the vast majority of ferrets used in research are reared conventionally, histories should include birth dates, sex identification, immunization dates, surgery dates if applicable, and any medications administered before shipment.

Transportation

Once selection criteria have been established, arrangements must be made for transportation of the ferrets to the laboratory. The delivery of laboratory animals in a safe non-stressful manner is extremely important for their health and well-being and for the generation of scientifically valid research. Virtually all laboratory animals are subject to some degree of transportation stress, and studies have documented the resultant impact (Fox et al. 2002, Ch. 29). For this reason, optimal transportation conditions are extremely important and warrant careful consideration.

Animal transportation is regulated domestically by the AWA and by the states of origination and destination (AWA/AWR, USDA-APHIS 2002). European transport is regulated by ETS 193, the European Convention on the Protection of Animals during International Transport, and by the countries of origin, transit, and destination (Swallow et al. 2005). Globally, the Live Animal Regulations of the International Air Transport Association (IATA[1]) have been formally adopted by many countries including the United States. These regulations have also been accepted by the Convention on International Trade in Endangered Species of Wild Fauna and Flora, the Office International des Epizooties, and the European Union (IATA-LAR 2005). In addition, the Laboratory Animal Science Association recently formed a Transport Working Group with the goal of defining standards that ensure good practice and full compliance with national and international legislation governing laboratory animal transport (Swallow et al. 2005). This Working Group addressed all aspects of transport that may affect welfare including route plans, vehicle and container design, driver training, travel duration, and food and water supplies.

It is often necessary to transport ferrets long distances, including internationally, because the number of research ferret breeding operations is limited. In light of this situation, it becomes especially important to optimize transportation arrangements and ensure adequate quantities of feed, water, and bedding. Although the vendor is responsible for making appropriate arrangements, it is advisable for the researcher to be aware of the details of the transportation process.

Delivery Method

Ferrets are delivered by ground transportation, air, or a combination of the two. Ideally, ground transportation includes direct door-to-door delivery from the vendor to the research institution. This situation, however, is often confined to transport distances of several hundred miles or less. Cross-country or international shipments invariably include air transport carriers and intermediate handlers. Delivery from the vendor to a local airport typically takes place in a vendor-owned dedicated vehicle, but an intermediate handler may provide ground transport from the airport to the final destination. In this circumstance, it is especially beneficial for the receiving institution to be aware of the specifics of airport pick-up and delivery.

Occasional unanticipated delays are inevitable, but these instances can be minimized through effective communications among the vendor, the carriers and intermediate handlers, and the research facility. Timely awareness by the research facility of delays in the process will minimize any negative impact.

Transport Containers

The functional design elements of transport containers are regulated by the organizations described above. These design elements include the following: viewing ports, ventilation areas, feed and watering devices, structural components that promote hygiene, and proper labeling. Stocking densities are not specifically regulated by USDA and IATA for ferrets, but the animals must be able to turn around, lie down, and make normal postural adjustments.

Because young ferrets are social animals, it is preferable to ship them in groups under most circumstances. Exceptions are sexually intact adult or breeder male ferrets (Staton and Crowell-Davis 2003). In my experience, 5 to 6 wk of age should be considered the minimum age for shipment of young ferrets without the mother. Twenty-eight days of gestation, which is the end of the second trimester, should be considered the latest time-point for shipment of pregnant jills.

Shipment containers for conventionally reared ferrets should be lined with contact bedding and equipped with containers for feed and water. Wire mesh or vent openings must allow for sufficient exchange of air but not compromise safety or hygiene from spilled bedding and excrement. Animals must be readily visible, and containers must be injury and escape proof.

Regardless of container design, additional safeguards must be applied during very warm weather (e.g., > 85°F) due to the heat sensitivity of ferrets (Fox et al. 2002, Ch. 13). Precautions include reduction of stocking densities, provision of additional water sources, inclusion of gel packs, and possibly even delay of the shipment. Filtered containers designed for specific pathogen-free rodents are sometimes used for ferrets to prevent exposure to influenza virus while in transit. In warm weather, particular attention must be paid to ambient temperatures and stocking densities because of the reduced airflow within these containers.

Laboratory Husbandry and Care

Arrival and Acclimation

Regardless of the duration of transportation, it is imperative to assess the health and general well-being of the ferrets as soon as they arrive. This assessment is especially important for young or pregnant animals, during times of climatic extremes, or in the event of shipment delays. Early intervention is key to addressing potential complications such as dehydration and stress-related diarrhea or anorexia. An appropriate acclimation interval should reflect the particular research protocol as well as the age and status of the ferrets. Young adults are resilient, but weanling and pregnant ferrets require close attention during the first 1 to 2 wk after arrival.

For ferrets, a lethargic or dehydrated condition is a medical emergency. In such a condition, a balanced electrolyte solution may be administered subcutaneously. Fluid consumption may be encouraged by offering highly palatable oral electrolyte products such as Pedialyte®, or by using multiple methods of water supply such as a water bowl in addition to automatic watering. Most ferrets are raised on automatic watering systems, so specific acclimation should not be necessary except in the case of complications or undue stress in transit.

Use of a familiar diet will ease the transition, especially with young or pregnant ferrets. It is beneficial to offer a moist supplement in addition to a dry diet during the transition (Bell 1999). A thin gruel is made by adding warm water or milk replacer (e.g., kitten milk replacer) to the pelleted diet and offering it 2 to 3 times daily to encourage both food and fluid consumption.

Caging Systems

Questions regarding optimal caging systems for ferrets are common. In many circumstances, facilities desire to retrofit or adapt caging from other species for ferrets. Rabbit cages are most commonly adapted because the height and square footage are generally suitable. Rabbit and ferret cages may be different with respect to the grid size of enclosure surfaces, including walls and floors. To ensure the safety of weaned and older ferrets, grid openings for all surfaces should not exceed 1 inch square (Fox et al. 2002, Ch. 13). Preweanling ferrets require a grid that does not exceed ½ × 1 inch. This safeguard has prompted the retrofitting of some styles of rabbit caging.

Both suspended and solid flooring are suitable for ferrets. Because ferrets have a strong natural tendency to defecate in the back corners of the cage, suspended floors with 1 inch grids will facilitate passage of stools into the drop pan below. Cages with solid walls, especially those that are designed with a bevel along the bottom, will promote waste-build up and require frequent spot cleaning. Some solid bottom floors may be used if slippage can be avoided or minimized (e.g., using cage floor liners). In this circumstance, it is necessary to provide a litter box, which ferrets can be trained to use relatively easily (Quesenberry and Carpenter 2004, Ch. 1). Ferrets are curious and playful to the point of mischievousness, so it is important to ensure that the cage is escape proof.

Regardless of cage design, it is important to provide an area that accommodates a ferret's instinct for burrowing and hiding, which are examples of species-typical behavior, and its need for resting (Quesenberry and Carpenter 2004, Ch. 1). To accommodate these needs, it is necessary to provide an enclosure with a solid bottom and sides, with or without an overhead cover. Many options exist, including large dog bowls, 4- to 6-inch diameter polyvinyl chloride pipes, fabric ferret hammocks or sleeping bags, and ferret tunneling toys. Play toys may be provided, but due to the ferret's inquisitive behavior, it is imperative to scrutinize toys for safety and from potential ingestion.

As discussed above (see Arrival and Acclimation), automatic watering is optimal because most ferrets are already acclimated to this system, and mess and wastage are reduced. If bowls are used, they should be heavy crocks to prevent spillage. Feed may be offered via a J-feeder design. It is possible to minimize digging by providing a restricted access opening. If the feeder opens to the outside of the cage, the waist should be sufficiently narrow to preclude escape if the feeder is emptied. Open bowls may be used; however, their use inevitably results in digging and wastage. In addition, the use of a suspended floor design could result in inadvertent food restriction.

Social and Behavioral Needs

Although aggressive behaviors may sometimes be observed in group housing situations (Staton and Crowell-Davis 2003), ferrets in general are social animals and benefit from being in a group (Boyce et al. 2001). Ferrets should be housed in groups or in pairs, and solitary housing should be avoided. As mentioned above (see Transport Containers), exceptions to group housing are adult or breeder hobs (males) and whelping jills (females). Even nonbreeding sexually intact males may be housed together with some supervision. Although males have been observed grasping or biting each other by the nape of the neck, this is actually normal mating behavior and it should be tolerated if it is not excessive. If the animals develop lesions due to excessive biting, it may be necessary to separate them.

Social hierarchies exist, but usually not to the detriment of an individual animal. Ferrets sleep together in groups, so the rest area that is provided should be sufficient to accommodate all of the inhabitants of a given enclosure.

Properly trained care staff should handle ferrets regularly. This human contact will increase the ability of the animals to remain relaxed and comfortable during subsequent research procedures. Ferrets that are fed and watered daily but not handled regularly tend to become rambunctious and more easily frightened. Such fearful behavior may be evidenced by a vocalization best characterized as a screech. Chuckling or chortling sounds are more indicative of stimulation from play (Boyce et al. 2001).

Dietary Requirements

As with housing, knowledge of dietary requirements is an extremely important element of ferret husbandry. Ferrets have been described as obligate carnivores, and although their complete nutritional requirements are unknown, it is accepted that a high-protein, high-fat, low-fiber, meat-based diet is necessary to support good health (Bell 1999). Dog food is not suitable for ferrets due to the amount of cereal-based protein in most commercial diets. High-quality kitten food is acceptable, but there are also numerous commercially available ferret diets, including laboratory chows (Scipioni Ball 2002).

It is important to note that the ferret intestinal tract is short, with a rapid transit time and a relatively simple gut flora (Quesenberry and Carpenter 2004, Ch. 1). In addition, the energy requirements of ferrets are high and have been documented to range from 200 to 300 kcal per kg body weight daily (Fox 1998, Ch. 5). These factors underscore the need to select a palatable, high-quality diet. In addition, because ferrets may also resist dietary change, it is important to select an optimum diet at the outset, taking into account age, reproductive status, and experimental stresses.

Health Care

Immunization

An immunization program is an important aspect of preventive health care. Ferrets should be protected against canine distemper and rabies. A typical primary immunization protocol for weanling ferrets consists of canine distemper virus vaccination at approximately 6, 9, and 12 wk of age followed by annual booster immunization. Rabies vaccination takes place after 12 wk of age and is also followed by annual booster immunization (Quesenberry and Carpenter 2004, Ch. 2).

The safety and efficacy of commercial modified live canine distemper vaccines have been studied (Wimsatt et al. 2001). Although only a few vaccines are available, it is advisable to use only those that are licensed and approved for use in ferrets (Greenacre 2003). Presently, a modified live chick embryo vaccine (Fervac D, United Vaccines, Madison, WI) and a recombinant canary pox vectored vaccine (Purevax Ferret, Merial, Duluth, GA) are available. The first rabies vaccine approved for use in ferrets became available in 1990 (Niezgoda et al. 1997). Current recommendations regarding rabies prophylaxis in ferrets are described (Compendium 2006). Presently, there are two approved rabies vaccines available for use in ferrets (Imrab 3 and Imrab 3 TF, Merial, Duluth, GA).

One safety concern relative to immunization is the occurrence of anaphylactic reactions to one or both of the vaccines described above (Greenacre 2003; Moore et al. 2005). Potential sequelae include vomiting, diarrhea, hypersalivation, and collapse. For this reason, it is important to consider exposure potential and to maintain an available supply of emergency medicines including antihistamines, steroids, and epinephrine. Pretreatment with diphenhydramine has been used for ferrets that have demonstrated allergic signs to previous immunizations (Moore et al. 2005).

Physical Examination

In addition to immunization, the performance of routine physical examinations should be part of the health care program of any facility. The procedure with ferrets requires training in proper restraint methods and knowledge of baseline physiological parameters. The vital signs of these animals reflect their high metabolic rate. Normal body temperature ranges from 37.8 to 40ºC, normal heart rate is 200 to 400 bpm, and normal respiratory rate is 33 to 36/min (Fox et al. Ch 13, 2002). Published data are available regarding normal values for hematology, serum chemistry, and urinalysis (Fox 1998, Ch. 7; Morrisey and Ramer 1999).

During the physical examination, it is useful to be aware of several ferret behaviors that may confuse the assessment of an animal's state of health. For example, if ferrets are approached while sleeping, it often takes approximately 15 to 30 sec for them to become fully roused. To an inexperienced observer, it may initially appear that the ferret is severely depressed or even comatose. However, this behavior should be considered normal unless it persists. In addition, ferrets often express a shivering behavior that does not necessarily indicate ill health. Although the reason for this behavior is unknown, it may be observed when the ferret awakens from sleep or becomes excited. Finally, if a ferret is restrained by the scruff of the neck, it will often yawn as its body relaxes. Again, the significance of this particular behavioral response is unknown, but handlers should be aware that it is normal (Boyce et al. 2001).

Posterior paresis is a sign that is often interpreted as evidence of neurological disease. In ferrets, it is a presenting sign that may be observed in a variety of states of ill health. Ferrets normally maintain an arched position with their backs, typical of other mustelids such as weasels. When weak or lethargic, regardless of cause, ferrets will lose this arch causing the back to become parallel with the horizontal plane. This posture results in some degree of ataxia of the rear quarters and may be a nonspecific sign. However, it also may be due to central nervous system involvement from systemic illness such as Aleutian parvoviral disease (Quesenberry and Carpenter 2004, Ch. 11).

Common Diseases

Another integral part of the health care program is awareness of common ferret diseases. Various infectious and inflammatory diseases may be observed in ferrets in the laboratory. Most neoplastic diseases occur in older animals, but some are observed in young adults.

Ferrets are highly susceptible to canine distemper virus. This multisystem disease is virtually 100% fatal and necessitates immunoprophylaxis (Wimsatt et al. 2001). Ferrets are also highly susceptible to human influenza viruses, as described above (see Ferrets as Animal Models in Research). Signs may be similar to distemper but are less severe and with reduced mortality (Fox 1998, Ch. 15). Because of the zoonotic potential, care should be taken to protect workers and animals from influenza through use of appropriate protective clothing and practices such as vaccination (Maher and DeStefano 2004).

Enteric infectious disease in ferrets is commonly observed and may be either viral or bacterial. In neonates, rotavirus infection may cause diarrhea with significant morbidity and mortality if left untreated (Fox 1998, Ch. 15). These infections typically occur within the first month of life. Dehydration or secondary bacterial infections may complicate recovery. Coronavirus infection, also termed epizootic catarrhal enteritis or ECE, causes mucoid enteritis with a high level of morbidity and variable mortality. The disease tends to be mild or even subclinical in young kits, with older ferrets showing more severe clinical signs. Supportive care is the only treatment (Williams et al. 2000).

Aleutian parvoviral disease has been described as a wasting syndrome in ferrets. This disease should be included in the differential diagnosis of neurological disease with signs of ataxia, paresis, or paralysis (Palley et al. 1992; Welchman et al. 1993).

Helicobacter mustelae infection causes gastrointestinal disease of variable severity including subclinical, chronic, or acute gastritis, gastroduodenal ulceration, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma in ferrets (Erdman et al. 1997; Fox et al. 1990, 1997; Patterson et al. 2003). Clinical signs include inappetance, weight loss, tarry stools, severe deterioration, and acute death. Conventionally reared ferrets are presumptively *H. mustelae* positive, with disease becoming manifest as the result of stresses such as weaning, other illness, or experimental manipulations. Treatment includes use of amoxicillin, metronidazole, and bismuth subsalicylate, often referred to as "triple therapy" (Hillyer and Quesenberry 1997, Ch. 4).

Lawsonia intracellularis, previously described as *Desulfovibrio* spp., is the causative agent for a proliferative colitis syndrome in ferrets that is similar to syndromes observed in swine and hamsters (McOrist et al. 1995). It presents as a wasting disease with progressive weight loss and deterioration and has been noted to respond to chloramphenicol (Fox et al. 1994).

Otodectes cynotis, a common ectoparasite in ferrets, causes ear mite infestation. Treatments that have been described and compared include topical use of ivermectin in propylene glycol (Patterson and Kirchain 1999). Although infestations are usually subclinical, consequences such as otitis may occur.

Ferrets appear relatively resistant to helminth infestation; however, routine fecal examinations are useful to verify endoparasite status. Protozoa including Coccidia and *Giardia* have been described but are usually present subclinically (Fox 1998, Ch. 16). Ferrets are susceptible to heartworm infestation; however, preventive treatment with ivermectin is highly effective (Quesenberry and Carpenter 2004, Ch. 6).

Various neoplastic diseases may be observed in older ferrets and include adrenal cortical hyperplasia/neoplasia and insulinoma (Wagner et al. 2001; Weiss et al. 1998). Thymic lymphosarcoma occurs in young (usually < 1 yr of age) adult ferrets and has been proposed to have a viral etiology (Batchelder et al. 1996). Other neoplastic diseases have been described (Li et al. 1998).

Urinary tract diseases have also been observed and include cystitis and obstructive uropathy (Coleman et al. 1998; Li et al. 1996; Orcutt 2003). Splenomegaly is a relatively common finding in ferrets and may reflect extramedullary hematopoiesis, anesthetic administration, lymphoid hyperplasia, or lymphoma (Morrisey and Ramer 1999). Gastrointestinal obstruction from foreign bodies, including hairballs, has been described. Conservative treatment with laxatives may be curative; otherwise, surgery is indicated (Quesenberry and Carpenter 2004, Ch. 3).

A polymyositis syndrome has recently been noted in ferrets. Although there are no published studies in the scientific literature to date, various reports have characterized this disease by fever, leukocytosis, hind limb weakness, and death in ferrets less than 1 yr of age (personal communication, B.H. Williams, Department of Telemedicine, US Armed Forces Institute of Pathology, Washington, DC, 2006). As a proposed immune-mediated event, several potential triggers have been suggested including vaccination, viral disease, and genetic predisposition.

To reiterate, maintenance of an immunization protocol along with regular physical examinations that reflect knowledge of ferret biology, behavior, and disease are integral to optimal health care. For some institutions, it is also necessary to develop a breeding program in support of their research. As described above (see Research-related Ferret Selection Criteria and Biology), some investigators may require ferret neonates for studies such as visual system development. In this circumstance, they may purchase timed pregnant jills, although another alternative is to maintain in-house breeding. If the latter option is chosen, personnel must be knowledgeable and trained in all aspects of ferret breeding and neonatal care.

Reproduction

Breeding

Several aspects of ferret reproduction are unique. For example, it is well documented in the literature that a long day photoperiod is necessary for successful breeding, gestation, and lactation (Fox et al. 2002). A short day photoperiod suppresses onset of estrus in females and prevents development of breeding condition in males. When the light cycle is reversed to produce a long day photoperiod, breeding activities are initiated. Estrus is determined by marked vulvar swelling in females; in males, breeding condition is determined by enlargement and full descent of the testes into the scrotum.

Estrus jills may be induced to ovulate through breeding or by chemical methods using gonadotropin-releasing hormone or human chorionic gonadotropin. Ovulation is not reliably induced unless the jill has been in estrus for approximately 7 to 10 days, when the follicle responds (Fox 1998, Ch. 8). Successful ovulation is evidenced by a gradual decrease in vulvar swelling over a period of several days.

As mentioned above (see Research-related Ferret Selection Criteria and Biology), jills that remain in heat for longer than 3 to 4 wk develop risk for estrogen-induced bone marrow suppression. Clinically, loss of body condition and pale mucous membranes are noted. Clinicopathologically, leukopenia, anemia, and thrombocytopenia may be observed. The eventual mortality rate is significant and necessitates early intervention.

Care of Pregnant Ferrets

Whether ferrets are bred in-house or purchased as timed pregnant jills, researchers should be aware that pregnant ferrets are subject to pregnancy toxemia. The disease is characterized by anorexia, depression, ketosis, and fatty liver (Batchelder et al. 1999; Dalrymple 2004). This metabolic syndrome is a medical emergency, and mortality of both the jill and kits can occur. Anything that results in anorexia of the jill can precipitate this syndrome through development of a negative energy state, especially in late gestation or with a high fetal burden. In my experience when neonatal ferrets are required for research, pregnant jills are typically requested in late gestation. It is strongly recommended that these animals be received *before* day 28 of gestation to avoid undue stress.

It is also very important to ensure that the transition to a new environment is nonstressful. A change of diet is contraindicated during this period. The diet should be made available ad libitum; offering a wet mash supplement of the diet mixed with warm water to a soft consistency will encourage food and water consumption. This supplement may be offered twice daily and should be continued throughout lactation because it will also serve to initiate the weaning process for the kits. If pregnant jills are received at the end of the second trimester, they should be housed in a cage that is suitable for parturition and equipped with a bedded nest.

Whelping and Neonatal Care

The gestation period for ferrets is 41 to 42 days, with little variation. If parturition begins earlier than 40 or later than 42 days, the prognosis for a successful outcome is guarded. Little can be done to prevent early delivery, other than avoiding stresses and subsequent complications such as pregnancy toxemia. Surgical intervention should be considered if gestation reaches 43 days.

It is critical to have quiet surroundings during delivery, and the whelp nest should provide privacy for the jill. It is important to monitor parturition to prevent dystocia or entanglement of umbilical cords (Quesenberry and Carpenter 2004, Ch. 5). At the same time, monitoring should involve minimal disturbance to avoid causing the jill to leave the nest and, in extreme cases, to cannibalize her young.

Even once parturition is complete, it is best to continue to minimize disturbances for the next several days. The progress of the litter may be sufficiently ascertained without removing the jill from the nest or handling the kits. After several days, it is safe to perform a closer inspection that includes weight and sex determination of the young. Cage changeover and other noisy or disruptive activities should be planned accordingly.

Ferret jills have strong maternal instincts, which make cross-fostering a simple procedure even when delivery dates are not coincident. Cross-fostering is especially useful given the difficulty of hand rearing neonates. Lactating jills tend to be very protective of the kits, so care should be exercised in their handling. Lactation failure may occur but is usually secondary to problems such as inadequate food or water intake, stress, or illness. Mastitis may also occur and is usually caused by β-hemolytic *E. coli*. Systemic illness in both the jill and kits may occur. Antibiotics and supportive care are often required, and cross-fostering may be necessary (Marini et al. 2004). Vendors should provide only pregnant jills that have a history of successful delivery and lactation to minimize complications.

Weaning

Ferret kits are altricial at birth. They weigh only 8 to 10 g, and their eyes do not open until approximately 30 days of age (Quesenberry and Carpenter 2004, Ch. 5). Nevertheless, their growth is rapid, and ferrets weigh approximately 150 g by 4 wk of age. At this time, kits should be offered a moist supplement that is sufficiently diluted with water to achieve a soup-like consistency. This supplement will allow the young kits to begin eating solid food, which initiates weaning. After 4 to 5 wk of age, the supplement may be made with less moisture but should continue to be offered along with the dry diet for several weeks. Weaning is complete by 6 wk of age, when body weights are approximately 250 g. As stated above (see Ferret-related Research Selection Criteria and Biology), a significant divergence in body weight gain between sexually intact hobs and jills is observed later (Figure 1).

Research Methodologies

Handling and Restraint

Proper handling and restraint techniques are crucial not only for husbandry (see Social and Behavioral Needs) but also for the conduct of experimental procedures (Moody et al. 1985). Ferrets are used in a wide variety of research applications, so handling requirements may vary. For example, when ferrets are used in an acute surgical procedure, it may be necessary to handle them only for a preanesthetic physi-

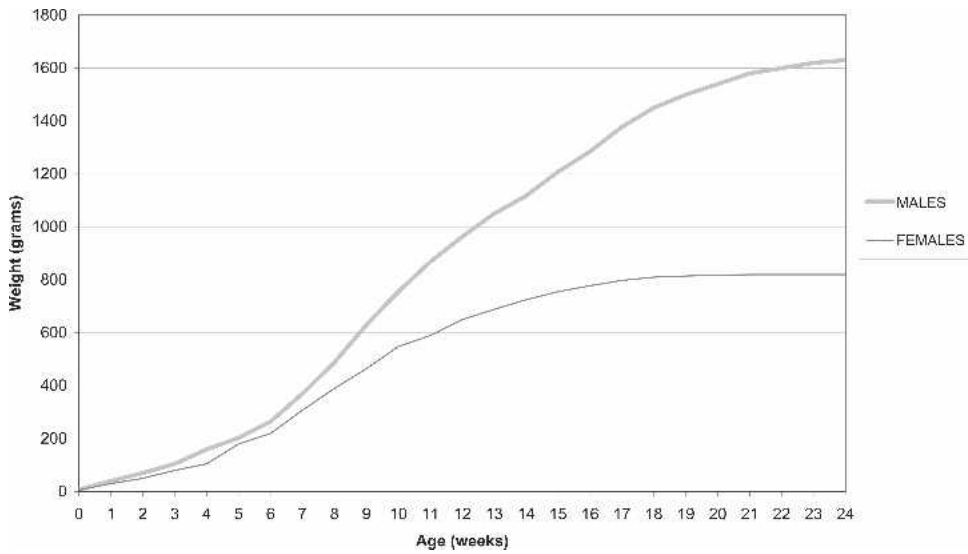

Figure 1 Ferret growth curve, obtained from Marshall BioResources, approximately 50 ferrets per age group.

cal examination and for injection of a sedative or anesthetic induction agent. For survival surgery, especially for major invasive procedures, extensive handling is required, which includes preoperative blood work and daily postoperative observations and treatments. Regardless, the key to successful nonstressful handling is acclimation of the ferret to whatever method of restraint will be required. This goal should be accomplished well in advance of initiation of the experimental procedure. Otherwise, especially with young ferrets, their rambunctious behavior may make handling difficult.

Ferrets are easily removed from their cage or other enclosure by being grasped by one hand around the thorax, although large males may require the use of both hands. For more complete restraint, the ferret may be grasped by the skin at the base of the neck, a procedure called scruffing. Ferrets may be retrieved from their cage in this manner or carried to a treatment table and then scruffed because the flat solid surface of the examination table facilitates grasping the ferret. As stated above (see Physical Examination), this method of restraint often produces a relaxation response that is accompanied by a yawn. It is useful for examination, oral dosing, or administration of injections. Another effective and nonstressful method of restraint involves tucking the ferret "head first" against one's waist using the crook of one's arm. Hugging the ferret in this manner appears to offer them security, which minimizes resistance. This method of restraint is particularly useful for taking a rectal temperature or giving an intramuscular injection (Scipioni Ball 2002).

Methodology

Even with optimal handling methods, peripheral vascular access is more difficult in ferrets compared with other large laboratory animals due to the short appendages, small ears, and thick hair coat of ferrets. Accessible blood vessels include cephalic, jugular, cranial vena cava, and lateral saphenous veins, as well as the ventral tail artery (Morrisey and Ramer 1999). When larger quantity sampling is required (greater than approximately 1 mL of whole blood), jugular or cranial vena cava sampling is preferred (Fox 1998, Ch. 19; Quesenberry and Carpenter 2004, Ch. 2). For small sample quantities or injections, including catheter placement, cephalic or lateral saphenous venipuncture is indicated (Hem et al. 1998; Morrisey and Ramer 1999). Access to these vessels may be achieved when the animal is either sedated or conscious. Surgical placement of vascular access ports in the jugular vein and femoral artery has also been described (Etheridge et al. 2002).

Anesthesia and Euthanasia

As outlined above (see Ferrets as Animal Models in Biomedical Research), ferrets are used in a variety of research applications that require anesthesia for surgery or other experimental procedures. Research includes cardiovascular, respiratory, and skeletal studies, among others. Ferrets have been described as hardy animals that make excellent surgical candidates (Mullen and Beeber 2000). Isoflurane anesthesia is often preferred, but numerous sedative and anesthetic protocols, both injectable and inhalation, have been reported (Fox 1998, Ch. 19; Mullen and Beeber 2000; Quesenberry and Carpenter 2004, Ch. 33).

Perioperative considerations for ferrets are similar to those of other laboratory species, albeit with some exceptions. Because of ferret gastrointestinal physiology (see Dietary Requirements), the preoperative fast interval should be short (~ 3 hr), especially for compromised subjects. Due to their small body size, pediatric endotracheal tubes are needed as well as a nonrebreathing system for inhalational anesthesia. Intraoperative monitoring (e.g., pulse oximetry and capnography) has been described (Olin et al. 1997). Postperative analgesics should be used preemptively and routinely, and a description of analgesic options is available (Fox 1998, Ch. 19). Techniques such as epidural narcotic administration have been assessed (Sladky et al. 2000). Ferrets are prone to hypothermia, especially during prolonged procedures. Use of warmed fluids and circulating warm water heating pads help prevent loss of body temperature (Fox 1998, Ch. 19).

Humane euthanasia may be achieved by using injectable sedation or anesthesia followed by barbiturate euthanasia. The 2000 Report of the American Veterinary Medical Association Panel on Euthanasia (AVMA 2001) should be utilized for guidance on appropriate and humane methods of euthanasia.

Conclusion

The domestic ferret has been used in biomedical research since the early 1900s. Although the number of animals used may be small compared with other laboratory animal species, they occupy a distinct niche in research. Ferrets are used to study the pathogenesis and treatment of a variety of important human diseases, including influenza, SARS, peptic ulcer disease, and cystic fibrosis to name only a few.

Despite the possibility that researchers may be less familiar with ferrets than with other laboratory species, it is important for investigators to realize that ferrets may be selected, purchased, and transported in a manner similar to other animals. Caging systems are readily accessible either commercially or through modification of pre-existing equipment. Knowledge of ferret behavior will guide the use of social housing and environmental enrichment devices that encourage species-typical behaviors. High-quality ferret diets are commercially available and satisfy the unique nutritional needs of ferrets, including young or pregnant animals. As with other conventionally reared species, a good health care program is composed of an immunization protocol, routine health assessments, and knowledge of common ferret diseases. If breeding the animals is required for the research, all aspects of ferret breeding may be successfully accomplished in the laboratory with sufficient training in the particulars of ferret reproductive biology.

Successful use of the ferret in the laboratory also requires familiarity with their unique behaviors, training in proper methods of restraint, and a regular routine of handling. Acclimation to research procedures is crucial to minimize stress and promote well-being.

Ferrets are friendly, curious, and playful. They have distinct behaviors and a unique biology. Efforts to prepare ferrets as research subjects in the laboratory are successfully achieved only through in-depth study. Such study ensures a thorough understanding of the needs of these interesting animals.

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