US Fish and Wildlife Service Black-footed Ferret Managed Care Operations Manual (BFFMCOM)

January 16, 2017

Adopted as the official

Black-footed Ferret Species Survival Plan® Animal Care Manual

PREFACE

This manual represents a compilation of information regarding the management of black-footed ferrets (*Mustela nigripes;* hereafter, BFFs). As BFF management is a dynamic process, an annual addendum for this document will be developed to address any new issues and concerns. Any association by any party with BFF captive breeding may only be conducted pursuant to an endangered species permit issued by US Fish and Wildlife Service (Service).

PURPOSE OF THIS BFF MANAGED CARE OPERATIONS MANUAL (BFFMCOM)

This document provides guidance to parties holding current Service permits to work with BFFs during captive operations involving husbandry and breeding efforts. The BFFMCOM and ongoing consultations with the Service are intended to address specific coordination needs between the Service BFF Recovery Program and permit holders, but both of these efforts remain subject to more general permit revisions.

ACKNOWLEDGEMENTS

This document was drafted by Joanne Luyster (retired, Louisville Zoological Garden) at the request of the Service based on her extensive knowledge and experience with captive BFF husbandry and breeding. The content of this final document (dated January 16, 2017, but likely to be periodically revised) is solely a product of the Service's BFF Recovery Program for which no other parties are responsible. The BFFMCOM benefited greatly from review by and contributions from many partners actively involved in BFF recovery, especially participating institutions of the Association of Zoos and Aquariums (AZA) Species Survival Plan (SSP) program. Photographs are from many unaccredited, but much appreciated BFF Partners. The cover photo is by Angie Cox.

COORDINATION WITH THE SERVICE

At the present time the primary Service contact for all BFF captive-related activities is Robyn Bortner. Questions regarding techniques and procedures described in the BFFMCOM, permit requirements, and general BFF captive activities should be directed to Ms. Bortner at robyn_bortner@fws.gov or 970-897-2730 x226 (office).

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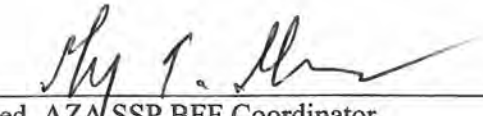
Approved, Service BFF Recovery Coordinator



Adopted, AZA SSP BFF Coordinator

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NATURAL HISTORY OF THE BLACK-FOOTED FERRET

INTRODUCTION

The black-footed ferret (BFF), one of the most endangered mammal species in North America, stands at a crucial point in its survival. Efficiently adapted for life as a predator in prairie dog towns, BFFs were an integral component of an extensive prairie/steppe ecosystem for tens of thousands of years. The BFF's small size, nocturnal and fossorial (underground) habits, and low density, resulted in it being one of the last mammal species identified in the continental United States (Gilbert 1980). However, the BFF's obligate relationship to prairie dog species spelled near disaster for the BFF when prairie dog eradication programs and conversion of native prairie habitats to cropland reduced prairie dog populations by 98–99 % (Miller et al. 1996). Vulnerable yet resilient, BFFs are now conserved as a captive population supporting reintroduction to the wild.

TAXONOMY

The BFF, *Mustela nigripes* (Audubon and Bachman, 1851), has no recognized subspecies and is the only ferret native to North America. BFFs evolved 30,000 – 45,000 years ago from a race of the Siberian or steppe ferret, *Mustela eversmanni berengii,* that crossed the land bridge connecting Asia and North America about 100,000 years ago (Anderson, et al. 1986). The Siberian ferret, or polecat, evolved from European ancestors and still exists today, as does the European ferret, or polecat, *Mustela putorius.* (The domestic ferret is the subspecies *Mustela putorius furo).*

The earliest known ferret, Stromer's polecat (*Mustela stromeri*), arose about a million years ago during the middle Pleistocene epoch (Anderson, et al 1986). Ancestral ferrets preyed on ground-dwelling rodents of grasslands, and may have used burrows to help survive the ice ages. The Mustelid family, to which ferrets belong, evolved about 25 million years ago as forest hunters. The mustelid family also includes weasels, mink, badgers, wolverines, and otters.

PHYSICAL CHARACTERISTICS

BFFs have long, thin bodies and short legs. Their size is similar to other ferrets; nose to tail tip length of adult males is around 50 centimeters (cm) (20 inches (in)); females average 45 cm (18 in). The hind foot is 6–7 cm (2.5 in) long. The 17-vertebra tail is the same for both sexes, 13.5 cm (5 in) long. Wild males average 1035 grams (g) (2.3 pounds (lb)) and females 710 g (1.6 lb) (Miller, et al 1996). Because of their size, shape, and musculature, a BFF's strength is disproportionately high compared to its body mass. Articulation of the vertebra allows the BFF extreme flexibility. Even a large male can turn around in a tunnel with a diameter of 10 cm (4 in). BFFs possess anal glands that, when expressed, emit a noxious odor.

BFFs have a blunt head with a short, broad skull (Hillman and Clark 1980). Their eyes are wide set and they sport rounded ears approximately 3 cm (1 in) long. A sagittal crest on the skull anchors massive jaw muscles. Their 34 teeth consist of incisors (3/3), canines (1/1), premolars (3/3), and molars (1/2). Each pair of carnassial (shearing) teeth consists of the last upper premolar and the first lower molar.

BFFs' short pelage (1 cm (0.3 in) is buckskin with dark guard hairs dorsally, and white to cream ventrally. Their legs, nose, and tail tip are black, and their face is embellished with a dark, expressive mask. Their ear margins and muzzle are white. Many white vibrissae (whiskers) grow from the muzzle, and two or three bristly hairs also sprout from the elbow. BFFs' coat colors do not change seasonally.

Size is the principal sexual dimorphism. Males have a wide black midline over the penile sheath; females have a narrow ventral midline in the same spot that extends to the perineum. Additionally, males possess a baculum, or penile bone. Females have six to eight mammary glands, though some variation in numbers has been noted.

A BFF's vision covers a broad arc and is especially sensitive to movement (Miller, et al 1996). A tapetum lucidum allows night vision and gives the BFF its distinctive, intense emerald-green eyeshine. While a BFF's hearing is acute, its sense of smell is probably its most developed faculty.



ONTOGENY & LIFE CYCLE

Deep within a prairie dog burrow in May or June, a litter of one to eight (average is three) altricial (helpless) kits are born to a solitary female. Typically three or four kits per litter survive to leave the nest (Hillman and Clark 1980). At birth each neonate weighs only 5–10 g (1/6 to 1/3 ounce (oz)) and has a coat of fine white hair on a long pink body. Kits are born with eyes closed, toothless, unable to thermo-regulate, and capable only of uttering little cheeps and using their forelegs to reach a teat. Individuals in small litters may claim a particular teat (Miller et al. 1996). For the first 5 weeks the dam consumes the kits' urine and feces, constantly grooming them and herself with her soft tongue.

Kits grow fast and at 2 weeks have deciduous canine and premolar teeth. Pigmentation starts in week 3, and at 4 weeks deciduous carnassials (molar teeth) help them eat solid food. In week, 5 the ears rise away from the head and the eyes open. At 6 weeks, kits are walking and are being weaned. Kits appear above ground at about 2 months. During the third month, permanent

teeth replace deciduous teeth. The time from 60–90 days of age is a critical period for developing sensory preferences; kits are exposed to prey that they will kill and eat for the rest of their lives. At this age kits accompany their mothers on forays and at 3 months are attacking live prey. By 15 weeks, females reach 95% of their adult weight, but males do not do so until 18 weeks of age (Vargas and Anderson 1996).

Males venture out on their own before females, and by September they may be visiting other prairie dog colonies. At 5 months, by October, all are independent, dispersing in search of a home of their own. Females tend to stay near their natal burrow, while males wander farther afield. If they can secure a territory by winter, they have a good chance of surviving until spring. (Miller et al 1996). In March, males begin searching for females in estrus. Seven years is the average life expectancy in captivity, but it is believed that BFFs seldom survive more than 3 years in the wild.



BEHAVIOR

BFF breeding encounters usually start with ano-gential sniffing and sniffing the substrate. A ‘chuckling’ sound indicates a friendly encounter. Agonistic (combative) encounters usually involve hair rising on the body, tail flaring, and back arching. Vocalizations include hissing, barking, chatter-barking, and shrieking. On offense, females typically arch, lunge, bite at the neck or flank, and then roll over the antagonist; or they might just back into him to displace him. In defense, females rise to meet the attacker, roll over onto their back, bicycle-kick, scratch, bite, and release musk stored in their scent glands.

Males, more than females, scent-mark their territory by defecating, urinating, pelvic rubbing, or anal dragging on the ground. Males also mark territory with their sebaceous glands surrounding the prepuce by straddling rocks and bushes. These activities increase in the breeding season.

When BFFs are hunting, they travel at night in a zigzag pattern, typically covering 1.4 kilometers (km) (1 mile (mi)). Opportunistic juveniles apparently learn the efficiency of going from burrow to burrow in search of prairie dogs. BFFs kill smaller prey by delivering pithing bites to the head, neck, or back; they kill adult prairie dogs in their burrows by suffocation from a throat bite.

RANGE & HABITAT

BFFs historically ranged across the North American Plains from southern Canada to the southern United States and northern Mexico. Their range coincided with the three major species of prairie dogs *(Cynomys*) that occupied the short-grass prairies of the Great Plains as well as the inter-montane basins of the eastern Rocky Mountains and mid-grass prairies. Their range was so vast that some estimates put a maximum prairie dog population at 5 billion and BFF population at 500,000—1,000,000 individuals.

BFFs are solitary, nocturnal, semi-fossorial, specialized hunters who live in nearly obligate association with prairie dogs, keystone species of the western prairies and steppes. The prairie dog ecosystem supports a variety of plant and animal species, such as swift fox, burrowing owl, and mountain plover.

The BFFs' diet primarily consists of prairie dogs, but they also eat deer mice (*Peromyscus*), voles (*Lagurus* and *Microtus*), and cottontails (*Sylvilagus*). BFFs hunt at night, when it is easier to avoid predators and find prairie dogs asleep in their burrows. BFFs seldom kill prairie dogs above ground where they are at a decided disadvantage since their prey can outweigh them. When attacking a prairie dog, they typically bite the throat and suffocate it, perhaps using the wall of the burrow for support and traction. Lacking the ability to store much fat on their body, they cache uneaten food. An adult may kill more than 100 prairie dogs annually. They have never been observed drinking water in the wild; however, they may secure fluids from their prey.

Individuals stake out home ranges that they defend from members of their own gender. Female home ranges seem to be prey-density-dependent, covering enough territory to include about 900 prairie dogs. Male home ranges seem to be female dependent, large enough to include several females.

BFFs use prairie dog burrows for shelter, breeding, and raising their young. Multi-chambered burrows are typically selected and provide BFFs with shelter from the wind, the cold of winter, and the heat of summer. Underground shelter is essential because the BFF's body shape and short hair are poor protection. Climate on the short-grass prairie is dry, but a BFF can conserve moisture in a burrow. Temperatures in the burrows stay between 4–13degrees Celsius (°C) (39–56^{o} Fahrenheit (F)); the humidity is usually higher than above ground. Burrows also protect BFFs from predators such as coyotes, swift fox, great horned owls, badgers, golden eagles, and ferruginous hawks.

THREATS TO SURVIVAL

The disease sylvatic plague is the greatest threat to BFF survival. It is an exotic, introduced disease which kills both prairie dogs and BFFs. Two lesser threats are the loss of prairie dog habitat and the control of prairie dogs. The BFF's specialized dependence on prairie dog colonies for food and shelter made them extremely vulnerable to these issues. Prairie dog habitat has been reduced to only 1–2 % of its former range. Small prairie dog towns limit BFF population size and the large distances between fragmented habitats inhibit BFF dispersal.

Other natural threats to BFF survival include predators and diseases such as canine distemper, which is 100% fatal in BFFs. Finally, potential reintroduction sites are often complicated by political resistance and cautious ranchers, who fear that once an endangered species is located on their property, restrictions will be placed upon the use of their land.

STATUS IN THE WILD

During the first half of the 20th century, prairie dog eradication by poisoning and conversion of prairies to cultivation decimated BFF populations. Only 70 BFFs were observed between 1943 and 1959. In 1964, wildlife biologists discovered a small group of BFFs in Mellette County South Dakota. Nine BFFs from this group were captured and taken to the U.S. Fish and Wildlife Service's (Service) Patuxent Wildlife Research Center in Maryland for a captive breeding attempt before the group disappeared in the wild. An effort to protect the BFFs against canine distemper proved disastrous. Biologists used the modified live canine distemper vaccine that provided safe and effective immunization in Siberian ferrets. It was the only vaccine available to protect against distemper. However, four of the six BFFs vaccinated died as a result. Although some births did occur among the captive BFFs, the Patuxent population dwindled, and the last remaining specimen died in 1971. No other wild BFFs were documented in the 1970's.

In 1966, the BFF was placed on the Service's Endangered Species list. Wildlife biologists thought the species might be extinct. However, on a September night in 1981, a ranch dog killed an adult male BFF near Meeteetse, Wyoming. The Wyoming Game and Fish

Department (WGFD) was notified and became the lead agency in the recovery program. WGFD managed the BFF population in the wild for several years. Their numbers improved and the population grew from 57 animals in 1982 to 129 in 1984; however, in 1985, only 38 individuals were observed; none born in the previous year. The prairie dog population around Meeteetse was dying out from sylvatic plague. Trapping for captive breeding began in the winter of 1985, and the last BFF, "Scarface," was captured in February 1987. A total of 18 animals were rescued from the wild, and although 15 of the 18 bred, only seven successfully reproduced and served as founders for the current world population of BFFs.

In 1991, Shirley Basin, WY became the first reintroduction site for BFFs. Although the first year survival rate of only 10% was anticipated, biologists were pleased to discover two wild born litters the following spring.

There have been 28 (2016) specific BFF reintroduction projects with varying success since 1991. Reintroductions sites include: Shirley Basin, Wyoming, 1991; Badlands National Park, South Dakota, 1994; UL Bend National Wildlife Refuge, Montana, 1994; Conata Basin, South Dakota, 1996; Aubrey Valley, Arizona, 1996; Ft. Belknap Indian Reservation, Montana, 1997; Coyote Basin, Utah, 1999; Cheyenne River Indian Reservation, South Dakota, 2000; Wolf Creek, Colorado, 2001; BLM 40-complex, Montana, 2001; Janos, Mexico, 2001; Rosebud Indian Reservation, South Dakota, 2004; Lower Brule Indian Reservation, South Dakota, 2006; Wind Cave National Park, South Dakota, 2007; Espee Ranch, Arizona, 2007; Logan County, Kansas, 2007; Northern Cheyenne Indian Reservation, Montana, 2008; Vermejo Park Ranch, New Mexico, 2008; Grasslands National Park, Saskatchewan, Canada, 2009; Vermejo Park Ranch, New Mexico, 2012; Walker Ranch, Colorado, 2013; Soapstone Complex, Colorado, 2014; North Holly Complex, Colorado, 2014; Liberty Complex, Colorado, 2014; Rocky Mountain Arsenal National Wildlife Refuge, Colorado, 2015; Crow Indian Reservation, Montana, 2015; and Meeteetse, Wyoming, 2016.

It is difficult to estimate the BFF population in the wild. The BFF Recovery Plan calls for 3,000 individuals in populations of 30 or more adults, with at least 10 populations of 100 plus, and at least one population occurring in at least 9 of 12 states within the species' historical range in order to meet delisting goals. Monitoring of populations reintroduced to date is difficult and irregular, but there are likely several hundred individuals at a portion of these 28 (2016) sites located in eight western states, Mexico, and Canada. Importantly, BFFs have a high reproductive rate and even small populations can expand rapidly to fill adequately managed habitat where disease management is in place.

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MANAGEMENT OF INDOOR FACILITIES

QUICK FACTS

- **BLACK-FOOTED FERRETS (BFFS) ARE HOUSED IN QUARANTINE FACILITIES**
- **BFFS ARE HOUSED INDIVIDUALLY (EXCEPT MOTHERS WITH KITS)**
- **ENCLOSURES SHOULD INCLUDE A TUNNEL AND NEST BOX (BURROW)**
- **NEVER RESTRAIN ADULT BFFS BY HAND UNLESS UNDER ANESTHESIA; HANDLE BFFS IN RESTRAINT CAGE**
- **BFFS ARE HIGHLY SUSCEPTABLE TO CANINE DISTEMPER**
- **ROOM TEMPERATURES SHOULD BE KEPT BETWEEN 15.55°-23.58° C (60°-75° F)**
- **HIGH INTENSITY OR LED SUPPLEMENTAL LIGHTING SHOULD MIMIC BFF'S NATURAL LIGHT CYCLE**
- **FEED WHOLE CARCASS DIET 1-2 TIMES A WEEK TO MAINTAIN ORAL HEALTH AND MIRROR WILD DIET (PRAIRIE DOG)**
- **VISIUALLY INSPECT BFFS DAILY**
- **MAINTAIN REGULAR SCHEDULE OF WEIGHING BFFS TO MONITOR BODY CONDITION**
- **PERFORM ROUTINE FECAL EXAMINATIONS TO TRACK INTESTINAL ISSUES (COCCIDIOSIS, CRYPTOSPORIDIOSIS, ETC.)**
- **ALPHA-DRI® IS THE PREFERRED BEDDING MATERIAL FOR BFFS**
- **BFFS ARE SENSITIVE TO DISTURBANCE, ESPECIALLY DURING BREEDING SEASON**

MANAGEMENT OF BLACK-FOOTED FERRETS IN INDOOR FACILITIES

INTRODUCTION

Ex situ management of black-footed ferrets (BFFs) varies according to the participating institution, its available resources, geographic location, and in-house policies and procedures. The specifications described below incorporate aspects of a number of current breeding facilities (primarily those located at zoos) and offer a broad overview of housing and husbandry methods. The U.S. Fish and Wildlife Service's (Service) National Black-footed Ferret Conservation Center (NBFFCC) protocols and procedures utilized at the National Center are included as Addendum A**.**

HOUSING REQUIREMENTS

Present BFF ex situ breeding facilities utilize either prefabricated aluminum pole barns with concrete floors and drywall interior walls or modified existing buildings. Such buildings include a locker room and, in some instances, shower-in facility, a kitchen for diet preparation, laundry facilities, a room or area for video monitoring, office space for record keeping, and a separate, distinct room or rooms that house BFFs. In the BFF room, high intensity fluorescent or LED supplemental lighting on a timer ensures that BFFs cycle correctly. Enclosure design incorporates the unique needs of the BFF. Heating and cooling systems maintain temperatures between 15.55–23.58^{o}C (60–75ºF) year-round. Ideally, a viewing window allows visitors, such as media, to view the BFFs and/or video monitors of BFFs in their enclosures from an area outside of this quarantine room. SCBI, which has a unique set up, is described in **Addendum B**.

QUARANTINE FACILITY

Per Service and Species Survival Plan (SSP) recommendations, all breeding facilities maintain a quarantine protocol for BFF buildings. Anyone entering the building must remove their shoes and put on shoe covers, or use a footbath. Surgical masks must be worn in rooms housing BFFs. Other specific protocol required before entering rooms housing BFFs varies between breeding facilities. Some facilities require everyone to change into designated clothes and shoes and wash hands. Other facilities also require everyone to shower and wash his/her hair. These procedures are designed to protect BFFs from contagious diseases, principally canine distemper virus and human influenza.

ENCLOSURES

The most common type of BFF enclosure is an elevated freestanding enclosure supported by 1 meter (m) (3 feet (ft)) high legs, with two distinctly separate sections each measuring 1 m deep x 1.2 m deep x 0.6 m high (3 ft deep x 4 ft wide x 2 ft high) with tubes that attach to floor level nest boxes (see discussion below). The sections are divided by sealed plywood, often with a pass-through connecting the two sections. Enclosures are constructed of 1.3–1.9 centimeter (cm) (0.5–0.75 inch (in)) sealed plywood and 1.3 x 2.5 cm (0.5–1 in) vinyl coated mesh wire. The plywood surfaces are either painted or treated with epoxy, urethane, or fiberglass resin. Enclosure furniture consists of lengths of 10 cm (4 in) diameter corrugated black plastic tubing, and enrichment items. Food is placed in the "upper" enclosure, (either in a bowl or on the surface) and water is provided via heavy crock bowls or rabbit-size water bottles.

New enclosure designs utilized by some facilities are made of aluminum supports, heavy gauge stainless steel wire and molded plastic floors (see photos of enclosures below).

Enclosures at Louisville Zoo

Enclosures at NBFFCC

(If a facility is exploring designing new enclosures other than what is provided here, the facility should contact BFF SSP and the Service for approval before beginning any construction.)

NEST BOXES

Nest boxes are connected to the bottom of the upper enclosure via a length of 10 cm (4 in) diameter corrugated black plastic tubing. There is an 8–10 cm (3–4 in) hole in the bottom of the upper enclosure and in the side of the nest box to allow the BFF access. The BFF can be closed into either the upper section or the nest box with the use of sliders. [**Note**: while some facilities use metal sliders, it is recommended that opaque Plexiglas or HDPE sliders be used to prevent tooth breakage with BFFs that are chronic slider biters.] The nest boxes are roughly 41 cm deep x 41 cm high x 61 cm wide (16 in deep x 16 in high x 24 in wide) and are comprised of 1.3–1.9 cm (0.5–0.75 in) plywood that is either painted or coated with an epoxy, fiberglass resin,

or polyurethane finish. Some facilities use stainless steel nest boxes with removable molded plastic inserts, which are easily removed for cleaning.

The nest box is divided into two sections of roughly equal size, ostensibly a den side and a latrine side, that connect via an 8–10 cm (3–4 in) pass-through hole. The top of each compartment is hinged and secured with a latch to allow easy access to the nest box. Nest boxes contain ventilation holes to keep them cool and to allow ammonia fumes from urine to escape. Some facilities increase ventilation by incorporating a second hole in the outside of the nest box that houses a small computer fan with a protective metal mesh cover. In some cases, the top of the nest box contains an opening to mount a camera (and infrared light source if needed) on the nest box to monitor breeding and whelping activities. The holes are covered with Plexiglas® or wire mesh to prevent animal access to the camera or light source. This camera connects via a coaxial cable to a monitor and a recorder in another area or room.

Several facilities also elevate the lower nest box off the floor slightly, either by attaching it to the legs of the upper enclosure or by installing 1 inch "feet" on the bottom of the nest boxes. Being off the floor allows better regulation of the temperature within the nest box and BFFs can more easily maintain their body temperature. In addition, it facilitates floor cleaning; when floors are hosed, nest boxes do not stand in water. Also, if a nest box leaks, excess urine can drain freely from the box.

Some facilities offer BFFs an upper nest box attached to the enclosure. This second nest box is especially advantageous during kit season for dams with large litters.

Elevated nest box

Nest box den side


Nest boxes connected to upper enclosures

HOUSING ARRANGEMENTS

Since BFFs are solitary animals, all BFFs are housed singly, except when paired for breeding and when a dam has kits. The upper two separate enclosure sections are divided as described above. Breeding facilities often house a female on one side and a male on the other. If possible, institutions will try to house a female's primary choice breeding male opposite that female to allow them to become accustomed to the presence of each other.

CLEANING PROCEDURES

Enclosures are cleaned every morning. (NBFFCC cleans BFF enclosures every other day: half the animals (two rooms) one day and the other half the next). When cleaning enclosures, all solid waste material should be removed with a clean scraper or cloth, and the floor of the enclosure wiped down daily with a virucide (e.g., chlorhexadine) mixture that is applied with a spray bottle. Dry enclosures with either a cloth or paper towels. **[NOTE: to reduce chances of spreading infectious disease and internal parasites, use one scraper, scrubby, and cloth per animal; do not use from enclosure to enclosure.]** Some facilities run all scrapers and sponges through dishwasher daily for disinfection. Also clean, disinfect, and rinse enclosure furniture and enrichment items that are contaminated with food, feces, or urine.

Remove feces and dirty bedding from the nest box with a metal scraper or scoop and, as needed, disinfect nest box with virucide solution. Dry nest box with paper towels or designated cloth for that enclosure. Place clean bedding in the nest box. The recommended type of bedding is Alpha-Dri®. Some facilities use a different type of bedding (e.g. shredded paper) in addition to the Alpha Dri® as enrichment. Even if a BFF does not urinate or defecate in its bedding, strip and disinfect nest box routinely.

Check tunnels and remove food and feces accumulation by shaking, as needed. Maintain a regular cleaning and disinfecting schedule (e.g., once a month) for tunnel sections. When cleaning tunnel sections, replace the dirty tunnel with a spare clean tunnel section, giving the BFF access to all areas of its enclosure, while allowing ample time to soak the dirty section. To clean dirty tunnels, immerse them completely in a trough of virucidal disinfectant and soak for at

least 20 minutes, and then agitate to help remove debris. Rinse tunnels well by hosing them and store to dry.

Clean and disinfect water and food bowls daily. If BFFs use water bottles, clean, disinfect, and change water once a week.

Sweep, mop, and disinfect floors in the BFF room on a regular basis, (once a week or as needed). The mop and other cleaning utensils used in the BFF room should **not** be used for cleaning the rest of the building. Maintain separate cleaning supplies for the quarantine and non-quarantine areas.

FEEDING REGIMES

Always wash hands before and after handling BFF diet. Some facilities require that gloves be worn when handling any meat products or rodents. BFFs receive a diet of Toronto Zoo Small Carnivore Mix Diet (prepared by Milliken Meat Products). The amount given to each BFF varies slightly depending on the animal's size and age. The amount of diet fed each BFF is calculated so that all diet should be consumed daily. **Any BFF that fails to eat 100 % of its diet warrants attention.** Adult BFFs are fed once a day late in the afternoon to mimic their natural nocturnal behavior. This also affords keepers an opportunity to observe an animal's appetite and behavior a second time each day. BFFs may also be fed small rats once or twice a week in place of Small Carnivore diet. Likewise, most facilities offer live hamsters or mice weekly for nutritional and behavioral reasons. Protocols for husbandry and care of a quarantine hamster colony are included as **Addendum C**.

When available, kits 50–90 days of age should also receive processed prairie dog on a schedule of 50 grams (1.75 ounces (oz)) of prairie dog per BFF, three times a week. This is extended for the release candidates until they leave for pre-conditioning pens. All release kits will be fed prairie dog during pre-conditioning. When available, the NBFFCC may supply frozen processed prairie dog to SSP captive-breeding facilities, contingent on the facilities' regulations and restrictions (with regards to the change in prairie dog quarantine procedures). [See Body Condition Chart **Addendum D**]

ORAL MEDICATION

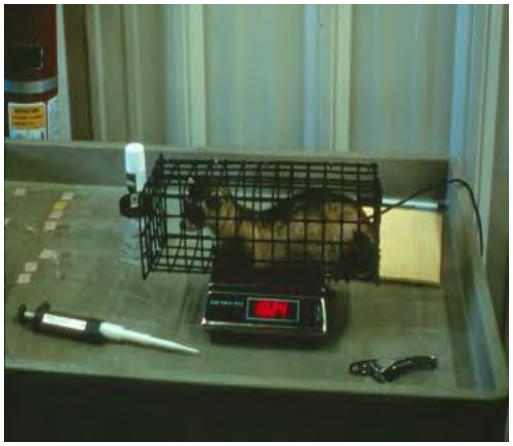
The least stressful way to administer oral medication to a BFF is to place the medication in the animal's diet. To insure all medication is consumed, place the medication in a small meatball of diet mixture and offer the medicated meatball before giving the BFF the remainder of its diet. If the BFF is not eating well, the medication can be injected into a pinkie mouse and offered before feeding time. If the BFF is completely anorexic, catch it in a restraint enclosure and place the medication directly into its mouth. With litters, it may be necessary to catch animals individually in a restraint for medicating.

RESTRAINT

When restraining BFFs, all institutions use vinyl coated wire mesh restraint enclosures that the animal can be secured in after being removed from its nest box. BFFs are placed in restraint enclosures for medication, weighing, medical examination of teeth, wounds, sedation, vaginal flushes, and testicular evaluations during breeding season.

BFFs are quick and are good at escaping, especially when they are being transferred from enclosure to restraint device. After scouting the area, an escaped BFF will naturally seek out a small, dark, closed space in which to hide. Therefore, to catch a loose animal, keep a 0.6–1 m (2–3 ft) piece of corrugated black plastic tubing on the floor, and in most cases the BFF will run into it. It is also advisable to keep an open empty nest box in a corner of the quarantine room, as this is another location in which the animal may hide.

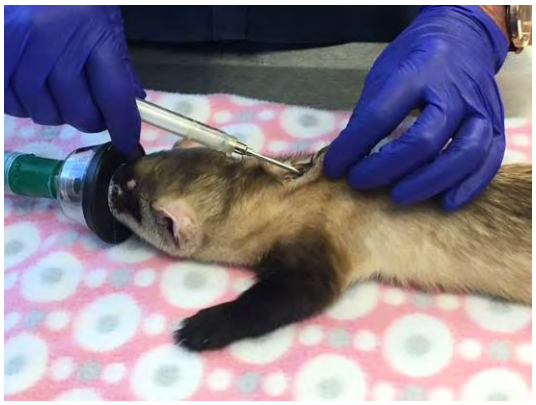
Never restrain adult BFFs by hand unless they are under sedation. BFFs experience high stress if handled and become hyperthermic, possibly to the point of death (exertion myopathy).



IDENTIFICATION

The BFF SSP studbook keeper assigns each animal a studbook number at birth. Studbook information also includes date of birth, sex, sire, dam, birth location, present location, name, and local ID number. Most facilities assign each BFF an in-house number, such as the Species360 or ZIMS number system. Furthermore, individual identification of each BFF is accomplished by the use of AVID® brand passive microchip transponders that are inserted under the skin in the scruff between the BFF's shoulder blades. Since this neck region is prone to biting trauma, especially in females during breeding, some of the glass transponders break or malfunction, and may need to be replaced prior to release. Within litters, many facilities shave a patch of fur in a distinct location on each kit to distinguish same sex littermates from each other, especially in the event of cross-fostering. Alternative methods include applying Nyanzol-D® dye, permanent, non-toxic markers to kits to differentiate them from one another.

Transponder chip



Inserting transponder chip

RECORD KEEPING

Record keeping is done at all facilities on a daily basis with each institution having its own form for recording data. Information such as type of diet fed, amount consumed, medications given, weight, etc. are recorded. Medical and census information is recorded on a form at each institution so that information can be entered into the ZIMS computer program. Individual sheets should be kept for each adult BFF. In addition, maintain separate forms for recording pertinent breeding information, as well as kit information [see examples of forms in **Addendum E**]. The Service and/or SSP will ask for particular information from facilities throughout the year and will provide data forms when needed.

BREEDING SEASON

If the facility does not use an astral timer, begin in December to advance the light cycle per schedule:

Dec 21: 10 hours Light: 14 hours Dark

Jan 21: 11 hours Light: 13 hours Dark

Feb 21: 12 hours Light: 12 hours Dark

Mar 21: 13 hours Light: 11 hours Dark

Apr 21: 14 hours Light: 10 hours Dark until last litter is 30 days old

- All facilities should have a light meter and staff should check light readings in the enclosures to ensure that each enclosure is receiving adequate lighting. Readings should be a minimum light reading of 50 foot-candles.
- Before breeding season starts, thoroughly clean all enclosures and tubing. Make sure all upper nest boxes are clean and ready for use. Check BFFs weight in the fall, and if overweight, begin weight loss program by reducing diet; monitor until individual is at ideal weight parameters.

- In December or January, pre-breeding physical exams should be performed by the veterinarian to correct any problems (e.g. sub-clinical tooth problems).
- Around the first of the year, weekly monitoring of BFFs for breeding development should be initiated. Males will exhibit testicular enlargement and increased testicular firmness.
- Females will show signs of vulvar swelling. Once a female's vulva begins to swell, start vaginal flushing for cytological examination. As swelling increases and cytology indicates the female is nearing breeding readiness, perform vaginal flushing more often.
- Ideally all males, but especially yearling males, should be electro-ejaculated to check for the presence of viable sperm.
- When an SSP designated breeding pair reaches breeding readiness, either take the female to the male's enclosure, or the male to the female's enclosure (depending on the facility's determined protocol) and record breeding activity.
- After a successful breeding, flush the female within 30 minutes after copulation to check for the presence of sperm.
- Following a successful sperm check, leave a compatible breeding pair together for two to three days, but monitor their behavior. Separate the pair once they start squabbling [see **Reproduction** chapter].
- As whelping time nears, attach nest box fans (if applicable) to lower nest boxes and to accustom the female to fan noise.
- Replace metal or Plexiglas sliders with wire sliders in end of nest box so air can easily pass out of nest box.

CARE OF PREGNANT FEMALES

Care of pregnant females follows protocols for non-pregnant females in all areas except two. First, pregnant females have special nutritional needs. Specifically, they require more digestible protein (DP) and a higher caloric intake. Most breeding facilities accommodate this elevated need by steadily increasing the standard diet serving size by 25% every other week as the female progresses through gestation. Ideally, the female should be consuming approximately 1.5–2 times the normal amount of diet by the time she reaches her due date. Some breeding facilities accommodate this increased nutritional need by supplementing the diet with a more natural food source such as 50 g (1.75 oz) of hamster, mice, rats, and/or prairie dog. Care should be taken to ensure that the female does not get excessively overweight, which can interfere with lactation. However, a female should not be weighed or otherwise handled after her follow-up cytology indicates pregnancy, as this will elevate stress levels. A visual assessment of the female's body condition is usually sufficient.

Secondly, **noise and activity should be minimized around females both in late gestation and when with new kits**, as reduced stress will minimize neonate fatalities. However, several facilities have found that "white noise," such as that generated by exhaust computer fans, may aid in reducing extraneous noises and associated stress levels in dams. Whelping and Kit Care are covered under a separate chapter of this manual.

TRANSPORTING BFFS

When transporting BFFs between facilities, ensure that animals are transported under quarantine conditions as similar as possible to those at breeding facilities. Keep BFFs in a cool environment as they overheat quickly. For complete information, see the **Transport Protocol** chapter in this manual. Transport of BFFs is coordinated by the Service. No BFFs may be transferred without the Service's approval.

PEST CONTROL

Pest control varies from facility to facility depending on local concerns. All efforts are taken to keep vermin and other animals away from BFFs by using either fencing or traps. Raccoons and dogs are of particular concern since they carry canine distemper. All refuse should be disposed of in a timely manner.

GENERAL MANAGEMENT ISSUES

- ✓ Visually check all BFFs daily, (twice if possible—at cleaning and at feeding).
- ✓ Maintain a regular schedule for weighing BFFs (once a month during non-breeding season).
- ✓ Perform routine fecal examinations on a regular basis, e.g. every 1 to 2 months.
- ✓ Keep the facility in as clean a condition as staffing allows. Ideally, conduct an annual thorough cleaning of the facility, especially the quarantine area.
- ✓ Participate in SSP conference calls as scheduled by the Service.
- ✓ Attend the annual BFF SSP Working Meeting and bring formalized annual report. If unable to attend, provide information to the Service or SSP Coordinator prior to the meeting.

PITFALLS AND PROBLEMS ENCOUNTERED IN MANAGEMENT OF BFFS

ITEM	PROBLEM
Use of aspen shavings as bedding	Dental issues caused by pieces lodged between teeth, perforations of oral cavity or intestines
Gristle in meat diet	Can become lodged in roof of mouth especially in kits and prevent nursing
Use of wire mesh between enclosures	Injuries from neighboring BFFs

Wire enclosures (outdoor facilities)	Be aware that BFFs do climb wire and, if stressed or upset, may fall and injure themselves
Dirt pens (outdoor facilities)	BFFs dig in dirt and digging may cause tunnels to cave-in, trapping or crushing the BFF
Retained litters	Dams who pass whelp date and do not regain their appetite may require x-raying to ensure they do not have retained (dead) kits

PRODUCT INFORMATION

Alpha-dri®	Shepherd Specialty Papers, Inc., Kalamazoo, MI 49005
Astronomic timer	Dayton Electric Manufacturing Co., 5959 West Howard St., Chicago, IL 60648

Toronto Carnivore Diet Milliken Meat Products, Scarborough, Ontario, (416) 299-9600

Nyanzol-D® dye	J. Belmar Inc., P.O. Box 145, 200 Sutton St., Room 210, N. Andover, MA 01845 (508) 683-8726
Video equipment	Fuhrman Diversified, Inc., 905 South Eighth St., LaPorte, TX 77571
Vinyl coated wire	Valentine Equipment Co., 4259 South Western Blvd., Chicago, IL 60609-2276
LED lights	Philips T8 32w replacement bulbs. 17w LED, 48 inch, Medium Bi-Pin Base, compatible with ballasts. 2100 lumens/4000k.
SCBI LED lights	Sorra Premium LED PAR 38 3000K 18.5 w; multiple lights per pen
FCC enclosures	Lab Products, Inc. 742 Sussex Ave. Seaford, DE 19973 800-526-0469 http://labproductsinc.com/
Phoenix enclosures	Kornegay Fabrication LLC. www.kornegayfabrication.com

HDPE sliders [same as enclosure manufacturer]

ADDENDUM A

NBFFCC INDOOR FACILITY MANAGEMENTPROTOCOLS

The U.S. Fish and Wildlife Service's (Service) National Black-footed Ferret Conservation Center (NBFFCC) is unique among black-footed ferret (BFF) breeding facilities in that BFFs are its sole focus. Consequently, it houses a large portion of the BFF captive population. Four indoor breeding rooms house 42 individual enclosures each for a total of 168 holding spaces. In general, NBFFCC manages for full capacity heading into a new breeding season. Most protocols specific to NBFFCC are geared toward managing the large population of BFFs in the most efficient way possible, while still paying close attention to animal welfare.

MAIN BREEDING BUILDING PROTOCOL

Any employee may enter the breeding building entryway in normal clothes and shoes. If a person goes beyond the locked doors they must wear shoe covers or change into "indoor only" designated shoes to be in the main hallway.

The locker room is the only area where indoor and outdoor shoes and clothes should mix. Indoor shoes stay on the rack by the hall door south of the line. Outdoor shoes stay near the changing rooms north of the line.

If a person is going to work in a BFF room they must have on "indoor" clothes and shoes. Gloves are provided for staff to use when cleaning BFF enclosures and associated items.

If a person wearing indoor clothes must go into the breeding building entryway, they must change shoes/shoe covers. Personnel should change into outdoor clothes if they need to be outside for extended periods.

During breeding season, non-husbandry employees are discouraged from accessing the breeding building. Tours are also discouraged. Talking in the breeding rooms should be kept to a minimum and not above a whisper. Talking loudly in the areas of the building outside the breeding rooms should be avoided.

Masks must be worn at all times when in direct contact with BFFs, especially litters or other sensitive individuals. Any person possibly exposed to or suffering from influenza is NOT ALLOWED to have any contact with a BFF. Staff exhibiting flu-like symptoms, especially a fever, should stay at home.

The holding building is considered "outside." Outdoor clothes and shoes worn in pens can be worn when working with BFFs in holding. When holding contains animals that have been outside for a length of time, employees should assume plague may be present and wear a surgical mask.

Anyone accessing Prairie Dog Holding while prairie dogs are present **must** shower out if they are planning on going beyond the keypad doors in the Breeding building. BFF rooms should not be entered on the same day after having been in prairie dog holding even if you showered out, unless extenuating circumstances require it.

DAILY SCHEDULE

- AM Walk-Through
- Clean BFF Enclosures
- Feed Indoor BFFs
- Check (or Clean) Hamsters
- PM Walk-Through
- Clean & Feed BFFs in Holding
- Feed Outdoor BFFs
- Clean/Feed Prairie Dogs in Prairie Dog Building

The purpose of the AM walk-through in the main breeding rooms is to check that each BFF ate all of its diet from the day before and scan for any obvious problems. Check the surface of each enclosure for left-over food. Check the nest box of any animal receiving special diets, medical treatment, or other special attention. Inform the breeding manager or veterinarian immediately of any BFFs leaving diet.

The purpose of the PM walk-through in the main breeding rooms is to ensure all BFF enclosures are properly secured and that duties in BFF rooms are finished for the evening. Sliders should be in the up position so animals have access to the entire enclosure, and door and nest box lids should be latched.

CLEANING OF BFF ENCLOSURES

Upper nest box (insert): If bedding material is soiled in the upper nest box, dump all bedding intro trash bucket. Wash rag(s) soaked in dilute chlorhexadine should be used to remove all feces and/or soiled bedding that stick to the insert. Clean Alpha-dri® (1/2 scoop-to mark on small red coffee can) should be placed in upper nest box. Also, make sure the pass-through leading from the upper nest box to cage floor pan is wiped clean.

Lower nest box (inserts): Removal of soiled bedding material and food items is identical to upper nest box insert cleaning. Treat each half of the lower nest box individually—if bedding is at all soiled on either side, remove all bedding on that side and replace with new. All feces, urine and food items should be wiped from the insert with chlorhexadine soaked wash rag(s). One and a half scoops of clean Alpha-dri® should be placed on each side of the lower nest box. Also, make sure the pass-through leading from the lower nest box to the tunnel and the O-ring connecting the two lower nest box inserts are wiped clean. The two lower nest boxes must be securely connected to each other via the O-ring. The insert on the tunnel side of the box does not have to fit snugly to the metal connector. The same wash rag used to clean upper nest box insert

can be used to clean lower nest box inserts, if it is not overly soiled. Change soiled wash rags as often as needed. Alpha-dri® bedding amounts can be adjusted to accommodate BFFs that are particularly dirty (add more) or drag it up the tunnel to the cage floor (add less).

For particularly soiled inserts, plastic scrapers can be used to remove dried-on fecal material, or the inserts can be switched out for clean ones and the dirty ones soaked in dilute bleach in the sink or stock tank.

Latches: All spring-loaded latches should be pulled back in order to open and close nest box lids. Nest box lids should not be forced shut.

Cage floor pan: Clean wash rag(s) soaked in dilute chlorhexadine should be used to pick up fecal material and urine (including dried urine). Cage floor pan as well as sides of pan and metal lip should be wiped down. Use a fresh rag if they soil quickly (more than one rag per cage can be used if needed). Use the spray bottle (dilute chlorhexadine) to apply more cleaning solution to heavily soiled areas. If animals are known to defecate or urinate in the same location on the cage floor pan, place some loose Alpha-dri® (about a handful) in these areas. Any fecal material on the metal cage walls should be wiped clean.

Hammocks: Check hammocks for fecal material and/or urine. Most BFFs do not defecate in their hammocks, but if they do, remove the hammock and replace with a clean hammock. Remove carabineers from the dirty hammock before placing hammock in the back hallway stock tank for soaking.

Sliders: Before closing slider to clean lower nest box, make sure BFF is entirely up the tunnel; mind the whiskers. When finished cleaning, sliders should be fully pulled up until the spring-loaded support pops back into place. The upper nest box slider should remain in the fully open position unless otherwise instructed (pre-whelping, kits, pairing).

Tunnels and elbows: Tunnels and elbows will be replaced when soiled. If animals are known to defecate and/or urinate in their tunnel/elbows these items need to be replaced with clean items. Dirty tunnels and elbows should soak in dilute (3%) bleach solution in the back hallway stock tank. Both elbow-tunnel rubber suspenders (for upper and lower elbows) should be attached in order to prevent escapes and to extend the life of the cage material. Heavy vinyl gloves are provided for use when using bleach solution.

Room floor: The room should be swept on cleaning days. All stains (urine, feces, medicine, and blood) should be wiped or mopped clean.

Water bottles/dishes: Water bottles should be filled if they fall below the half-way mark. Water dishes should be cleaned and filled completely. Use 0.473 liter (l) (1 pint (pt)) water bottles between cages; 0.946 l (2 pt) water bottles can be placed on end cages.

Enrichment: All cages get a 15 centimeter (cm) (6 inch (in)) boomer ball, hammock, and sometimes a tunnel or Nylabone®. Additional enrichment items may be added at the supervisor's discretion. No item is added without approval due to safety concerns.

FEEDING

Feeding can be very complicated. BFF's staple food is a ground horsemeat diet from Canada, Toronto Small Carnivore Diet, which we refer to as "Toronto or TOR." BFFs also get whole carcass rations (usually rat) twice a week. Animals should be observed during feeding for body condition and general appearance. A healthy BFF will not leave any food if it is given the appropriately sized ration. If leftovers are seen, the animal should be visually observed, and the vet and supervisor notified. Most animals that are fed food daily on the top of their cage will come up for food. If changes in who comes up are observed, the BFF should be visually inspected (if a BFF that comes up every day at feeding time suddenly does not, open box and verify it's alive.)

Indoor BFFs receive:

- ¾ scoop Toronto for females, 1 flat full scoop for males daily
- Twice weekly rat/hamster/or prairie dog on the day prior to when the cage is cleaned), 60 grams (g) (2 ounces (oz) for females, 80 g (3 oz) for males

Some BFFs are picky about what they will eat or have specific medical reasons for needing a specialized diet. These animals are on "Special diets" and will have a YELLOW FLAG on their cage. Their current diet will be listed on the small white board in the prep room. Some get rat, hamsters, or combos. When feeding a whole carcass, the ration should be placed in the lower nest box instead of on the cage floor.

All animals on special diets need to have their lower nest box checked for leftovers at AM walk-through. Additionally, it is imperative that any animals given a whole carcass have any leftovers removed at the AM walk-through, unless their cage is being cleaned that day. Leftovers of any kind will rot and can cause BFF mortality.

During breeding season, paired animals will have two index cards on the cage and a BLUE flag. They need rations for both animals. A cage containing a BFF paired with a special diet animal will receive double rations of the special diet.

Pregnant females will have an index card indicating the dates their food gets increased and by how much. They will get an ORANGE flag once they are on increased rations.

The night prior to their due date, females will start getting a half scoop, which will be indicated on their index card. This will continue until they are considered "Did Not Whelp" or until they have completely eaten their half scoop after whelping.

Females with litters will have a color wheel on a clipboard attached to the bottom of their cage. Once it is confirmed they have eaten the half scoop, their ration will be increased by a half scoop every time it is confirmed they have eaten the entire last ration. Increase rations only on full-cleaning days. Look to the color wheel to determine how much to feed. **The flags on the litter cages relate to their age, not their feeding schedule.**

ADDENDUM B

SCBI FACILITY DESCRIPTION

Smithsonian's Conservation Biology Institute (SCBI) consists of a "hybrid" facility, utilizing and incorporating both indoor and outdoor facility aspects. A description of the facility follows:

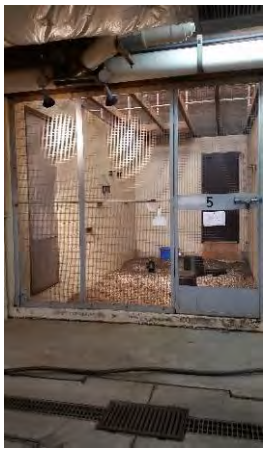
The SCBI facility is comprised of two separate housing areas for BFFs. Both groups are housed under isolation conditions and are off limits to visitation, unless authorized by a supervisor.

The indoor wing has 24 total enclosures, 12 per side, separated by a central corridor. The outdoor wing has 12 enclosures located side-by-side and serviced by a single, outside alleyway. The top of each outdoor enclosure is covered to prevent entry of rain or snow. Each enclosure contains a nest box and several lengths of corrugated black plastic tubing serving as artificial burrows). Each enclosure is labeled with the animal's name, studbook number, accession number, sex, transponder number, and date of birth.

In the outdoor wing, each nest box sits on a heating pad, which is thermostatically-controlled, providing heat when the external temperature decreases below 40ºF. Heating in the indoor wing is thermostatically-controlled by a computer. A rise or fall in temperature causes the computer to send an alert to the maintenance staff. Indoor wing temperature in the winter should be maintained at 18–20 degrees Celsius (°C) (64–68° Fahrenheit (F)); indoor wing temperature in the summer should be 20–22°C (68–72°F). Indoor wing temperature should not exceed 23°C (74°F).

Within the complex, there is an office area, food preparation area, restroom, and a storage closet available to the BFF keepers. The facility also includes a second indoor wing that houses birds and an adjacent storage closet.

BFFs are highly susceptible to stress (including noise, disturbance, and high temperatures). Unnecessary noise (music radios, loud speaking, and heavy equipment) are avoided, including posting signs on the perimeter of the facility to avoid unnecessary disturbance to the outdoor wing.



Indoor Enclosure

Indoor service area

Outdoor enclosure

Outdoor service area

ADDENDUM C

HAMSTER PROTOCOL

CLEANING

- Alpha dri® in large breeding / housing bins are to be stripped and bins cleaned weekly.
- Alpha dri® in small weaning bins are to be stripped and bins cleaned only after young are weaned from females at 21 days of age.
- Water and feed are to be checked daily and replenished as needed.
- Water bottles are disinfected weekly.
- Service hamsters at the end of the day after the keeper is finished with BFFs.

BREEDING AND FEEDING

- Place five female hamsters (more than 21 days old) in each of three large breeding bins.
- When females are 10 weeks old, place two males in each breeding bin.
- After 10 days, check females for bulging bellies and remove females suspected to be pregnant; house these females individually in small weaning bins. Check remaining females daily after 10 days until all females have been impregnated and placed in small weaning bins.
- Feed male hamsters to BFFs after all female hamsters have been impregnated.
- When young are 21 days old, wean:
    - Save 15 juvenile females to be used as replacement breeder females. Place five each into three breeding bins. Select juveniles from several litters.
    - Save six juvenile males to be used as replacement breeder males. Place them in a breeding bin. Select males from different litters than females.
    - House remaining juveniles (up to 10 animals) in large feeder bins. These are to be used as feeder animals. The ideal size for hamsters fed to BFFs is around 6 weeks old and 50 grams (g) (1.75 ounces (oz)).
    - When juveniles are separated from females, feed adult females immediately. Adult females will fight if housed together, even for short periods of time.
    - Breed females in breeding bins as per steps 2–5.

MISCELLANEOUS

- Feed hamsters to BFFs at least once weekly.
- All diseased hamsters should be removed. Dead hamsters should be removed and necropsied.
- Replenish breeding stock periodically.

NBFFCC HAMSTER CARE PROTOCOLS

A hamster colony is maintained at the National Black-footed Ferret Conservation Center NBFFCC in order to supplement the standard diet of both adults and kits. Typically, if animals are sick, they are provided a live or euthanized hamster. Generally, BFFs will consume the head of the hamster quite readily. Feeding of live hamsters to kits is dictated by litter size and age of kits (as well as availability of hamsters).

Daily Care

- Hamster chow is kept in trash cans located either in the storage bay or in the hamster room in the Breeding Building.
- Lights are set to turn off in the evening and turn on prior to the workday. Timing should not be adjusted, as hamsters need to be on a summer light schedule to stimulate breeding. The hamster light schedule is 14 hours Light/10 hours Dark year-round, unless otherwise noted.
- On temperate days, windows can be opened, but ensure they are closed at the end of the day! Also, if windows are left open, it should be only slightly, as the wind tends to blow bedding around.
- The exhaust fan should be turned on as needed; typically only during weekly cleaning. The exhaust fan should be turned off at end of day.

Large Bins

- Top-off water bottles daily (ensure all water bottle holders have solid bottoms to minimize escape).
- Food should be provided *ad libitum*. Usually 1 scoop is adequate.
- Clean corners as necessary and replace soiled pine shavings bedding with clean bedding. Replace with the approximate amount removed. Bedding is stored in trash cans either in the hamster room, main hallway, or storage room. Gloves are provided for use during all cleaning procedures.
- Provide pups with elevated platforms (small water bowl upside down) so they can reach water bottles. Make sure platforms are under water bottle nipples and that water bottle nipples are within reach of pups.
- Young pups are allowed 1-2 huts, but these should be removed when they get big enough to reach the water bottle without a stool. Place huts away from the edges of the bin and water bottles, otherwise they will crawl over the side to neighboring bins.
- All empty bins, adjacent to those that are occupied, need to have food and water bottle(s).
- Pregnancy check females (all bins need to be checked if sexes are not separated)

Maternity Bins

- Check for pups, if present, write date of birth and circle on tape.
- Top-off water bottles. Expectant females are currently on the rack in the SE corner of the room. They should receive a small glass water bottle with a short nipple.
- Feed (large pellets) as needed.

- Feed small (a handful is adequate) pellets when pups begin walking or as required. Bins being fed small pellets are currently placed on the rack on the western wall. When the bin is transferred to this rack, they should receive a large glass water bottle and long nipple. If the nipple is too long, bedding will get soaked, but if it is too short, the pups may not be able to reach and quickly become dehydrated. Adjust nipple length as necessary but always make sure pups can reach the water!

Cleaning of bins (During weekly cleaning or as bins are emptied)

- Remove soiled bedding and all leftover food from large bins.
- Clean interior of bins using rags/sponges with dilute chlorhexadine. Pay particular attention to corners where hamsters urinate.
- Once bin is dry, provide clean bedding material.
- Add a handful of shredded paper to all bins. Cardboard dowels and small boxes are also provided when available for enrichment.
- Remove bedding from empty maternity bins and immediately wash with soap and water using a coarse green scrubby. Scrub entire bin to eliminate pinworm eggs. Leave in bin cleaning area to dry. Do not let dirty bins sit in hallway overnight.
- Replace all plastic and glass water bottles as needed. Dirty bottles/nipples should soak overnight in a 10 % bleach solution and be cleaned and rinsed the next afternoon.

Weaning

- Wean young hamsters when large enough (mouse-sized) and eating on own (3–4 weeks old). Eyes should be completely open.
- Place young hamsters in large bins (maximum of 30 hamsters/bin), keeping track of date of birth.
- Smaller hamsters may need a "stool" (upside-down water bowl) to reach water bottles.
- Provide huts, enrichment, and large and small pelleted food for their first feeding in the bin.
- Make sure water bottle nozzles are turned downward so that pups can reach them.
- Place dam in feeder bin, or leave in small breeding tub to reduce fighting until processing/feeding out.
- Separate males from females as soon as sex can be determined, keeping track of date of birth. This is critical!

Breeding

- There should always be about 15 males in the male breeder bin. Males are used multiple times for breeding (move old/scarred males to feeder bin as necessary).
- Place 12-15 females (about same age) in breeding bin, and label tape as follows:
  Number of females_____ Date to be bred_____
  Number of males_____ Date to pregnancy check_____
- Date to be bred is at 8 weeks or older, when they are large enough to breed.

- On ‘date to be bred’, place 3–4 large males from male bin into female bin, and check off date to be bred on tape. Write in number of males in bin on tape.
- Stagger breeding events to avoid all pups being born at once.
- In October, increase breeding to begin stockpiling for the next kit season. Approximately 1,500–2,000 hamsters are needed in the chest freezer by June 1.
- In May, begin filling all bins with pups in anticipation of feeding live hamsters to BFF kits. See hamster feeding schedules for different litter sizes and ages.

Pregnancy Checks

- Begin pregnancy checks about 10 days after breeding (gestation is 14 days).
- When hamster is clearly pregnant, place her in maternity bin half full of wood shavings, record date on tape.
- Give water (small glass bottle), food (large pellets), and shredded paper to nest in.
- Check daily for pups.
- If no pups are born within 14–16 days, or female eats pups, place her in feeder bin.
- Males should be returned to male breeder bin (unless scarred; see above)

Processing

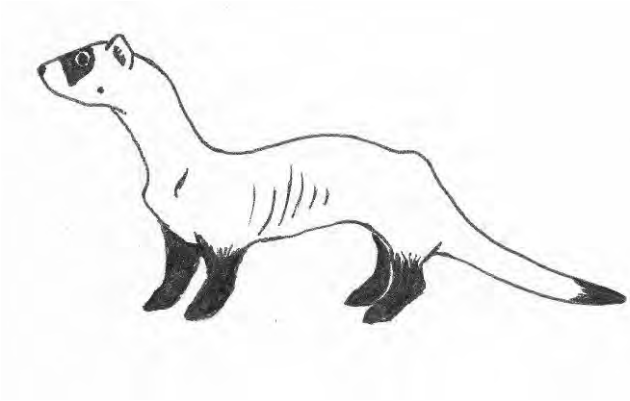
- During the fall, winter, and summer months it is necessary to stockpile processed hamsters in anticipation of the upcoming kit season. (Approximately 1,500–2,000 are needed by June 1).
- Only process hamsters if there are enough adult-sized hamsters for feeding for the week.
- Hamsters to be processed should be of adult size (80–100 g/2.8–3.5 oz)
- Process the oldest hamsters first (except those you plan to feed out).
- Process one bin of hamsters at a time; do not leave several half-full bins.
- Water bottles and food should not be removed from hamster bins prior to processing.
- Processing of hamsters will take place in the Food Prep room only.
- Animals should be euthanized using cervical dislocation or CO_2. Only euthanize the equivalent of one layer of hamsters in the chamber at a time (usually about 20, no more than 24).
- Use clean surgical scissors. Wear gloves when processing hamsters.
- To use CO_2, place the hamsters in the CO_2 chamber, attach the hose, and turn the CO_2 on. A CO_2 regulator must be used and should be set to slowly trickle gas into the chamber. It usually takes about 10 minutes for the hamsters to stop breathing. Remove them promptly after the last one stops breathing.
- Check for a heartbeat before making the first incision by placing your hand on the chest. If you feel a heartbeat, place back in chamber and turn on CO_2 for 2–3 more minutes.
- Pinch the skin of the belly and pull up making a midline incision from the neck to the pubis, being sure not to cut any portion of the intestinal tract.
- Cut open the thorax (ribcage).
- Use two fingers to grasp the stomach and pull back from the liver. Cut the esophagus just below the diaphragm between the stomach and liver without nicking the liver.

- Pull intestinal tract up and remove the stomach and intestines. Cut as close to the rectum and anus as possible.
- Dispose of the stomach and intestines in the trash.
- Squeeze out any feces that may be left in the rectum.
- Remove the bladder at its base and dispose of it in the trash.
- It helps to cut open all euthanized hamsters before "gutting" them to help avoid nicking the intestines; they bloat after sitting a while.
- If you notice any abscesses or other signs of infection, dispose of the hamster. If you see something abnormal or are not sure, please ask your supervisor or the vet.
- Food Prep should be thoroughly cleaned following processing of hamsters. This includes:
  - Wiping down all stainless steel counters;
  - Wiping down all walls;
  - Mopping floor;
  - Cleaning all utensils (tongs, scissors, etc.) and buckets; and
  - Throwing out the trash bag after processing hamsters regardless of how full it is.
- Processed hamsters should be placed in labeled (date and number of hamsters circled) zip-loc freezer bags; typically 10–12 hamsters per bag.
- Bags should be placed on racks located in walk-in freezer.
- Total number of animals processed should be recorded on cumulative "Processed Hamster Log" located in prep room next to chest freezers or hallway outside of room 3 when hamsters are put in walk-in freezer.
- Once frozen (the next day) hamster bags should be stored in chest freezer until needed

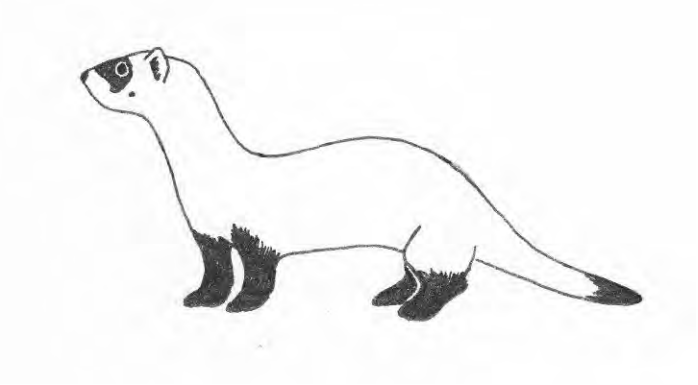
ADDENDUM D

BLACK-FOOTED FERRET BODY CONDITION SCORE

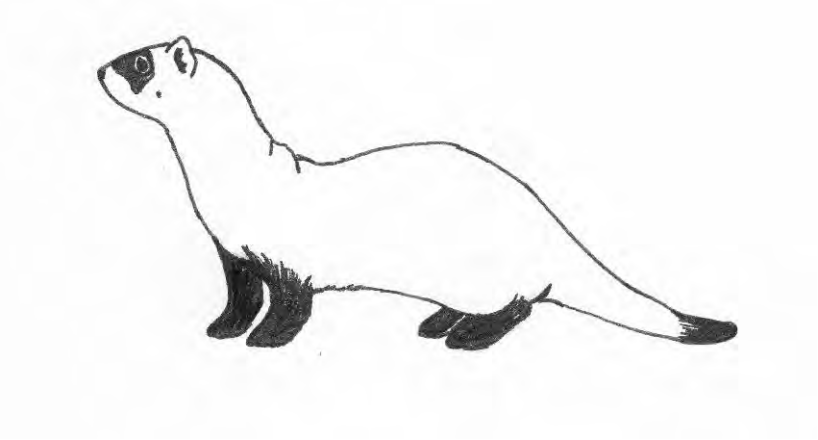
1: Emaciated	General appearance is bony with no muscle mass. Hips, ribs, and points of shoulders very prominent. Often accompanied by dehydration.
2: Thin	Ribs, hips, and points of shoulders noticeable. Some muscle mass loss.
3: Ideal	Overall smooth contour. Ribs may be seen when stretching or turning.
4: Overweight	Fat covering over ribs and shoulders with a wide waistline.
5: Obese	Heavy fat covering over ribs, shoulders, and pelvis. Waistline very wide, often unable to lift abdomen off the ground.



#1 Emaciated



#3 Ideal



#5 Obese

Drawings by Heather Branvold

ADDENDUM E

FORM EXAMPLES


2013 Male Repro.xls


2013 Female Repro.xls


2015 FCC Summary MALES.xls


2015 FCC Summary FEMALES.xlsx


Blank Pairing Log.xlsx


Blank Breeding Whelping Summary.xls


Kit Summary 2016.xlsx


Blank SSP shipment spreadsheet 2016.xlsx


Medical History Form pg1 2015.doc

OUTDOOR PEN MANAGEMENT

QUICK FACTS

- **ALL BFFS UNDERGO PRE-CONDITIONING IN OUTDOOR PENS PRIOR TO RELEASE.**
- **OUTDOOR PENS CONSIST OF EXTENSIVE PRAIRIE DOG BURROWING SYSTEMS.**
- **BFFS RECEIVE WHOLE CARCASS PRAIRIE DOGS WHILE IN PRE-CONDITIONING.**
- **BFFS MAY RECEIVE LIVE PRAIRIE DOGS DURING PRE-CONDITIONING.**
- **BFFS MAY BE HOUSED IN OUTDOOR PENS OVER THE WINTER FOR MANAGEMENT PURPOSES.**

BLACK-FOOTED FERRET MANAGEMENT IN OUTDOOR PENS

INTRODUCTION

The U.S. Fish and Wildlife Service (Service) manages a total of 48 outdoor pens for preconditioning and overwintering of black-footed ferrets (BFFs), as well as possible future outdoor breeding and whelping. In regard to BFFs, the term "preconditioning" means exposure to a realistic prairie dog burrow system and life outdoors. At this time, all BFFs suitable for release are housed in outdoor pens located at the National Black-footed Ferret Conservation Center (NBFFCC). Pens also exist at FE Warren Air Force Base (Cheyenne, Wyoming) and Brown's Park National Wildlife Refuge (Maybell, Colorado), but are currently not being used. In addition, the National Zoo's Conservation Research Center maintains outdoor pens.

HOUSING STRUCTURE

The main prerequisite to preconditioning BFFs is the establishment of an extensive prairie dog burrow system. To reduce the possibility of BFFs and prairie dogs escaping, construct pens using 2.5 centimeters (cm) (1 inch (in) steel welded wire to form an apron on the bottom and sides of the pens, and construct slurry walls approximately 2.4 meters (m) (8 feet (ft)) deep around each pen. Fill pens with substrate (soil) to a depth of approximately 2.4 m (8 ft). Some settling may occur as a result of weather events and prairie dog activity. While pens will ideally contain native vegetation, this aspect of pen infrastructure is not necessary for the preconditioning of BFFs. The interior walls of welded wire should be bordered at a height of 1.2 m (4 ft) with 46 cm (18 in) metal flashing, which prevents BFFs and prairie dogs from climbing out of the pen. Gaps in the flashing, as well as non-rounded rivets and caulking, should be minimized as animals have been observed using these as a means of climbing past the flashing, thereby increasing the likelihood of injury and escape. Mesh netting is stretched across the top on the pen to prevent predatory raptors or other animals from entering the pen. Each pen also has a single 1.5 m x 1 m (5 ft x 3 ft) door to allow staff access to the pen. At NBFFCC, there are currently four blocks consisting of 12 pens each, with each pen measuring approximately 12 m x 12 m (40 ft x 40 ft)

In the past, BFFs fighting between the welded wire pen barriers resulted in injuries and even death. To avoid fights between neighboring animals, do not share walls between pens. Instead, leave a gap of at least 10 cm (4 in) between shared walls of the pens. In the case of injury or apparent illness, trap the animal(s) and house in isolation rooms maintained elsewhere. A veterinarian should assess the condition of any animals before returning them to the outdoor burrow system.

At NBFFCC there are currently three distinct models of preconditioning pens: Natural, Medusa, and Culvert. A description of each type follows:

- A *natural* pen is simply a pen filled with soil and burrows dug by prairie dogs. Prairie dogs are introduced to a pen and remain for months at a time to allow for construction of

an intricate and stable natural burrow system. In this situation, prairie dogs must be fed and watered to maintain the health of the animals. The prairie dogs are removed from the pen before BFFs are introduced. This method has also allowed for captive breeding of prairie dogs which can be used for feeding BFFs.

Natural Pen

- *Medusa* pens are artificial prairie dog tunnels installed underground by NBFFCC staff or contractors. The tunnel system is made of 10 cm (4 in) diameter corrugated black plastic tubing and is in various designs and depths of approx. 1–2 m (3–7 ft) deep. There are also 20 cm (8 in) diameter plastic nest boxes installed underground in these systems connected to the corrugated black plastic tubing using reducer couplings.

Medusa Pen

- The final design is known as a *culvert* pen, which is a modified and simplified medusa pen. These pens have two 46 cm (18 in) diameter culverts installed vertically to a depth of approximately 2 m (7 ft) with 10 cm (4 in) diameter corrugated black plastic tubing exiting the culvert perpendicularly approximately 20 cm (8 in) from the bottom. The corrugated black plastic S tubing slopes downward slightly for a length of approximately 1 m (3 ft) to create a cold air and moisture trap, then slopes upward to ground level into a modified sprinkler box, which is used as a latrine for the BFFs and as an access point for staff. Another section of corrugated black plastic tubing is attached to the sprinkler box, which leads above ground to allow the BFFs to surface. A Y-coupling is installed below the sprinkler box and leads directly to ground level; this allows BFFs to enter or exit the burrow system without having to pass through the latrine. Latrines should be cleaned (fecal material removed and sand substrate replaced) at least once a week while BFFs are present. This design can be very useful as the nest box (culvert bottom) can be accessed by staff to assess animal health even when the animal is underground. However, there is some anecdotal evidence that these systems may lead to a higher likelihood of disease (especially *Clostridia*) outbreak in BFFs; for this reason weekly cleaning when BFFs are present is critical.

Culvert Pen

Every year, many weeks before the first BFFs are introduced to the preconditioning pens, a general maintenance check should be done on all pens using the NBFFCC pen maintenance checklist (see **Appendix A**). When assessing the condition of the pens, it is necessary to check for plugs in the burrow system, gaps in the netting over the top of the pens, holes or gaps in the walls of the pens, detaching or bowing out of metal flashing and wire apron, the condition of the wooden frame of the pens, or anything that may injure animals that are in the pen. All necessary repairs should be completed as soon as possible as it is vital to have repairs completed before animals are placed outside. These maintenance matters should continually be monitored throughout the preconditioning season.

STAFF CONDUCT

Human imprinting on wildlife has shown to be perilous to wildlife as well as dangerous to humans. It is very possible that human imprinting could lead to wild BFFs looking for people and/or vehicles as a source of food or care. Imprinting could also lead to staff facing a larger risk of being bitten by an animal that no longer exhibits its natural behaviors.

Although it is necessary for staff to spend significant portions of time in the pen area during preconditioning, it is essential to remember that preconditioning should be viewed as teaching the animals to be wild, not just simply moving captive animals to an outdoor cage. It is crucial to monitor animal well-being during the preconditioning phase, but it is also important to minimize human interaction to the fullest extent. Counting animals daily and performing general visual health checks is a necessary step for staff to conduct, but disturbing the animals can condition them to humans and reduce the animal's chances of survival in the wild. If there is not a specific concern in regard to animal care, staff should not enter the preconditioning area.

When working in or near the pens, it is important to keep unnecessary noise (loud talking, operation of tools or equipment, etc.) to a minimum, so as to not disturb the animals and condition them to human activities. Do not talk directly to the animals and keep human interaction to an absolute minimum. Human participation in live prairie dog kills (see below) should be avoided unless deemed necessary; even steps such as limiting eye contact with BFFs should be taken into consideration.

FEEDING

Feed all BFFs in outdoor pens whole carcass food once daily between 1200-1400 hours. This provides staff the opportunity to observe each animal's condition. Pen animals receive disease-free prairie dog (*Cynomys* sp.), euthanized and processed on site, as well as processed rat and live or processed hamsters. Serving size is typically 80–100 grams (g) (2.8–3.5 ounces (oz)) per BFF. However, this amount should be increased if one or more animals appear skinny or malnourished, or in the case of pen whelping and kit raising. Feeding frequency and the amount offered may vary due to climactic conditions such as freezing foodstuffs in winter and quick food spoilage in summer, or due to the physical condition of the animals. It is common for single animals in cold winter conditions to require up to 120 g (4 oz) daily to maintain body weight. It is also common to give half-rations, or have a fast day, to simulate wild conditions, encourage full consumption of rations, or before trapping.

Personnel should attempt to observe each individual daily. While minimal contact should be made with the animals, feeding every afternoon (or every other afternoon) provides ample opportunity to observe an animal's condition. To ease catching and handling (when required), BFFs can be fed in live traps that are wired open to prevent accidental trapping (do this only for animals that are **not** being pre-conditioned for release). Always provide water. A brick or rock

may be placed in each water tub to prevent drowning, or a Lixit® water bottle can be placed on the outer wall of the pen.

When feeding live prairie dogs to BFFs, place no more than two prairie dogs in a pen at a time, and only one if BFFs do not kill the first prairie dog. Live prairie dogs should be fed to BFFs once per week and, when possible, should be fed no earlier than 1800 hours. Prairie dogs will often "plug" BFFs within the burrow system. Do not give large or aggressive prairie dogs to inexperienced BFFs. All pens should be checked the next day after live feeding for evidence of live prairie dogs. Any live prairie dogs should be removed (by lethal means if necessary) no later than 1600 hours the day after live feeding has taken place. All prairie dogs, live and processed, must be collected in accordance with the NBFFCC Prairie Dog Protocol (see **Appendix B**) and show no signs of weakness, illness, sluggishness, etc.

Currently, "feeder portals" are being experimentally installed in some of the preconditioning pen walls, which will allow staff to feed the animals without having to physically enter the pen. This may prevent possible animal escapes and injuries that can occur through opening and closing a heavy door. It should also decrease the amount of direct human interaction with preconditioning BFFs, which may reduce un-natural behavior of BFFs and therefore increase wild survivorship.

BREEDING

BFFs in the breeding population may be housed in outdoor pens over winter for management purposes. These include space, animal behavior, and reproductive response to natural lighting conditions. Often juvenile females are chosen because they may respond better, reproductively, to natural lighting than to indoor artificial lighting conditions, but males may also be housed outdoors. Animals should ideally be outdoors by late November/early December to allow adequate response time to the natural photoperiod for the following breeding year.

Animals housed outdoors generally begin to enter reproductive readiness in March or April. Beginning in March, trap males and females every one to two weeks to determine reproductive readiness. BFFs fed in "dummy" traps (live traps without doors or securely locked open) in the preceding months may be easier to catch on a regular basis. Start vaginal lavages when a female BFFs vulva begins to show signs of swelling. However, relying solely on vulva size as a determination of breeding readiness affords an extremely poor indicator of BFF estrus. Once a BFF's cytological findings indicate the number of keratinized superficial cells surpasses 50%, take the female into an isolation area. If performing vaginal lavages outdoors is challenging, females can be brought into an indoor holding area for vaginal lavages after they appear "washable," or definitely swollen enough to perform a lavage. Females can then stay indoors on an appropriate light cycle. Continue vaginal lavages until the female's cytology reveals $\geq$90% highly keratinized superficial cells. Males can be brought indoors after they are firm (See **Reproduction chapter** for photo examples of cells).

Five to seven days after the female reaches breeding readiness (as indicated by a greater than 90% keratinized superficial cell count), introduce an appropriate, reproductively ready male,

(testes large and firm, for a month or more) to the female's cage. Often females placed in outdoor pens are of unknown paternity or have a high mean kinship. Selection of mates, therefore, does not always follow standard SSP criteria. All pairings take place during the day. Vocalizations such as soft "chuckling" or "cooing" indicate the animals are getting along, whereas persistent "screaming" or loud and continuous "chattering" may indicate a potential problem requiring separation of the pair.

Sperm checks are conducted fifteen minutes after the pair completes copulation (1–1.5 hours after initial introduction if not using cameras). If a positive sperm check is determined, the pair remains together for three consecutive nights. However, if the male exhibits poor positioning and a positive sperm check is not obtained within 48 hours, introduce a different male to the female. Upon completion of a successful breeding, return the female to her pen after first performing a follow-up lavage to determine if she ovulated. If the female is not intended to whelp in the pen, she can be returned to the main indoor breeding building. Conduct follow up vaginal washes 5–7 days following a positive sperm check to determine if the female ovulated. Males are typically given a minimum of 3 days rest between pairings.

WHELPING IN PRECONDITIONING PENS/KIT CARE/WEANING

This section refers to pens at Sybille, where breeding and whelping took place in outdoor pens. NBFFCC currently does not have accommodations for pen breeding. However, this information is included for reference.

One week before her due date, trap the female and confine her to the vault system/nest box, which allows staff to determine if and when whelping occurs, as well as the number of kits born. No video monitoring is conducted in the pen. Without cameras, personnel listen for "squeals" and opportunistically view the nest side of the box. Boxes should not be cleaned or litters disturbed until five days post-whelping. Use separate cleaning tools for each litter. Aspen shavings are the standard bedding material used on the nest side and Alpha-dri® on the latrine side.

Nest boxes are cleaned on the fifth and tenth day post-whelping. After the tenth day, nest boxes are cleaned every other day until the onset of kit care on day 30. Beginning day 30, offer regular Toronto diet and eviscerated or live hamsters to litters. Continue this feeding regimen until the dam and her litter are given full pen access (about 40 days). At this time, furnish live prairie dogs (if available), processed prairie dog, live hamsters, and Toronto diet ad libitum.

Once kit feeding begins, determine the sex of the kits and clean every day. Litter size and kit condition may warrant twice-daily kit feeding. Clean nest boxes after every kit feeding. Monitor litters for giardia, coccidiosis, and cryptosporidiosis. Litters may be treated for these diseases while maintained in the vault/nest box system. A litter is typically given pen access at 40 days post-whelping, but this may vary depending on the kits' condition and health.
Kits are not trapped until 120 days of age, as dictated by transfer dates to reintroduction sites. Once trapped, give kits ponazuril prophylactically prior to transfer.

RECORD KEEPING

As with indoor management, it is important to keep accurate, orderly, and up-to-date records on all BFFs housed in outdoor pens. Maintain data sheets on foods offered, weights, medications, breeding activities, kit care, etc. [See data forms in **Appendix C**].

PREDATOR CONTROL

Monitor the exterior of pens for signs of predators or structural defects on a regular basis. Monitor the exclusion fence for gaps and holes as well as debris piling up on either side of the fence, which could allow predators to access the pen complex. Also monitor the flashing of the border fence and make sure that access gates are always closed when not in use. Monitor nets over tops of pens for gaps which would allow for raptors or other predators to enter the pen. Watch for rattlesnakes and large bull snakes in or near pens and safely (ideally non-lethally) remove them from the premises. Stay vigilant for sign (tracks, scat etc.) of predators in or near the pens.

APPENDIX A

NBFFCC PRECONDITIONING PEN MAINTENANCE CHECKLIST

Evaluated By: _____

Date: ____/____/____

Pen	Type	Occupancy	Ground / Veg.	Burrows / Tunnels	Sprinkler Boxes	Skirt	Door	Walls / Flashing	Nets	Other
A1	RN									
A2	RN									
A3	RN									
A4	N									
A5	M									
A6	M									
A7	N									
A8	C									
A9	N									
A10	N									
A11	N									
A12	N									
B1	M									
B2	C									
B3	N									
B4	N									
B5	N									
B6	N									
B7	N									
B8	M									
B9	N									
B10	N									
B11	RN									
B12	N									

Notes:

Pen	Type	Occupancy	Ground / Veg.	Burrows / Tunnels	Sprinkler Boxes	Skirt	Door	Walls / Flashing	Nets	Other
C1	N									
C2	N									
C3	M									
C4	RN									
C5	RN									
C6	RN									
C7	RN									
C8	C									
C9	C									
C10	RN									
C11	N									
C12	N									
D1	RN									
D2	N									
D3	M									
D4	RN									
D5	N									
D6	N									
D7	C									
D8	C									
D9	C									
D10	C									
D11	C									
D12	N									

Notes:

APPENDIX B

NBFFCC PRAIRIE DOG PROTOCOL

INTRODUCTION

The National Black-Footed Ferret Conservation Center (NBFFCC) conducts a preconditioning program in order to properly imprint captive black-footed ferrets (BFF) on their primary food source, prairie dogs (*Cynomys spp.*). Imprinting takes place via feeding of euthanized and live prairie dogs during a 30-day preconditioning period at the NBFFCC, and requires the coordination of several partners to provide an adequate supply of prairie dogs while ensuring the health and safety of NBFFCC staff and captive BFFs. Research has shown that preconditioning greatly improves the survival of BFFs released into the wild, and prairie dog management and husbandry is an important aspect of the NBFFCC's preconditioning program. This document describes the prairie dog trapping, receiving, processing, and husbandry procedures followed at the NBFFCC.

TRAPPING PROCEDURES

1. All prairie dogs used at the NBFFCC (processed and live-fed) will be captured from colonies in Colorado that have been dusted with Deltamethrin. Capture via trapping or burrow flushing will not take place until at least 21 days post-treatment.
2. The majority of prairie dogs supplied to NBFFCC in 2016 were collected from Boulder County Parks and Open Space, and a small number may have been provided by private pest control contractors that adhere to the burrow dusting protocol described above.
3. All prairie dogs will be sprayed with pyrmethrin immediately after capture.
4. If prairie dog activity levels at potential capture sites appear suspiciously low during moderate weather conditions (Menken and Anderson 1993), staff from Boulder County Parks and Open Space and any private pest control contractors will actively survey for plague population impacts during capture activities using visual counts (Menken and Anderson 1993) conducted on three successive days. If counts decline by more than 25% over the three-day observation period, the following procedures will be implemented:
    a. Capture activities will immediately cease and NBFFCC staff (John Hughes @ 970-305-1158, Robyn Bortner at 505-228-2744, or Mary Wright @ 970-231-9722) will be notified.
    b. Burrows will be swabbed for the presence of fleas, and fleas will be analyzed for the presence of plague via PCR analysis performed by Colorado Parks and Wildlife.
    c. Any dead prairie dogs found above ground will be collected by trapping personnel and placed in plastic bags. Plastic bags will be labeled with collection date and time, collection site, and names of trapping personnel. These prairie dogs will be transported to the NBFFCC by trapping personnel, frozen by NBFFCC staff, and sent to the University of Wyoming Veterinary Laboratory to be analyzed for the presence of plague. The NBFFCC will pay any laboratory analysis costs.

d. If plague is detected, capture activities will cease and all prairie dogs captured from the site will be euthanized and submitted to the University of Wyoming Veterinary Laboratory or Colorado State Veterinary Diagnostic Lab to be analyzed for the presence of plague.

RECEIVING PROCEDURES

1. Prairie dogs will be received from approximately June 1–October 31 throughout the work week (Monday–Friday), with staff from Boulder County Parks and Open Space and any private pest control contractors contacting the NBFFCC (John Hughes @ 970-305-1158, Robyn Bortner @ 505-228-2744, or Mary Wright @ 970-231-9722) a minimum of two days prior to their arrival at the facility. Prairie dogs will be received on the west side of the prairie dog holding building, and NBFFCC staff receiving prairie dogs will wear personal protective equipment (PPE) as directed.
2. Prairie dogs that will be processed for food will be unloaded from traps and placed in hanging cages prior to placement in CO_2 induction chambers for euthanasia. Three days per regular work week will be identified for processing activities. Prairie dogs that will be processed for food that arrive on non-processing days will be placed in 0.6 meter (m) x 1 m x 2 m (2 feet (ft) x 3 ft x 6 ft) galvanized steel bins provided with food, water, and bedding material. These prairie dogs will be captured by hand and placed in CO_2 induction chambers for euthanasia on the next available processing day.
3. Prairie dogs that are being kept alive for live-feeding to BFFs in preconditioning pens will be housed in 0.6 m x 1 m x 2 m (2 ft x 3 ft x 6 ft) galvanized steel bins in the prairie dog holding building for no longer than 21 days. Live-feeding in preconditioning pens will take place from July 10–October 21.

PROCESSING PROCEDURES

1. Euthanized prairie dogs will be processed for future feeding and frozen.
2. All health and human safety protocols, including personal protective equipment, will be addressed by the NBFFCC Safety Plan.
3. Only personnel trained and fitted for PAPRs will euthanize and process prairie dogs.
4. If ≤30 prairie dogs arrive from a provider, they will be placed in CO_2 induction chambers for immediate humane euthanasia and processing for future feeding to BFFs. If >30 prairie dogs arrive from a provider, excess prairie dogs will be kept in hanging cages and placed in CO_2 induction chambers for euthanasia as soon as possible. Prairie dogs will not be kept in hanging cages overnight under any circumstances.
5. During processing, each prairie dog will be evaluated for gross clinical lesions or signs of disease. Any suspicious prairie dog carcass will be submitted to the University of Wyoming Veterinary Laboratory for evaluation. On-site training for NBFFCC staff on the identification of signs of disease will be provided by NBFFCC contract veterinarian Dr. Mary Wright. If disease is suspected, the entire cohort that contained the suspicious individual will be processed, frozen, identified, and stored until determined to be disease free or disposed of if disease positive. Disease-positive carcasses will be sent to the

Centers for Disease Control and Prevention Division of Vector-Borne Diseases in Fort Collins, Colorado for incineration. No prairie dogs from such group will be fed out to BFFs unless they are determined to be disease free.

HUSBANDRY PROCEDURES

1. Each 0.6 m x 1 m x 2 m (2 ft x 3 ft x 6 ft) galvanized steel bin will contain ≤10 prairie dogs, one nest box, two water bottles, two food containers, and proper bedding. Initially, assigned staff will monitor the prairie dog holding building daily and determine the appropriate intervals for feeding, watering, and bedding changes. It is anticipated that these activities may take place every other day or every three days. Assigned staff will wear protective equipment, including PAPRs, Tyvek® suits, and latex gloves during monitoring activities.
2. Bedding material will be wood chips, and will be changed as needed.
3. Live-feeding in preconditioning pens will take place from July 10–October 21 each year. Ideally prairie dogs will be kept in bins for <3 days prior to feeding to BFFs in preconditioning pens. In the event of a large influx of trapped prairie dogs, large adult prairie dogs will be euthanized and processed for food (see procedures described above), and smaller adults and pups will be kept in bins until ready for use in preconditioning pens. Prairie dogs will not be kept in bins longer than 21 days.
4. In the event of a prairie dog death that cannot be attributed to intraspecific aggression or other non-disease related causes, the dead prairie dog will be sent to the University of Wyoming Veterinary Laboratory to be analyzed for the presence of plague. If the carcass tests positive for plague, the entire cohort in the holding building will be euthanized in CO_2 induction chambers and sent to the Centers for Disease Control and Prevention Division of Vector-Borne Diseases in Fort Collins, Colorado for incineration. If the carcass tests negative for plague, decisions will be made on the fate of the remaining prairie dogs on a case-by-case basis.
5. During transfer of prairie dogs from the prairie dog holding building to the preconditioning pens, prairie dogs will be housed in cages and transported via utility vehicles.

REFERENCES

Menkens, G. E. JR., and S. H. Anderson. 1993. Mark-recapture and visual counts for estimating population size of white-tailed prairie dogs. Pages 67-72 in J. L. Oldemeyer, D. E. Biggins, and B. J. Miller, editors. Proceedings of the symposium on the management of prairie dog complexes for the reintroduction of the black-footed ferret. Biological Report 13. U.S. Fish and Wildlife Service, Washington, D.C.

Updated May 16, 2016

Point of Contact: John Hughes, phone (970) 897-2730 x229 or (970) 305-1158

APPENDIX C

OUTDOOR MANAGEMENT FORMS


OD Pen Log weekly.xlsx


OD Pen Feeding Guide.xls


Preconditioning Form.doc

NUTRITION

QUICK NUTRITION OVERVIEW

- **FEED BFFS TORONTO SMALL CARNIVORE MIX DIET (45–100 G/1.6–3.5 OZ) ONCE A DAY, AS LATE IN THE DAY AS POSSIBLE.**
- **FEED WHOLE CARCASS ANIMALS TO BFFS 1–2 TIMES WEEKLY.**
- **PRAIRIE DOG IS FED TO ALL PRECONDITIONED KITS PRIOR TO RELEASE.**
- **FEED LIVE PREY (HAMSTERS, MICE, OR SMALL RATS) ONCE A WEEK, AS KITS NEED THE EXPERIENCE OF CATCHING AND KILLING LIVE PREY.**
- **KEEP COMPLETE, ACCURATE, UP TO DATE DAILY RECORDS ON EACH BFF'S FOOD CONSUMPTION, WEIGHT, HEALTH, ETC.**

BLACK-FOOTED FERRET NUTRITION

BACKGROUND RESEARCH EFFORTS

Under natural circumstances in the wild, black-footed ferret's (BFF's) primary prey item is the prairie dog. Only occasionally does a BFF supplement its diet with mice, voles, or rabbits. In captive management facilities, BFFs previously received a homemade mixture of mink pellet and ground rabbit as their primary source of nutrition. This diet was analyzed by several nutritionists who were concerned that the 60/40 diet had an excessive level of polyunsaturated fatty acids (PUFAs). This diet was subsequently changed to the current commercially available diet developed by Dr. Eduardo Valdes for the Toronto Zoo. The Milliken Meat Company of Ontario Canada, manufactures the horsemeat based Toronto Carnivore Mix that was tested on BFFs through breeding and weaning. After successful results of the feeding trial, all BFFs in the breeding program were converted to the Toronto Carnivore Mix in 2001. The soft nature of the captive diet, though advantageous for several reasons, necessitates the supplementation of the diet with whole carcass food items for better dental and gingival health.

RECOMMENDED NUTRITIONAL REQUIREMENTS

The chart below displays acceptable levels of nutrients in BFF rations, based on the nutritional requirements of mink, as published by the National Research Council (NRC, 1982):

Nutrient	**Adult (maintenance)**	**Kits (growth)**
Protein (%)	21.8 to 26.0	32.6 to 38.0
Fat (%)	20 to 30	44 to 53
Ash (%)	<10	---
Energy (kcalME/kgDM)	3600	3930 to 4080
Calcium (%)	0.3 to 1.0	0.4
Phosphorus (%)	0.3 to 0.8	0.4
Ca:P ration	1:1 to 2:1	1:1 to 2:1
Iron (mg)	20 to 30	---
Zinc (mg)	59 to 66	---
Vitamin A	200 IU/day	145 IU/100kcalME
Vitamin E (IU/kgDM)	25	27

Nutritional Toxicities and Deficiencies

Because the nutrient requirements of BFFs are based on restricted data, it is difficult to determine if a particular nutrient is being over or under represented. Oyarzun et al (1994) found that the nutrient concentration of the 1994 Metro Toronto Zoo (MTZ) 60/40 diet met or greatly exceeded dietary recommendations established for mink (NRC, 1982) as well as nutrient levels reported in prairie dog (Dierenfeld and McGuire, 1989). Of particular concern to the researchers was the possible excessive dietary mineral and PUFA content. The report states that even though mineral levels are not high enough to cause acute toxicosis, the feeding of higher than normal recommended levels over an extended period of time may cause adverse effects.

The 1997 MTZ 60/40 diet, which met or exceeded dietary recommendations established by the NRC (1982) for mink (Oyarzun et al, 1994), was analyzed by Valdes and Dunstan (1997) as follows:

Nutrient	**1997 MTZ 60/40 Diet**
Dry Matter (%)	37.40
Crude Protein (%)	46.52
Crude Fat (%)	17.65
Ash (%)	9.09
Gross Energy (kcal/kg)	5351
Calcium (%)	2.35
Phosphorous (%)	1.39
Ca:P ration	1.69:1
Vitamin A (IU/kg)	5694
Vitamin E (IU/kg)	174
Iron (ppm)	501
Zinc (ppm)	139

The new diet of Toronto Small Carnivore Mix is a quality controlled diet based on human quality horsemeat approved by the Canadian version of the USDA. Its use in the SSP has met with success in all facilities. Adult male BFFs are provided with 70–100 grams (g) (2.5–3.5 ounces (oz)) and adult females 60–90 g (2–3 oz) on average. Females are offered increased amounts (to ad-libitum) during gestation and lactation. Generally, staff feed BFFs weighed

amounts of the Toronto diet once a day. Because of their nocturnal nature, BFFs are fed as late in the day as possible.

Lactation Diet

A dam's caloric needs gradually increase during gestation and lactation and are at the maximum during peak lactation, (just before weaning of her kits). Dams are offered increasing amounts of Toronto diet and should be offered on an ad libitum basis at peak lactation. To ensure she is getting all that she needs during lactation, a small amount of diet should be left over each day.

Prairie Dog

A comprehensive study on prairie dog carcass composition was conducted by Dierenfeld et al. (1997) and summarized by McGuire (1989). Results were as follows.

	Black-tailed Prairie Dog				**White-tailed Prairie Dog**		
Analysis (DM)	**Summer**	**Fall**	**Winter**	**Spring** no juveniles	**Summer**	**Fall** only juveniles	**Spring** no juveniles
Dry Matter (%)	30.3 - 45.6	35.6 - 43.1	42.6 - 50.3	27.0 - 32.8	24.9 - 47.6	36	29.7 - 31.3
Protein (%)	41.8 - 58.5	35.2 - 44.4	27.9 - 36.0	51.4 - 64.0	28.4 - 68.8	42	52.5 - 59.3
Fat (%)	35.9 - 55.6	42.7 - 48.8	49.2 - 61.8	21.8 - 37.1	22.7 - 66.7	49	34.0 - 34.3
Ash (%)	4.1 - 8.0	5.4 - 6.2	5.1 - 7.5	11.7 - 13.4	3.3 - 8.3	8	11.2 - 11.3
***Gross Energy (kcal/g)**	3.10 - 4.61	3.48 - 4.11	4.46 - 5.11	3.64 - 5.31	3.91 - 4.58	4	4.11 - 4.29

*GE values are minus waste portions: anterior skull, feet and lower legs and hide

Since BFFs develop a preference for a food source at approximately 50–90 days of age (Vargas and Anderson 1996), kits 50–90 days of age should receive processed prairie dog (when available) on a schedule of 50 g (1.8 oz) of prairie dog per BFF, three times a week. This is extended for the release candidates until they leave for pre-conditioning pens. All release kits receive prairie dog upon arrival at FCC for pre-conditioning. When available, U.S. Fish and Wildlife Service (Service) National Black-footed Ferret Conservation Center (NBFFCC) may supply frozen processed prairie dog to Species Survival Plan (SSP) captive-breeding facilities,

contingent on the facilities' regulations and restrictions (with regards to the change in prairie dog quarantine procedures).

BFF kits are also afforded early hunting and killing experience by being offered live hamsters or mice. Young kits do best when confronted with younger, smaller live prey. The ideal hamster size for feeding young BFFs is approximately 6 weeks of age (at about 50 g/1.75 oz of weight). Older kits and adult BFFs can handle the much larger, more aggressive hamsters. A kit's first experience with prey should be in the presence of its dam so she can demonstrate how to catch and kill prey. BFFs intended for release must go through preconditioning where they live outside in large pens that include active prairie dog burrows. During this time (30–60 days or more) they continue to be fed processed prairie dog as well as having access to live (quarantined) prairie dogs to practice hunting skills.

Hamsters and Mice

The nutrient composition of hamsters, analyzed by Dierenfeld (unpublished), and mice, analyzed by Valdes and Dunstan (1997), are as follows:

Nutrient	**Hamsters**		**Mice**
	Males	**Females**	
Dry Matter (%)	34.05	29.83	33.70
Crude Protein (%)	49.12	53.30	51.63
Crude Fat (%)	20.80	31.12	18.99
Ash (%)	4.08	10.22	8.61
Gross Energy (kcal/kg)	---	---	6050
Vitamin A (ug/gDM)	7.54	7.90	164.39
Vitamin E (ug/gDM)	14.40	10.40	12.50

Depending on the breeding facility, one hamster or one mouse is fed per BFF kit one to two times per week, or as available.

Some BFF breeding facilities raise their own hamsters. [A hamster breeding protocol is included as an **Addendum B** in the **Indoor Facility Management** chapter.] It is important that all diseased hamsters in the breeding colony be removed and humanely destroyed. All dead hamsters should be necropsied.

Young ("pinkie") mice or hamsters serve as an excellent medium for stimulating appetite in BFFs. Small rodent carcasses can also be used as a means of administering medication to BFFs.

Record Keeping

All BFF captive breeding facilities keep comprehensive, accurate and up-to-date records in order to track BFFs' food intake. A BFF that does not eat its daily diet warrants examination. The first signs of an ailing animal are reduced food intake and weight loss. The two most important nutritionally-related items to record are daily husbandry records and body weight records. Daily husbandry records include notations on BFFs' food intake, diet items and amount offered, amount consumed health, behavior and any treatments. Body weights, taken regularly, also may be recorded on the daily record. Many facilities weigh BFFs once a month in non-breeding season, and more frequently prior to and when handled during breeding season.

Periodic Diet Analysis

The possibility of a lack of consistency in diet components, even the commercially available Toronto/Milliken diet warrants routine analysis. An annual analysis is justified.

Product Information

Toronto Small Carnivore Mix	Milliken Meat Products, Orlando de Rosa – proprietor, 3447 Kennedy Rd. Unit 1, Scarborough, Ontario M1V 3F1 ph: (416) 299-9600 fx: (416) 299-5305
Hamsters	Sasco, Inc., 251 Ballardvale St., Wilmington, MA, 01887, 800-228-4919; fax 1-800-992-7329 (New York facility)

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BLACK-FOOTED FERRET BEHAVIORAL ENRICHMENT

DISCUSSION

Environmental enrichment enhances the physical and psychological well-being of captive animals and promotes the development and expression of behaviors similar to those displayed in the wild (Carlstead 1992). In her dissertation, Astrid Vargas (Vargas 1994) cited numerous research projects that proposed play as a means of developing behavioral flexibility and physical fitness necessary to perform adult behaviors in black-footed ferrets (BFFs).

Studies conducted on BFFs indicate:

- BFFs raised in enhanced environments kill hamsters at significantly higher rates than those raised in non-enriched environments (Vargas 1994).
- BFF kits exposed to live hamsters during early developmental ages kill prey more readily than those deprived of that experience (Vargas 1994).
- Kits that perform chase and flight activities may be more likely to develop physical endurance and motor skills useful for avoiding predators and searching for prey (Vargas 1994).
- BFFs exposed to enriched environments exhibit increased activity over those housed without enrichment (Zhang 1995).

A study conducted by Poessel et al. (2011) demonstrated that enrichment [black plastic tube cap, a Nylabone® (Nylabone® Products, Neptune, NJ) or a plastic noise ball (larger ball containing a smaller ball with a bell inside of it)] lowered fecal glucocorticoid metabolites (used as a measure of stress) in juvenile male BFFs, while increasing it in adult females and having no effect on stress hormones in juvenile females and adult males. Behaviorally, juvenile males interacted more with the enrichment items than did adult females. Overall, the effect of environmental enrichment did not increase the incidence of disease or on the ability of BFFs to become reproductive during the following breeding season. However, the feeding of two prey animals a week was shown to decrease stress.

Enclosure size can also affect the size of BFFs. A study took morphological measurements on BFFs born in the wild and compared them to captive animals born in standard enclosures (1.2 meters (m) x 2.4 m 0.9 m/4 feet (ft) x 8 ft x 3 ft) and larger (6 m x 3 m x 3.6 m/20 ft x 10 ft x 12 ft) pens (Wisely et al. 2005). Adult BFFs born in the larger pens had similar tibia length, but animals from captive-breeding facilities had significantly shorter tibias (Wisely et al. 2005). These morphological changes seem to be related to the developmental environment and not to genetics. Most likely, long bone development and growth is reduced in captive BFFs due to the enclosure size (Wisely et al. 2005). The effect of shorter limbs on survival in the wild is not known, but should be monitored as the genes that control the growth and ossification of cartilage cells are no longer being selected for in the captive breeding program (Wisely et al. 2005). Providing larger habitat like the SCBI enclosures during development would help to increase limb length.

Since all BFF kits are potential release candidates, providing environmental enrichment is an essential element in preparing ferrets for reintroduction. In addition, furnishing enrichment to all BFFs may slow erosion of innate behaviors that could prove to be necessary for the ferrets' re-establishment in the wild.

When selecting enrichments, efforts should be undertaken to stimulate the following inherent behaviors and activities in BFFs:

* exploring
* searching
* play dancing
* play digging
* chasing objects
* pouncing
* moving objects
* hiding
* stalking
* caching
* wrestling
* scent marking
* awareness of environment-overhead as well as ground level

SUGGESTED ENRICHMENTS

ENRICHMENT ITEM	ACTIVITY ASSOCIATED WITH ENRICHMENT
• 10 cm/4 in diameter plastic ribbed tubing *tubes can be hung w/cable ties, carabineers	Hiding, exploring, caching, scent marking
• Plastic elbows & "Y" connectors	Create tunnel system with pieces, exploring
• Plastic tube cap	Caching, chewing, pulling
• Plastic noise ball (larger ball containing a smaller ball with a bell inside of it)	Pulling, caching, retrieving, stalking
• Plastic softballs	Digging, caching, retrieving, stalking
• Petite Gumabone^{R} wishbones	Caching, chewing, stalking
• Plastic colanders(small holes, bottom cut out)	Exploring, hiding (BFFs pull over tunnel to extend opening, watch goings on through holes)
• Plastic square milk crates	Hiding, exploring (add tunnel sections for

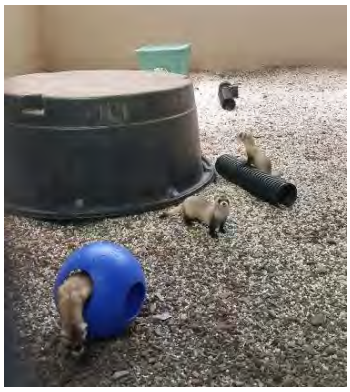
	variety)
• Hanging 11 cm/4.5 in Gumabones[R]	Stalking- attack object; alert to overhead movement; (drill hole one end, attach to cage top w/ cable tie, carabineer) promotes BFF "dancing"
• Paper bags	Shelter, exploring
• Newspaper balls	Caching, nesting, hiding
• Paper braids, paper mache balls	Hunting, stalking, grasping, caching
• Scents	Environmental awareness
• Toilet paper strung around enclosure	Environmental awareness, stalking, exploring
• Cardboard boxes (cereal, etc.)	Shelter, exploring, food cache site (*can also be used as item for BFF to chew up/shred in conjunction with Bitter Apple[R] sprayed on wood surfaces to curtail chewing enclosure)*
• Jolly balls®, sway N play®	Environmental awareness, stalking, exploring

Photos of Examples of enrichment items

PLASTIC POTS, TUPPERWARE[R] BIN W/ FLANGE

JOLLY BALL®

SWAY N PLAY®



CORRUGATED PLASTIC TUBING

ITEMS FOUND TO BE UNACCEPTABLE OR PROBLEMATIC

ITEM	PROBLEM
• Golf balls	Break teeth
• Rubber Tuffy^{R} or Kong^{R} chew toys	Able to chew apart, ingest rubber
• Regular style Nylabones^{R}	Some splintering; very difficult to clean imbedded feces from chewed areas
• Cardboard paper towel rolls	Kits can get head stuck in tube end *may be used if cut lengthwise or attached to cage*

BFF with head stuck in toilet paper roll

FOOD ITEMS USED FOR ENRICHMENTS

- Hamsters
- Mice
- Rats
- Rat pinkies or pups

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MANAGEMENT OF REPRODUCTION

QUICK FACTS

- **CHECK LIGHTS BEGINNING AFTER FALL TRANSFERS.**
- **BEGIN MONITORING MALES AND FEMALES AFTER THE BEGINNING OF THE YEAR.**
- **BFFS ARE SEASONAL BREEDERS THAT WHELP IN THE SPRING AFTER A 42 DAY GESTATION.**
- **LIMIT DISRUPTION, ESPECIALLY CONSTRUCTION, DURING THE BREEDING SEASON.**
- **FEMALE CYTOLOGY IS MONITORED USING PAPANICOLAOU® STAIN (recommended) OR DIF-QUIK® STAIN.**
- **A FEMALE BFF REMAINS IN ESTRUS FOR AROUND 30 DAYS UNLESS SHE IS BRED.**
- **MALE TESTES BECOME FIRM DURING BREEDING READINESS. MALES 2+ YEARS SHOULD BE FIRM 4 WEEKS BEFORE PAIRING. YEARLING MALES SHOULD BE FIRM SIX WEEKS BEFORE PAIRING.**
- **ALL MALE BFFS SHOULD BE ELECTRO-EJACULATED ANNUALLY; IF NOT POSSIBLE, ALL EFFORTS SHOULD BE MADE TO ELECTRO-EJACULATE UNPROVEN MALES.**
- **IF IT IS NECESSARY TO PAIR A FEMALE WITH MORE THAN ONE MALE, SEPARATE PAIRINGS BY 2-3 DAYS SO THAT PARENTAGE CAN BE VERIFIED.**
- **PERFORM A SPERM CHECK NO LESS THAN 30 MINUTES AFTER BREEDING FERRETS HAVE SEPARATED. IT MAY BE NECESSARY TO STAIN THE SLIDE USING DIF-QUIK® STAIN AND LOOK FOR THE PRESENCE OF SPERM OR SPERM HEADS. SAVE ALL SPERM CHECK SLIDES.**
- **PERFORM OVULATION CHECK ON FEMALES 5-10 DAYS AFTER BEING SEPARATED FROM MALE.**

INTRODUCTION

The primary goal of a breeding facility is the production of healthy animals for reintroduction and to retain genetic diversity of founder individuals. Therefore, understanding the black-footed ferret's (BFF's) reproductive biology and techniques necessary to promote BFF reproduction is paramount to the recovery of the species. (see National Black-footed Ferret Conservation Center (NBFFCC) protocols in **Addendum A**).

BASIC CHARACTERISTICS

Both the female and male BFFs follow a strict seasonal reproductive pattern. Increasing day length at the beginning of the year triggers the BFF's reproductive cycle in spring. Therefore, facilities initiate a light pattern that mimics the natural photoperiod. If the facility does not have an astral timer, use the following table:

Dec 21:	10 hours Light; 14 hours Dark
Jan 21:	11 hours Light; 13 hours Dark
Feb 21:	12 hours Light; 12 hours Dark
Mar 21:	13 hours Light; 11 hours Dark
Apr 21:	14 hours Light; 10 hours Dark until last litter is 30 days old

The female exhibits estrus with vulvar swelling while the male's testicular size and turgidity increase during this period. Studies indicate that males begin to undergo hormonal changes as early as November.

BFFs develop rapidly and are usually capable of breeding their first season (before they reach one year of age). However, female fecundity begins to drop off markedly at age four, and falls below 25% by age five. Therefore, it is important to take advantage of each female's prime breeding periods during the first three years of age. (see **Expected Production Range, Productivity Charts Addendum B**).

Male BFFs are also capable of breeding as yearlings, but they lag behind older males by two to four weeks for size, turgidity and sperm production. At age five, male fecundity drops sharply (Wolf et al. 2000).

Gestation for BFFs is 42 days. Litter size ranges from 1–10, but the average litter size is three–four kits.

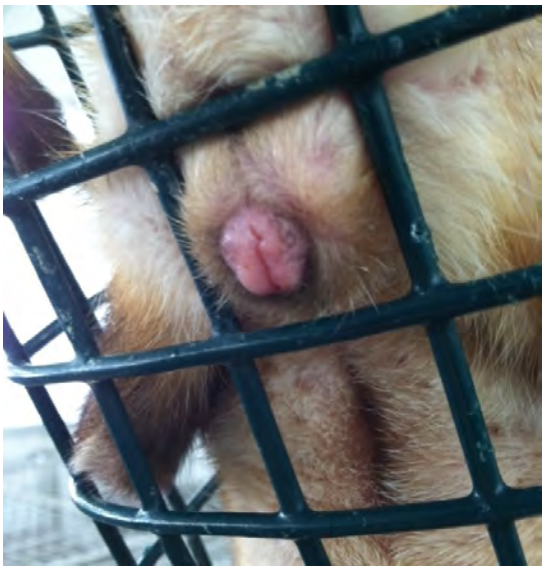
BEHAVIORAL CHANGES

Certain behavioral changes in BFFs indicate when animals are becoming sexually active. BFFs spend more time on the enclosure surface. They scent rub, urinate, and defecate in the corners of their enclosures, ostensibly staking territories and advertising for mates. If males and females are in adjoining enclosures, they will spend more time at the divider between sections, sniffing, defecating, or depositing bedding material. Males may exhibit orange tinged patches of fur at the back and sides of the neck in the area of their scent glands. Finally, diet consumption by the female may decrease as she approaches estrus.

DETECTION OF ESTRUS

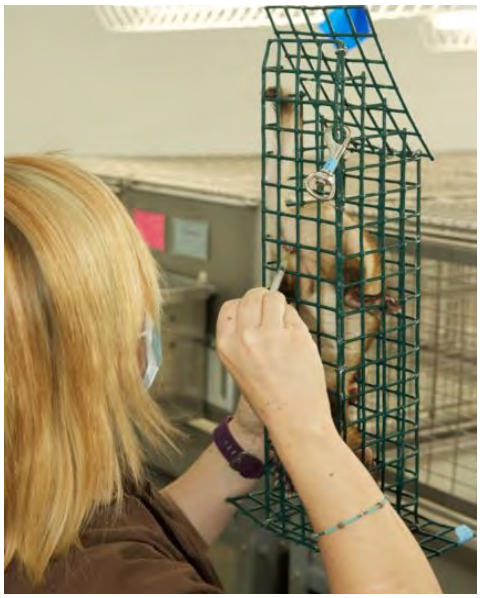
Monitoring of BFFs for estrus should begin in January. Catch females in a restraint device and examine their vulva for swelling. Catch females every 2 weeks until they exhibit obvious vulvar swelling. While BFFs are restrained, take the opportunity to weigh the animals and ask vet staff to check their overall health (measure and record vulvar swellings as estrus progresses). Once vulvar swelling is evident, begin weekly vaginal flushing for cytological examination.

Beginning vulvar swelling



Significant vulvar swelling

Perform vaginal flushes in restraint using a micro-pipet and infuse 50 micro-liters (uL) sterile saline into the vagina. Aspirate it back out and place wash solution in a thin film on a slide.

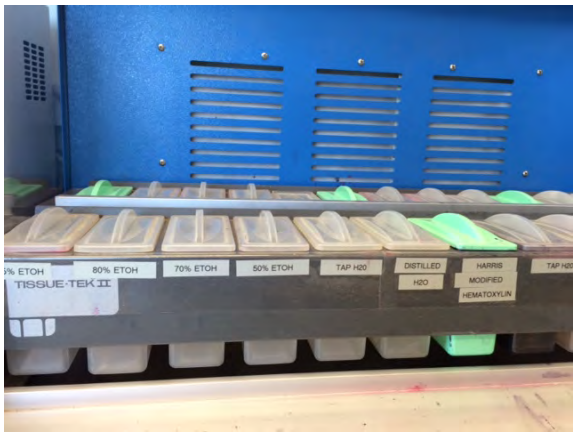


Performing vaginal lavages

Dry and spray the slide with fixative, and then stain the slide. The easiest stain to read for BFF cytology is a modified Papanicolaou® method devised by Dr. Beth Williams (see **Addendum C;** when using the Pap stain, it is advisable to wear protective clothing, gloves and stain slides under a fume hood). Slides may be stained using Dif-Quik®; however, identification of cell types is more difficult as the cornified cells are merely different shades of purple and do not achieve the orange tinged color for keratinized cells as they do when using the Papanicolaou® stain. View cells at 40x power and count 100 cells to get the percentage of cornified cells. You may want to do this several times and get an average.

Dif-Quik® stain

Fume hood manual

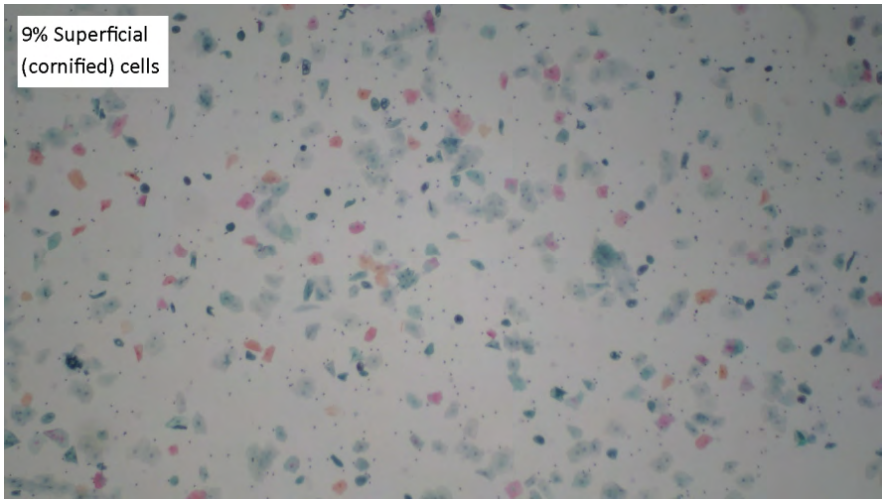


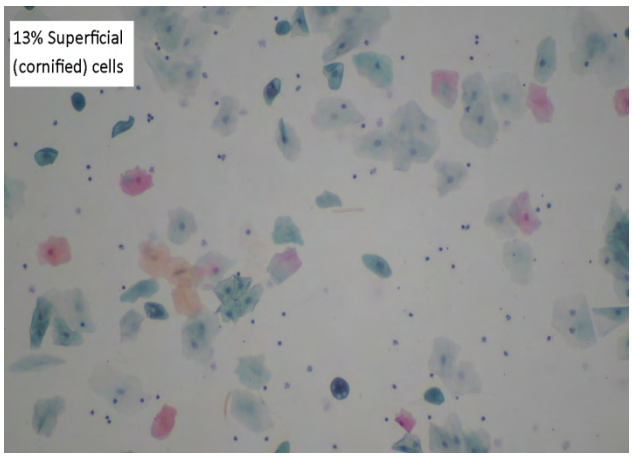
Pap stain

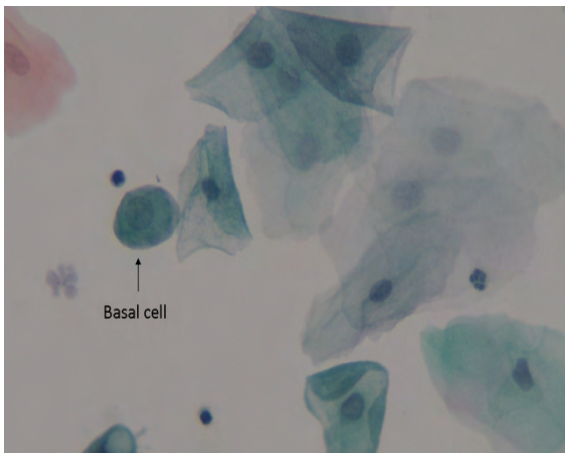
Pap stain setup under fume hood

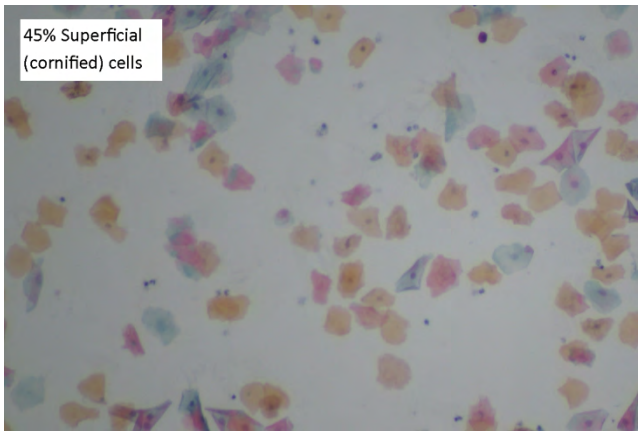
The onset of estrus causes the epithelial cells lining the wall of the vagina to change, eventually becoming cornified. The slide of a BFF in anestrus (not in estrus) shows small, round, nucleated cells. As estrus approaches, cells change getting larger and more irregular in shape and losing their nuclei. Fully cornified cells are large, irregular, flaky cells without a

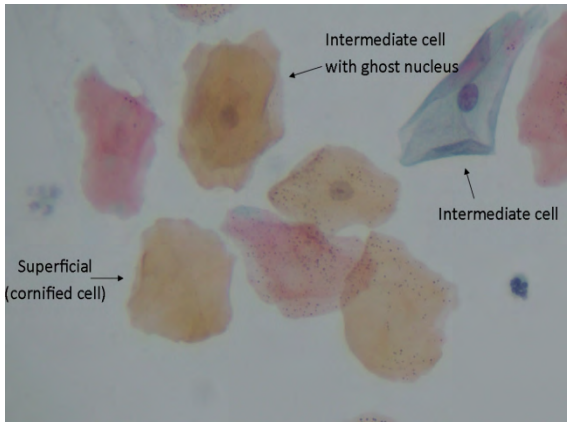
nucleus. Characteristically, the slide of a BFF in full estrus reveals 90% or more of the epithelial cells as cornified and, if using the Papanicolaou® stain, highly keratinized, or orange in color. See photos below:

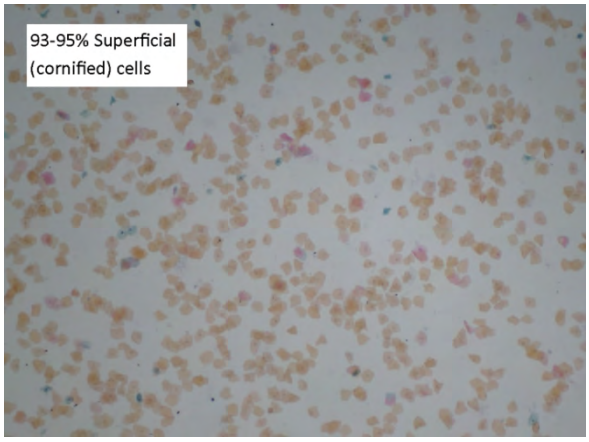


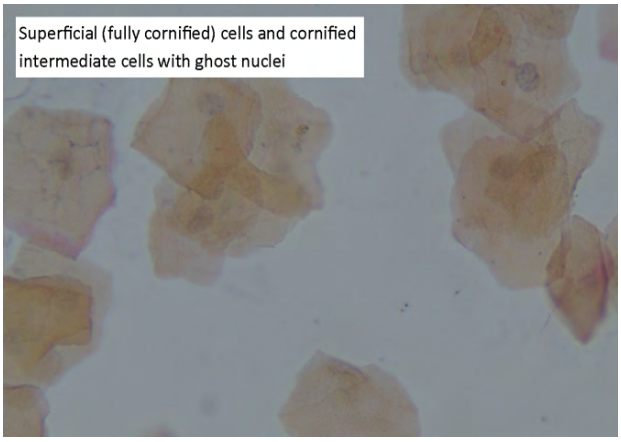


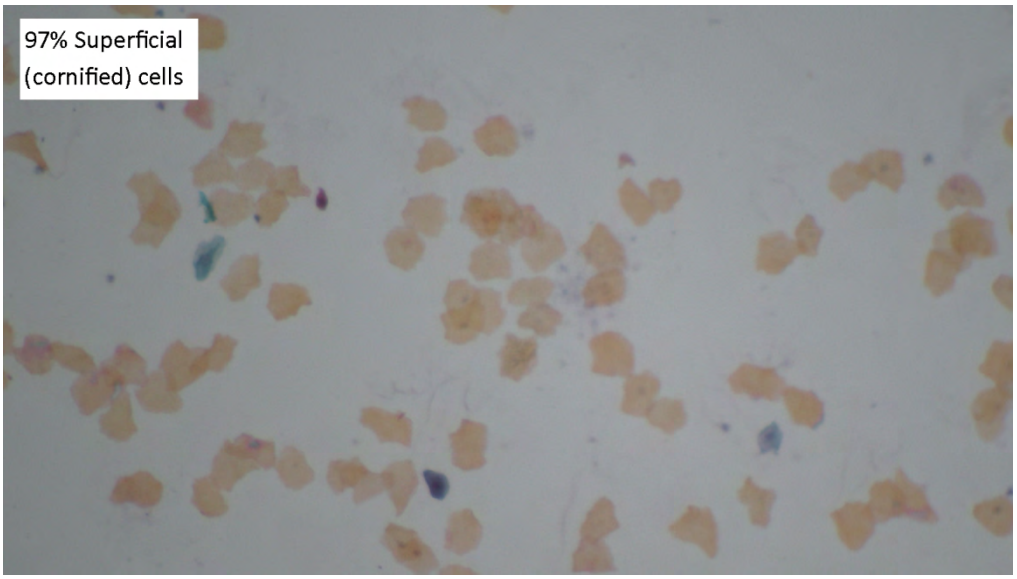



45% Superficial
(cornified) cells


Intermediate cell
with ghost nucleus
Intermediate cell
Superficial
(cornified cell)


93-95% Superficial
(cornified) cells


Superficial (fully cornified) cells and cornified
intermediate cells with ghost nuclei

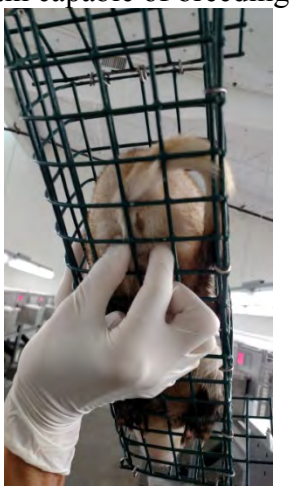

97% Superficial
(cornified) cells

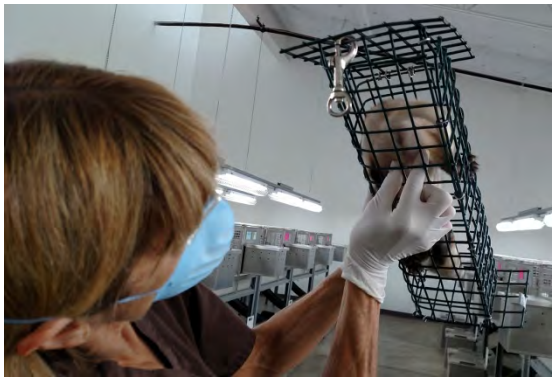
A female BFF usually remains at 90% cornified cells for approximately 30 days, unless she is bred. **Females can cycle a second time after losing a pregnancy or litter; however, these recycle estrus periods are usually much shorter** (for examples of data sheets for males and females, see **Indoor Management Addendum D**).

Important note: any drugs in the sulfa family, including **Albon®** and **SMZ/TMP**, may prevent uterine implantation of the embryo. The most crucial time to avoid these medications is during the 2 weeks prior to breeding and the 2 weeks immediately after breeding. **Any female that has vulvar swelling or is pregnant should not receive any sulfa based medications.** There is some evidence that sulfa drugs may also impair sperm development. The U.S. Fish and Wildlife Service (Service) and Species Survival Plan (SSP) have stopped using sulfa drugs as an anti-coccidial, using ponazuril instead (see **Veterinary Chapter**).

MALE READINESS

Evaluate males for breeding readiness by evaluating testicular length, width, and firmness. Most facilities rely primarily on firmness measurement. Beginning in January, catch males in a restraint device for testicular evaluation, body weight, and general health examination every other week. Record measurements and other pertinent information on each BFF data sheet. In addition, testicles are away from the body and exhibit a grape like firmness, or turgidity. Turgidity is recorded as soft, moderate or firm. Males often attain breeding readiness before females, but they remain capable of breeding for several months.





Because studies show that 1-year-old males are often aspermatic during their first reproductive month, (even though their testicles appear large and firm), it is advisable to use electro-ejaculation to evaluate the readiness of all yearling males. Once males are firm, wait 4 weeks before pairing adult males, and 6 weeks before pairing yearling males. It is recommended that all males are electro-ejaculated to measure sperm concentration (250×10^6 sperm/milliliter (ml)) prior to breeding, although occasionally this is not feasible. If so, prioritize BFFs concentrating on unproven males. Electro-ejaculation protocol is included at the end of the chapter in **Addendum E**.

PAIRING DETERMINATION

It is important to note that methods may vary from year to year depending on current needs of the program and updates/advances in the science and software used. Individuals are first evaluated based on reproductive condition. Once found to be in reproductive condition, managers can select any opposite sex individual also in reproductive condition with whom the first animal has an approved mate suitability index (MSI). Prioritize the best (lowest) available MSI for that individual.

Recommendations Using MateR$_x$

MateR$_x$ is analytical software developed jointly by the National Zoological Park and Lincoln Park Zoo. The primary output is a matrix of genetic ratings (Mate Suitability Indices = MSI) for every possible breeding pair in a population. MSIs allow managers to quickly discover how the genetic status of specimens in their collections compares to the rest of a managed population.

Each MSI represents the genetic consequences for the population if a given pair was to produce offspring. There are seven values for MSIs: offspring of pairs rated 1, 2, or 3 would benefit the population's genetic situation; pairs rated 4, 5, or 6 would be detrimental to the population's genetic situation. Pairs without an MSI value (i.e., a dash [--]) should not be considered under any circumstances without consulting an SPMAG advisor. These MSI values are defined as:

1 – very beneficial
2 – moderately beneficial
3 – slightly beneficial
4 – slightly detrimental
5 – moderately detrimental
6 – very detrimental

MateR$_x$ integrates four genetic factors to produce the Mate Suitability Index (MSI). These four components are currently used by SPMAG members to develop pairing recommendations for SSPs and PMPs. In decreasing order of "importance," they are:

1. the expected change in genetic diversity (increase, decrease) that would result if an offspring of a pair is added to the population;
2. the relative rareness or commonness of the parents genetic information (i.e., the relative dissimilarity of parental mean kinships);
3. the inbreeding coefficient of offspring that would be produced by a pair; and
4. the proportion, if any, of the dam and/or sire's pedigree that is of unknown origin.

Note: ANIMALS MISSING FROM YOUR MATRIX SHOULD BE PAIRED WITH NON FIRST-ORDER KIN (NO SIBLING, PARENT, OFFSPRING PAIRINGS)

MateR$_x$ bins have been customized for 2013 Black-footed Ferret SSP planning
Weighted Method 0.5:1.0:1.5

Modified F bins are as follows:

Breakpoint: 0.1200
No-way point: 0.1500

Bin	1	2	3	x	4	5	6	-
%	0	50	50	x	33	33	34	-
Value	0.000	0.0600	0.1200	x	0.1298	0.1400	0.1500	-
F MSI	0	0	1146	x	13412	4225	892	2042

Modified MSI	0	41	5899	x	12408	1327	0	2042

In 2013, bin distributions changed due to a change in the MateRx software, resulting in larger number "3"s and fewer "2"s and "1"s.

Example chart below:

LOUISVILL
Louisville Zoological Garden

	Females																	
Males V	6868	6870	6869	7401	7072	7452	7458	7476	7464	7073	6949	6948	7488	6690	7402	7403		
7432	4	4	4	3	3	3			4	3	3	3	3	3	3			From CARR
7376																		To CARR
6915	4	4	4	-	3	3			3	3	3	3	-	3	-			
7509	3	3	4	4	3	3			-	3	4	4	4	3	4			
6776	4	4	4	4	3	4			4	4	4	4	4	4	4			
6762	4	4	4	4	3	3			-	3	4	4	3	3	4			
6428	4	4	5	3	3	-			3	3	3	3	3	4	3			
6980	3	3	4	3	4	3			3	4	4	4	4	4	3			
6492	3	3	4	4	4	4			3	5	-	-	4	4	4			
7104	4	4	4	4	3	4			3	3	4	4	4	4	3			
6901	-	-	-	3	4	3			3	3	3	3	3	4	3			
6900	-	-	-	3	4	3			3	3	3	3	3	4	3			
							To CARR	To CARR								To SCBI	To PHX	

INTRODUCTIONS

Originally, BFFs were paired at night, ostensibly to mimic their behavior in the wild. Subsequent video monitoring of paired BFFs revealed that breeding did occur during the day and that breeding was typically undisturbed by daily work activities and moderate noise. All facilities now pair BFFs during the day.

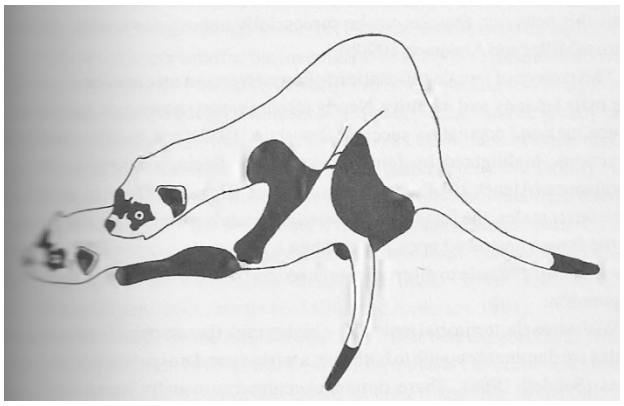
Prior to introducing a designated breeding pair of BFFs, clean the enclosure and set up a video camera on the nest box where breeding is expected to take place, if available. Breeding activity can then be monitored and recorded. Since BFFs are housed individually, a designated breeding pair must be introduced to each other by either taking the female to the male's

enclosure, or by taking the male to the female's enclosure (both methods have proven successful at different facilities). It may also be possible to introduce the pair by opening a door between their two adjoining enclosures. Alert the "resident" BFF to the incoming BFF by having the resident BFF come to the enclosure surface while you have the "visiting" BFF ready to release from a restraint, or as you open the slider between the two enclosures. Animals should have access to lower nest boxes. Release the resident BFF and evaluate the breeding activity via camera, if possible, or by listening.

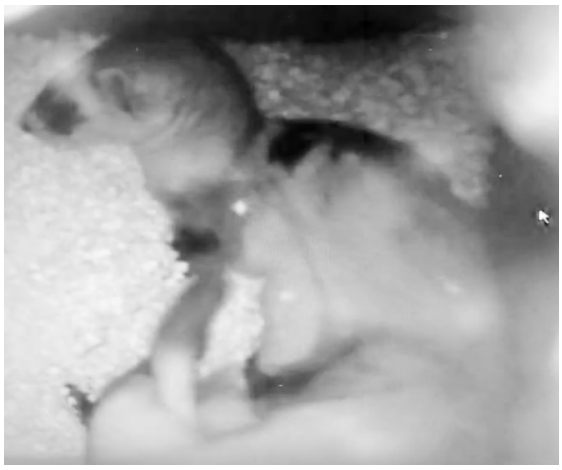
A pair is deemed to be compatible if the female is receptive to the male and allows the male to get into breeding position over the back of the female. A non-receptive female may show signs of aggression or fear. This may be marked by intense high- pitched vocalizations. Remarkably, a 600 grams (g) (21 ounces (oz)) female can hold off a 900 g (32 oz) male if she is not ready to breed. Experience suggests that even if a female's cytology indicates she is ready to breed, the BFF may not agree. In such cases, the animals should be separated and any further breeding attempts must be postponed for a day or two, perhaps with a different male.

If the female's non-receptive aggression is chronic, it may be her personality. In this case, it may be necessary to find a more confident male among her designated pairings. If the female is receptive to the male, but the male is disinterested or produces only short breeding attempts, then another male may be tried the next day. However, **before placing a female with another male, always perform a vaginal lavage on the female to check for the presence of sperm. It may be more prudent to wait a few days before using a new male in order to confirm sire, because it is possible to miss sperm, especially if there are only one or two heads on the slide.** Even if video monitoring indicates that a successful breeding is questionable, the possibility remains that the pair bred. From a genetic management standpoint, it is essential to know which male is the sire of the litter.

If a male is very aggressive with a female and attempts to injure her, **they should be separated immediately**. Some males display overly-aggressive behavior, which can include biting the female on the throat rather than the back of the neck, or outright attacking her. If it is an inexperienced juvenile male, often they can reform their behavior in subsequent pairings. However, some males will chronically attack females and cannot be bred.



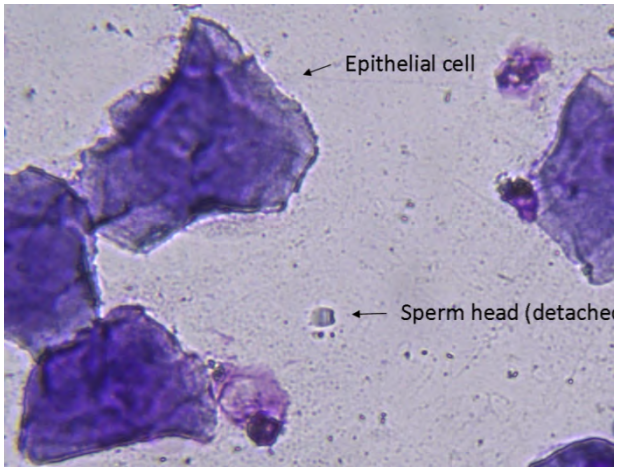
Drawing by Wally Van Sickle

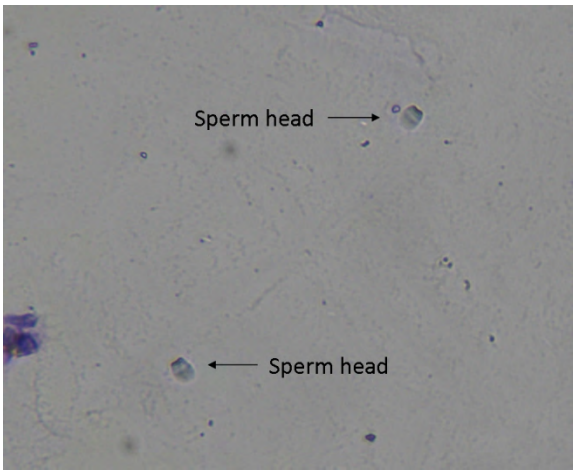


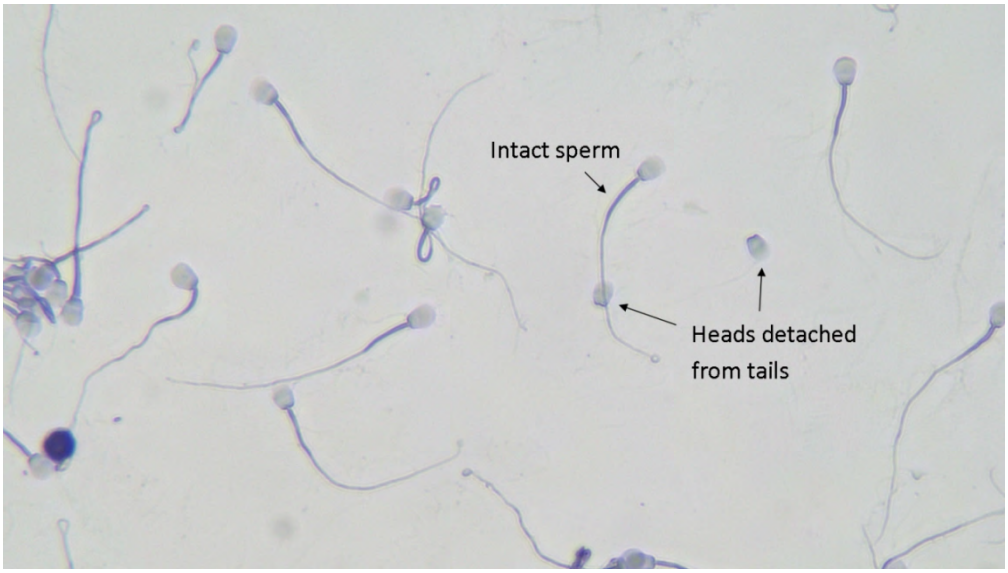
A promising breeding attempt is one in which the male mounts the female's back, and secures himself by grabbing the female around the flank with his front legs while holding the female's scruff on the back of her neck in his mouth. The female's tail is held to one side to allow breeding. Males and females usually remain in this coupled position for one–two hours. They must remain coupled for 15 to 30 minutes for the male to ejaculate. A successful breeding is one in which a post-coital lavage of the female shows the presence of sperm on the slide.

1. A post-coital lavage should be performed within 30 minutes after a promising breeding attempt has concluded.
2. Examine the slide at 10x power. It may take some time to find the tiny sperm heads as they usually dislodge from the sperm tail.
3. Note on their breeding sheets if it is positive or negative for sperm. (You can also record the number of sperm per field, motility and any abnormal sperm on his data sheet)
4. If no sperm are found on the lavage, these animals may be left together and re-checked, or a different male may be tried at the discretion of the attendant. Since BFFs are **induced ovulators**, it is important to have a positive sperm check as soon as possible after breeding. Females usually ovulate 30 hours post coitus.
5. If pairs are left overnight, and a positive sperm check was not accomplished on the first day, it will be necessary to flush the female the next morning and stain the slide to look for the evidence of sperm. Dif-Quik stain is an easy and quick method to look for the presence of sperm. Oftentimes, sperm heads may be the only evidence of a successful pairing.

Photos of lavages for evidence of sperm:







After determining that breeding was successful by identifying the presence of sperm on the post-coital lavage, keep the pair together for a period of about three days. Perform a follow-up vaginal lavage and examine cytology 5–10 days after the positive sperm check to verify that the female has ovulated. If she is still in estrus, pair her again. In rare cases where the female does not ovulate after multiple successful breedings it may be necessary to inject her with luteinizing hormone (LH) to get her to ovulate and come out of estrus. This injection should be timed with yet another pairing. LH may be used multiple years in BFFs to induce ovulation. **hCG, which also induces ovulation, is <u>not</u> recommended as it can only be used once in a BFF's lifetime due to antibody production** (**see Addendum D**).

GESTATION PERIOD

Females should not be handled or stressed after they have ovulated. The amount of diet offered to the bred female is normally increased incrementally to about 120–160 g (4–6 oz) during the last trimester. If a different type of bedding material will be used for the female after

she whelps, change to the new bedding material shortly before the expected whelping date. The gestation period should be 42 days. A few days before the expected whelping date affix a camera to the nest box for monitoring purposes (see **Care of Pregnant Females** in **Indoor Management** section).

BEHAVIORAL SIGNS PRIOR to WHELPING

A bred female BFF decreases her food consumption shortly before whelping. She also spends more time in her lower nest box. Camera monitoring often shows the female to be restless, repositioning herself and licking her sides and genital area. In addition, the attendant may see mammary development or see the female having contractions and/or straining. These behaviors occur throughout the whelping process until all kits are born. (See **Kit Development** and **Whelping and Kit Care** section of this manual).

PSEUDOPREGNANCY

In past years, high rates of non-whelping females were attributed to pseudopregnancy. However, it is unknown if these females were pregnant and aborted or were never pregnant. The lack of whelping in these females may be due to unsuccessful breeding, lack of ovulation, inadequate sperm checks following pairings, poor sperm morphology or concentration or a host of other pregnancy complications.

A pseudopregnant female exhibits the same signs as a pregnant female. She gains weight throughout the gestation period and performs the same whelping behaviors, even to the point of having contractions. Recent studies reveal lower levels of fecal progestin concentrations in the late luteal phase (days 12–40) in pseudopregnant female compared to pregnant females (Young et al. 2002), though not distinguishable to use for management purposes (i.e. a sample can't determine pregnant vs. pseudopregnant).

RECYCLING

If a BFF female loses her litter or ovulates but is not adequately inseminated early in the breeding season, she may recycle and can be rebred. **However, this second estrous cycle, if it occurs, has a rapid onset and short duration. Therefore, close monitoring is necessary in order to achieve a successful breeding during a second estrous cycle.**

ADDENDUM A

USFWS BFFCC PROTOCOL: REPRODUCTIVE READINESS CHECKS AND BFF PAIRING

REPRO CHECKS

Males

- Male assessment should begin in early January. Male reproductive readiness is determined by the firmness of their testicles. Categories are: firm, moderately firm, moderate, soft, nubs (usually juveniles, a hard testicle the size of a corn kernel), and too small (TS). A firm testicle will be about the size and turgidity of a freshly ripe grape.
- Adults can be bred 4 weeks from the date at which they are considered firm. Juveniles can be bred 6 weeks after their firm date.
- Electroejaculation on adult males can be done 4 weeks after firm date on indoor adults. 4 weeks after firm date for adults and juveniles living outdoors, and 6 weeks for juveniles living indoors. It is not necessary for EE to be done prior to breeding.
- After EE or a pairing, males should be given 3–5 days of rest.

Females

- Female assessment should begin the last full week of January. If all females are too small on their first repro check, wait 2 weeks before reassessment. If showing or washable, wash them the following week.
- Female repro checks will be conducted weekly as instructed until all females are out of season.
- When a female reaches 90% keratinized cells on her cytology she is in estrus, wait 5–7 days before breeding her. If the female is 5 years old or older wait 4 days.
- In early May, if there are lots of females in a particular room that are borderline on their cytology and not coming in and cannot be put outside into natural light, the lights in that room may be extended to 16 hours light/8 hours dark until they come into estrus.
- Females in outdoor pens will have repro checks done every 2 weeks starting in March, and then weekly once they start to show. They can be given half-rations the day prior so they will be easier to trap.
- Frequently, putting a female outside into natural light will trigger her to come into season. Whether to put a female outside in early spring or not will be determined by supervisor and vet.

Pairing protocol

- No one other than immediate staff will be permitted in the main BFF rooms. Access by select tours must be approved by supervisor.

- Male and female pairings will be based on Mate Suitability Index (MSI). There will be a matrix taped to the erase board in the main office. Additionally, information pertaining to MSI is also posted in the office.
- There can be two types of pairings (regular SSP [CARR] and possible line breeding of AI kits).
- MSI 1, 2 and 3 are desired, but if necessary, MSI 4, 5 or 6 will be used. A (-) pairing may occur when line breeding AI kits (we don't want to dilute them so that's why we're line breeding; similar to Annie (SB12) line).
- Readiness of both males and females will be determined by testes firmness, electroejaculation, and vaginal cytology counts only.
- Follow-up vaginal washes will be performed on all females 7–10 days following a positive sperm check.
- If a female is still in estrus at her follow-up wash (keratinized cells still >90%), she should be paired immediately (follow same MSI rules). Breeding manager will provide a list of animals for repairing after each cytology review is complete.

Pairing animals:

- Keep all unnecessary noises/talking to a minimum.
- Females will be paired 5–7 days after reaching 90% keratinized superficial cells (REMEMBER: time frame changes when females are still over 90% at follow-up and for recycling females).
- Pairings (male and female) and date will be on erase board in main office. Record all pairings on "Pairing Log" located on wall near alpha-dri. On first day pairings, record the studbook numbers of both animals next to their name. Update the "Pairing Log" as needed.
- All pairings will be done in the morning unless otherwise instructed. Morning pairs will be separated on the morning of the fourth day. Breeding manager will advise of any afternoon pairings after the vet checks the follow-up cytology slides. Afternoon pairings are separated on the morning of the fifth day.
- Close off upper nest box with slider, remove suspended tunnels and do a full clean on female's cage (or cage where animals will be paired) prior to pairing. ADS caps or balls can stay in the cage.
- Place BLUE flag on female's cage to indicate that 2 animals are present. All pairs will be cleaned daily (boxes, cage floor, fresh water for bowl), including the day they are separated.
- Check that selected male is still firm before bringing him to female's cage.
- Bring male to female's cage and release him into the cage thru the main cage door. It sometimes goes smoother if the female is "periscoping" in the tunnel so she can see the male coming.
- Standby and make sure no fighting occurs. Sometimes juvenile females scream although no fighting is occurring. If the male is chuckling that's always a good sign.
- If fighting occurs, female repeatedly tries to get away, or there is no interest after a few hours, proceed to next appropriate male for that female after calling person in charge.

- REMEMBER: females are induced ovulators and will release eggs w/in 25–30 hours of copulation. If paternity is in question, record as UNKnown.
- If compatible, conduct sperm check 1–1.5 hours from time of initial pairing.
- Males should be given 3–5 days rest before being paired with their next female.
- There will be times when pairings will occur in the male's cage or a neutral cage, if available, or on the floor (free-range).
- Clean male's empty cage that day, if possible.
- Conduct a full clean of cages with paired animals every day.

Since sulfa drugs (Albon®) may affect implantation and metronidazole may cause birth defects, **Bred females DO NOT get Albon® or metronidazole during gestation without a prior okay from the vet.**

Cage cards:

- Once a female has been bred, index cards containing dam (and SB#), sire (and SB#), whelp date (gestation is 42 days), food increase (and dates) should be posted on cage. On the back of the card will be written ½ scoop (or 40 grams (g)/1.4 ounces (oz)) rat and the due date.
- 14 days after the first day of pairing, food rations will be increased to one scoop of Toronto or 80 g (3 oz) of meat (whole carcass).
- 21 days after the first day of pairing, food rations will be increased to 1.25 scoops of Toronto or 100 g (3.5 oz) of meat.
- 28 days after the first day of pairing, food rations will be increased to 1.5 scoops of Toronto or 120 g (4 oz) of meat.

Sperm check:

- Label slide w/ female name, date, and sp√.
- Perform a vaginal wash.
- Look for neck stain and other signs that the male has been breeding or attempting to breed. You'll see orange stain on the back of the female's neck. This is where the male grabs her and his saliva turns the fur orange/brown.
- Do not put a cover slip on sperm check slides.
- Using the microscope at 10x power, check for up to 30 seconds to see if sperm are present or immediately spray slide w/ Spray-cyte® fixative and leave for Dif-Quik® staining.
- Make sure sperm check slide does not dry out (see below). If you can't see sperm in 30 seconds, then fix the slide and place on slide warmer for vet or breeding manager to review.

All sperm check slides should be fixed and left for staining even if sperm are present upon initial review!

- If sperm check is positive, record information on "Breeding/Whelping" data sheet, erase board, "Pairing Log", and schedule separation (fourth morning after initial pairing date). Breeding/Whelping data sheet is on wall near Room 3.
- If sperm check is negative, perform additional sperm check at afternoon walk-through. If still negative, do another check first thing in the morning.
- Sperm check slides will not be used to determine female readiness.
- If doing sperm check in holding, it is recommended to label the syringe with female's name and transfer the sample to the main building in the syringe instead of on a slide.

Animal weights:

- Keep track of male and female weights as instructed by the vet.
- Males will typically require increase in diet prior to and during breeding season.
- Females may need to drop weight prior to breeding season, but drastic changes are not encouraged.

ADDENDUM B

EXPECTED PRODUCTION RANGE

Per Paul Marinari August 2013
(Amended by Robyn Bortner 2016)

Expected Production Range (EPR) assessments were developed in the mid-1990s at the U.S. Fish and Wildlife Service's (Service's) request by Astrid Vargas and Ken Gerow in order to have a way to provide:

1) An estimate of the number of kits which should be available to reintroduction sites in a given year prior to any breeding taking place
2) A way to assess and compare how each captive facility is doing in terms of overall management of their collection. If a facility fell below their EPR minimum 3 years in a row, then they would be scrutinized a little more (protocols reviewed, visit by the Service, etc.)

EPR is a range based on the production of the prior 3 years **across all facilities**. It has a minimum, a maximum and an average. The spreadsheet will calculate all three numbers. A facility has "met" its EPR if it meets or exceeds the minimum.

EPR is based on the number of kits weaned per female in the age groups of concern (1–4 years) at the beginning of the breeding season and averaged over the prior 3 years. It includes all females in these age groups in the captive population (not just females from the facility whose EPR is being calculated) so all facilities are graded against the same standard. All females in an age group after fall transfers, even if they are not bred (e.g., juveniles that don't come in or adults that die) are included. Females that recycle during the season are only counted once.

$$\frac{\textbf{\# of kits weaned}}{\textbf{\# females in breeding age group}} = \textbf{kits weaned/female in that age class}$$

For example:

In 2013 there were 48 one-year old females in the SSP population (all breeding facilities)

$$\frac{\text{75 kits were weaned to one-year old females}}{\text{48 females}} = \text{1.6 kits/female}$$

Calculated by age class across the entire SSP population, you get a table such as provided below:

Productivity of BFFs at SSP facilities, 2013. Does not Include AI females.

Age	No. Females	No. litters (% whelping)	No. born	Mean kits born/litter	No. kits born/female	No. weaned (% weaned)	Mean no. weaned/litter	No. kits weaned/ female
1	48	22 (46)	90	4.1	1.9	75 (83)	3.4	1.6
2	60	46 (77)	219	4.8	3.7	195 (89)	4.2	3.3
3	48	24 (50)	92	3.8	1.9	73 (79)	3.0	1.5
4	8	2 (25)	7	3.5	0.9	3 (43)	1.5	0.4
Summary	164	94 (57)	408	4.3	2.5	346 (85)	3.7	2.1

The number of kits weaned/female is used for EPR calculations. Weaned=60 days. Entered into Excel you get the following table (calculated for NBFFCC for 2014). Remember, kits weaned per female per age class by year include all females in the population! You then multiply those averages by the number of females in a single facility to get their individual EPR.

	2011	2012	2013	AVG	SD	MAX	MIN
1-y-old	2.5	2.1	1.6	2.066667	0.450925	2.517592	1.615742
2-y-old	2.2	1.9	3.3	2.466667	0.737111	3.203778	1.729555
3-y-old	0.9	2.1	1.5	1.5	0.6	2.1	0.9
4-y-old	0.7	0.4	0.4	0.5	0.173205	0.673205	0.326795

FCC age	No. of females	avg	sd max	sd min
1	37	76.46667	93.15089	59.78244
2	22	54.26667	70.48312	38.05021
3	39	58.5	81.9	35.1
4	7	3.5	4.712436	2.287564

EPR for 2014

	192.7333	250.2464	135.2202

<---- THIS is the number to meet or beat.

This row represents the range for 2014 for FCC.

In 2014 FCC weaned 182 kits, which exceeds the minimum EPR.

There has always been the debate as to whether or not calculating EPR is a fair measure, and it by no means represents the biological potential of the species. For example, if a facility starts with 10 females, nine die, and the survivor produces a litter, does that facility have 100% or 10% whelping success? This is why the total number of females in the age group is used rather than only those bred in the age group.

2016 Productivity Charts

prodagesum2016.xlsx

ADDENDUM C

PAPANICOLAOU® STAIN

BLACK-FOOTED FERRET (BFF) VAGINAL SMEAR STAINING PROCEDURE USED BY THE U.S. FISH AND WILDLIFE SERVICE (SERVICE) AT THE NATIONAL BLACK-FOOTED FERRET CONSERVATION CENTER (NBFFCC).

The vaginal smear is fixed on the slide by spraying a cytology fixative, then is allowed to air-dry.

Staining Procedure

Dip the sprayed, dried slides into a gradient of ethanol.

3 dips into:	95% EtOH	Slowly
	80% EtOH	
	70% EtOH	
	50% EtOH	
	Tap Water	
	Distilled Water	
5-7 minutes:	Harris' Hematoxylin	
3 dips:	0.25% HCl (in distilled water)	
5 minutes:	Tap Water (slowly running)	
2 dips:	Distilled Water	
3 dips:	50% EtOH	
	70% EtOH	
	80% EtOH	
	95% EtOH	
5 minutes:	OG-6	
3 dips:	95% EtOH (3 separate containers)	
3-5 minutes:	EA-50	
3 dips:	95% EtOH (3 separate containers)	
1 minute:	100% EtOH	
10-20 min:	Xylene or Xylene-free tissue clearing solution	

ADDENDUM D

TO INDUCE OVULATION IN BFF

LH will induce ovulation if female BFF has mature follicles, so female needs to have >90% cornified vaginal epithelial cells. hCG is <u>not</u> recommended*

<u>LH for BFF (recommended for BFFs)</u>

Sioux Biochemical
140 19th St. SW
Sioux Center, Iowa 51250
Phone 712-722-4694; Fax 712-722-4649
siouxbio@mtcnet.net
www.siouxbiochemical.com item #925

LUTEINIZING HORMONE (LH) (PORCINE)
LH from Porcine (pig) Pituitary Glands
25 units=50,000 IU/ bottle, Lyophilized,
~$90/bottle, **keep refrigerated**
****DOSE = 1 UNIT = 2,000 IU**

<u>Conversion of Units to International Units</u>
1 bottle of LH = 25 UNITS
1 UNIT = 2,000 IU (International Units)
1 unit = 2,000 IU (International Units)
25 units LH /bottle = 50,000 IU/ bottle

- Dissolve 25 units in 10 ml sterile water for injection= 2.5units/ml,= 5,000 IU/ml **<u>1 unit = 0.4 ml; 2,000 IU = 0.4ml</u>**
- Give BFF female 1 Unit (= 2,000 IU) for ovulation in about 12 hours
- Intramuscular, one time
- Inject female in evening and pair her the next am (per FWS/Paul Marinari)

This is a lot of LH, but titers decline in less than 1 yr. (i.e., can use 2 consecutive years)

Lower dosages even 0.5 unit, (1,000 IU) may work.

Per Randy Meyer at Sioux Biochem:

OK to dilute aliquots (in sterile water) and store in Ultralow freezer for several years.

***<u>hCG is NOT recommended due to prolonged titers over years; very antigenic</u>** SIGA hCG, 2,500 IU/vial. If need to administer:90 IU im to female one time to ovulate. Will not work on her in following years.

ADDENDUM E

BLACK-FOOTED FERRET SEMEN COLLECTION PROTOCOL 2015
DR. RACHEL SANTYMIRE
LINCOLN PARK ZOO

SUPPLIES

-Electroejaculation box
-0.6 cm probe with 2 electrodes (2)
Box and probes can be purchased from **Carrol Platz at P.T. electronics:**

PHONE#: 503-663-7031; EMAIL: IK9SB@AOL.COM

-Fine sand paper
-KY jelly (NOT spermicidal)
-Gauze with 70% alcohol to clean probe in between black-footed ferrets (BFFs)
-Microscope with phase contrast
-3 Pipetman®® (20 µl for semen collection, 20 µl for fix sample, and 100 µl for TEST)
-Styrofoam box with holes for eppendorfs—or something to keep sample in away from light
-Sterile 1.5ml eppendorfs (May need 1 to 4 eppendorfs per male to avoid urine contamination)
-Hemocytometer
-RBC Unopette (OR Put 1.99ml of water (any type) into cryovial)
-Tips to fit Pipetman® (Sterilize tips in autoclave if not already sterile)
-Exam gloves

ANESTHESIA

-Ketamine (100mg/ml) and Valium (5mg/ml) mixture
-Add 1.5mg Valium (0.3ml) to 1000mg Ketamine (one 10ml vial)
=0.1456mg Valium/ml and 97.087mg Ketamine/ml

***NOTE: Do not make the dose in a syringe. Make an entire bottle of mixture by adding valium to 10 ml vial of ketamine
-Weigh the BFF that day to get the accurate dose.
-Squeeze animal and give intramuscularly.
-Male dose=40mg/kg. (Ex: if have 1.0 kg male, 1.0 kg*40mg/kg=40 mg or 0.40ml)
-Female dose=35mg/kg. (Ex: if have 0.8 kg female, 0.8*35mg/kg=28mg or 0.28ml)
-Animals should be fasted the night before.

MEDIA

-TEST egg yolk buffer with 0% glycerol

Irvine Scientific, 2511 Daimler Street, Santa Ana, CA 92705-5588
phone, 714-261-7800, 800-437-5706; fax, 714-261-6522

-Because TEST comes in 20 ml vials, thaw and divide into 0.5 to 1.0 ml aliquots in sterile vials and freeze for future use. Should not be thawed and refrozen once divided into aliquots.
-Rachel can provide if needed

FIXATIVE (0.3% GLUTARALDEHYDE IN PBS)

-Provided by LPZ. Must request before breeding season.

Rachel M. Santymire, Lincoln Park Zoo
Phone: 312-742-3520
Fax: 312-742-7220
Email: *rsantymire@lpzoo.org*

-Keep in refrigerator until collection. Should be at room temperature for semen collection.
-Can be put back in refrigerator if not used or after a sample has been put into the fix.
-Please label fix vial with BFF studbook #, BFF name, date and your facility.

PREPARATION

1. Thaw TEST 0% and place 100 µl into each eppendorf for collection. Set up 4 eppendorfs.
2. Place dime-size drop of KY jelly on gauze (to lube probe). Keep KY jelly away from penis and semen.
3. Make sure probe electrodes are shiny. If dull, gently clean with fine sandpaper.

Collection area: Electroejaculation box, probes, KY jelly, gauze, gloves, 20 µl Pipetman®, fixative, 20 µl FIX Pipetman®, sterile eppendorf with media and sterile tips. **Optional: You can measure testes with analog calipers.
Microscope area: Microscope (with phase contrast), glass slides with cover slips, Unopette® and hemocytometer.

SEMEN COLLECTION

1. Once the animal is on the table and determined to be in a stable plane of anesthesia, prolapse penis, make sure there is no urine (can dab with dry gauze). Do not use KY jelly.
2. Lubricate the probe well with KY jelly.
3. Make sure the box is turned off and the dial is all the way to 0. Insert the probe gently into rectum with steady forward pressure.
4. Position the two electrodes ventrally such that they are directly over the underlying accessory sex glands.
5. Determine that the animal is stable and that the health care staff is ready for the stimulations to begin. Signal the EE box operator to begin the procedure.

6. Series I generally consists of 10 stimulations at 2 volts, 10 at 3 volts, and 10 at 4 volts. Any series can be modified for any animal depending on size, age, level of anesthesia, semen output and other factors.

Example of Series I:

a. Increase from 0-to-2 volts over a 3 second period ("going up")
b. Hold at 2 volts for 2 seconds
c. Return abruptly to 0 ("going down") and pause for 2 seconds
d. Repeat 10 times
e. Increase from 0-to-3 volts over a 3 second period ("going up")
f. Hold at 3 volts for 2 seconds
g. Return abruptly to 0 ("going down") and pause for 2 seconds
h. Repeat 10 times
i. Increase from 0-to-4 volts over a 3 second period ("going up")
j. Hold at 4 volts for 2 seconds
k. Return abruptly to 0 ("going down") and pause for 2 seconds
l. Repeat 10 times
m. Rest the animal 3-5 minutes between each series while processing the sample.
n. Massage the probe in the rectum between each series.

- While stimulating the animal, apply gentle, ventral pressure to the probe.
- If the probe needs to be repositioned, do so between stimulations and not during.
- Good communication between the collector, the box operator, and the health care staff is crucial.
- Semen is collected off the tip of the penis using the 20 µl Pipetman®. Estimate the volume and put it directly into the TEST 0%. **To estimate this volume, it helps to have a separate tip to use as a reference. It should be pre-marked at 1, 2, 4 and 10 µl.** Make sure to slowly mix the sample in and out of pipette tip. Discard tip after mixing.
- During the collection, 1 to 2 µl of RAW semen should be placed into fixative for evaluation of sperm morphology to be sent to Rachel Santymire. Make sure to use the "**fix Pipetman®**". Place the semen immediately in 0.3% glutaraldehyde fixative at room temperature. Keep at room temp for at least 15 minutes, then store at 4^{o}C. **Prevent accidental use of the semen Pipetman® in the fixative vial and accidental use of the fix Pipetman® in the raw semen.**
- Discard the tip from the Pipetman® often during collection.
- If **urine contamination** occurs, the sample will be less viscous than semen and will have a yellow tinge. If an ejaculate is suspected to contain urine, place it into a separate eppendorf vial with TEST. This sample should be evaluated separately, because it could affect the overall sperm assessment. Remember to frequently change the tips used to collect the semen from the penis. (The TEST 0% is also yellow making urine contamination determination difficult.) Urine does not necessarily mean the male is not ready to pair with a female. It simply could be caused by the placement of the probe. Urine will dilute the sample and can kill the sperm.

7. 'Rest' the animal (~ 3-5 minutes) in between each series.
8. Series II generally consists of 10 stimulations at 3 volts, 10 at 4 volts, and 10 at 5 volts.

9. Series III generally is 10 stimulations at 4 volts and 10 at 5 volts. Again, this can be modified as needed.
10. Series IV generally is 10 stimulations at 5 volts and 10 at 6 volts.

****Staff can give some fluids and penicillin to the male after the procedure.**

SPERM MOTILITY EVALUATION

1. After each successful series, place a small volume (3-4 µl) of the diluted semen on a pre-warmed slide and place it on a warmed microscope stage. (Place a small bag of warm fluids on stage to pre-warm it to ~37°C/99°F).
2. Immediately determine percent motility and status of progression (0-5)
   Motility:
   Count the percentage of sperm moving in several fields and average.
   Status of Progression:
   0 = no movement
   1 = twitching with no forward progression
   2 = side-to-side movement with little forward progression
   2.5 = moving forward slowly or in circles
   3 = moving forward at a slow steady pace but with no circles
   3.5 = moving forward at a steady quick pace
   4 = moving forward rapidly
   5 = moving very quickly; hyperactive

OVERALL SEMEN ASSESSMENT

1. If all samples are comparable, they may be combined into one eppendorf at the end of the procedure. Keep and evaluate urine contaminated samples separately.
2. Slowly and gently add each aliquot to the container and mix well.
3. Determination of sperm concentration:
   -Remove 5 µl of diluted sample
   -Place this volume into Unopette® (OR cryovial…see recipe above) and mix well
   -Let Unopette® sit at room temperature for at least 5 minutes while sperm die
   -Mix well and load sample into hemocytometer (one drop on each side) and let sit for 5 minutes.
   -Count number of sperm in four corners (16 squares in each corner) on each side of hemocytometer and then average the number.
   4. Make sure to fill out the EEJ sheet completely. Send all fix and EEJ datasheets to Rachel at the end of the breeding season.

DETERMINATION OF SPERM CONCENTRATION

Example
Overall motility = 80%
Total raw semen volume = 20 µl
Total TEST 0% volume = 200 µl

Average sperm count on hemocytometer= 15
Concentration of sperm in the diluted sample = 15 x 10^6 / ml
Sperm Concentration:
(Raw semen) * (X) = (raw+Test volume) * (hemocytometer count)
20 * X = 220*15
X=165
165 x 10^6 sperm/ ml in raw sample

***A male can be used to pair with a female when his sperm concentration is at least 250 x 10^6 sperm/ ml.**

Total Sperm Count:
Total sperm count = (total volume of raw semen) * (Sperm concentration)
= (0.020 ml) * (165 x 10^6 sperm/ ml)
= 3.3 x 10^6 total sperm

Total Motile Sperm:
Total motile sperm = (total sperm) * (%motile)
Total motile sperm = (3.3 x 10^6 sperm/ ml) *(.80) = 2.64 x 10^6 total motile sperm

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ASSISTED REPRODUCTION IN BLACK-FOOTED FERRETS USE OF SEMEN ANALYSIS, ARTIFICIAL INSEMINATION, AND A GENOME RESOURCE BANK TO ENHANCE REPRODUCTIVE EFFICIENCY

INTRODUCTION

Reproductive biotechnology offers many advantages for enhancing reproduction and maintaining genetic diversity in small populations. The use of assisted reproductive techniques such as artificial insemination (AI: deposition of sperm into a female) provides an approach for improving reproductive efficiency in animals that demonstrate poor breeding performance. This strategy combats behavioral incompatibility between individuals and helps ensure reproduction in genetically valuable animals. These techniques especially benefit species like the black-footed ferret (BFF) that are propagated under the auspices of a genetic management plan such as the Species Survival Plan® (SSP). The SSP provides breeding recommendations in an attempt to equalize genetic representation of the few original wild-caught founders, and cooperating institutions breed animals on the basis of genetic value and how related an individual is to the rest of the population (termed 'mean kinship') (Ballou and Lacy, 1995). The use of AI offers an alternative to natural breeding when recommended pairings fail to reproduce.

The potential of assisted reproduction is enhanced further by sperm cryopreservation that saves valuable genetic material for future generations. The development of a Genome Resource Bank (GRB: a repository of cryopreserved sperm) offers a feasible strategy for infusing germ plasm into a genetically stagnant population or transferring sperm between geographically separated populations (Wildt et al. 1997). In species that have short life spans (like the BFF), the use of cryopreserved sperm extends the reproductive life of an individual. In the BFF recovery program, assisted reproductive techniques have been demonstrated to be effective and, currently, are being utilized in the management of this endangered species.

DEVELOPMENT OF ASSISTED BREEDING TECHNIQUES IN 'ANIMAL MODELS'

Since the beginning of the BFF captive-breeding program, the potential benefits of reproductive technology were recognized. A workshop facilitated by the Conservation Breeding Specialist Group (CBSG) in 1986 and the U.S. Fish and Wildlife Service's (Service) Black-footed Ferret Recovery Plan encouraged development of techniques for fertility assessment and assisted reproduction, including semen collection and AI using fresh or cryopreserved sperm. A high priority for protecting extant genetic diversity was to establish a 'Black-footed Ferret Genome Resource Bank' containing a frozen repository of sperm from the most genetically valuable males, especially those that had failed to reproduce.

The Smithsonian Institution's National Zoological Park (NZP) was requested to study the reproductive biology of BFFs, largely for the purpose of developing AI as an alternative strategy to natural breeding. The zoo's Reproductive Physiology Program began reproduction studies

using the domestic ferret (*Mustela putorius furo*) and Siberian polecat (*Mustela eversmanni*) as 'animal models' to first understand BFF biology, and then used that knowledge for developing assisted breeding techniques in BFFs. The justification for these specific research models was that the domestic ferret, its relative the Siberian polecat, and the BFF are taxonomically in the same genus and genetically similar as detected by molecular analyses (O'Brien et al., 1989). Most importantly, the reproductive cycles are similar among these species, with testes size, vaginal cytology, and vulvar tumescence changing throughout the year. Finally, these species are classified as 'induced ovulators' with ovulation occurring ~30 hours after a single copulation, or after an injection of human chorionic gonadotropin (hCG).

Extensive reproduction studies in domestic ferrets were conducted to develop a reliable approach for collecting, processing and maintaining fresh or cryopreserved sperm for AI. Over 300 semen samples from nine males were collected to address issues of practical application of assisted reproduction techniques in BFFs including: 1) development of an effective electroejaculation technique and optimal sperm processing methods; 2) the effect of temporal spermatogenesis patterns on sperm viability; 3) the comparative effectiveness of vaginal insemination versus uterine insemination using a laparoscopic technique for sperm deposition *in utero*; 4) the influence of sperm number, dilution medium and time of hCG injection (used to induce ovulation) on pregnancy rate, gestation interval, and number of offspring produced; and 5) the influence of semen cryodiluent, freezing method and thawing temperature on the biological competence of frozen-thawed BFF sperm (Wildt et al., 1989; Howard et al., 1991).

These efforts were highly successful in characterizing the basic reproductive biology of BFFs and developing reliable assisted breeding techniques. Vaginal insemination was determined to be an ineffective method for artificially breeding BFFs; none of the ten females became pregnant after sperm was deposited vaginally (Table 1). However, transabdominal intrauterine sperm deposition via laparoscopy resulted in a high pregnancy rate (Table 1). Seventeen of the 24 BFFs (70.8%) inseminated *in utero* laparoscopically became pregnant and delivered live young (Wildt et al., 1989). Comparative assessments of 12 cryopreservation methods determined that an egg-yolk/lactose cryodiluent (designated as 'PDV',) combined with the pellet method of sperm freezing and a 37°C thawing temperature, was effective for recovering viable BFF sperm after cryopreservation. Using this cryomethod, a high pregnancy rate was achieved; seven of ten females (70.0%) inseminated *in utero* with frozen-thawed BFF sperm became pregnant (Table 2) (Howard et al., 1991).

The large-scale, systematic research approach in the domestic ferret was important for generating knowledge leading to development of assisted reproduction. Because of the high success of AI in domestic ferrets, it was concluded that the same technology could be assessed in the Siberian polecat, and finally the endangered BFF. Comparative semen evaluation revealed differences in sperm quality among the three ferret species. Although sperm motility was similar, the proportion of structurally normal sperm was lower in BFFs than in polecats and domestic ferrets (Table 3) (Wildt et al., 1989; Howard et al., 1991, 1996).

Sperm survival after cryopreservation also varied among species. Percentages of post-thaw sperm motility and intact acrosomal membranes were lower in the BFFs than in the domestic ferrets and Siberian polecats (Table 3) (Howard et al., 1991, 1996). These differences

in sperm viability were attributed to the restricted founder base and reduced genetic variation in the BFFs. Nevertheless, laparoscopic intrauterine AI developed in the domestic ferret proved effective in its close-relatives (Table 2). Six of the seven (85.7%) Siberian polecats inseminated with fresh or cryopreserved semen became pregnant. This high pregnancy rate in polecats allowed for direct assessment of the AI technique in BFFs. In the initial study, four of six (66.6%) BFFs inseminated with fresh or frozen-thawed semen became pregnant and delivered live young (Howard et al., 1996). These results demonstrated that animal model research was beneficial for developing efficient reproductive biotechniques applicable to the endangered BFF.

Using Reproductive Technology to Enhance Reproductive Efficiency in BFFs

Despite the success of the captive breeding program, it was determined that the goal of the BFF Recovery Program (1,500 breeding BFFs in at least 10 free-ranging populations by the year 2010) would not be achieved at the current rate of propagation. To assist the Service and its numerous partners with recovery efforts, the Association of Zoos and Aquariums (AZA) was asked to organize a comprehensive program analysis and action planning process. This resulted in workshops focused on captive breeding, reintroduction/field conservation and education/funding/public relations.

The goals of the captive-breeding workshop, conducted in 1995, were to identify factors affecting reproduction success and develop a strategy to improve breeding efficiency, thus increasing the number of potential reintroduction candidates. Many issues affecting reproduction (such as neonatal mortality, husbandry practices, stress, nutrition, and disease) were evaluated. Interestingly, a detailed assessment of the breeding records revealed that a remarkably high proportion (>50%) of males considered to be prime breeding age (one, two, and three years old) failed to sire offspring in captive breeding situations for unknown reasons. Specifically, in the 1995 breeding season, 40 adult males (ages one to three years) did not sire young, representing 54.8% of the prime breeding age male population of 73 males.

Additionally, assessment of the genetic management program revealed that one of the original wild-caught BFFs was poorly represented in the modern population. Although the captive population began with 18 animals, only seven animals are considered 'founders' since many of the original 18 BFFs were related. The underrepresented founder lineage ("Annie" lineage) had only 43 descendants compared to more than 300 descendants from each of the six remaining founders. To help preserve original gene diversity in the small population, it was imperative to balance founder representation. This situation was exacerbated by the fact that the closest male descendant of the under-represented “Annie” lineage exhibited behavioral problems and severe aggression towards females during mating. Together, these issues prompted a recommendation to use reproductive biotechnology in the management of the BFF captive population.

The reproductive physiologists at the National Zoological Park's Conservation & Research Center (NZP-CRC) responded and agreed to: 1) assess reproductive traits and breeding behavior in male BFFs that failed to sire offspring for comparison with proven breeder males; 2) establish a BFF Genome Resource Bank containing a frozen repository of sperm from the most genetically valuable males, especially those with compromised reproduction; and 3) use AI to improve founder representation and enhance reproductive efficiency in non-breeders. In 1996

and 1997, a survey was conducted to determine the proportion of prime breeding age males not siring offspring and the reasons for failed reproduction. As observed in 1995, a high percentage of one to three year old males failed to sire young in 1996 (38 of 69 males, 55.1%) and 1997 (35 of 60 males, 58.3%; Wolf et al., 1998). Evaluation of ejaculate traits determined that sperm concentration, percent sperm motility, and the incidence of normal sperm were similar between proven and non-proven breeders (Wolf et al., 1998). Detailed review of breeding records revealed other causes for male reproductive failure including improper breeding positioning, behavioral incompatibility (such as aggression) and poor testes development (Fig. 1) (Wolf et al., 1998; Howard et al., 1998). Breeding records also revealed a high incidence (~40%) of pseudopregnancy each year in females.

Historically, male BFFs have been selected for breeding based on testicular size and tone. If testes were enlarged and firm, then males became candidates for mating. Recently, however, some males failed to produce spermic ejaculates. Results indicated that the onset of sperm production in one-year-old males was delayed in the breeding season compared with the older males (Howard et al., 1998). These young males appeared to be good breeders based on maximum testicular development and appropriate copulatory behavior, yet produced aspermic (no sperm) or oligospermic (few sperm) ejaculates. The asynchrony in sperm production among male BFFs may have influenced the incidence of pseudopregnancy since aspermic males can induce ovulation following copulation. This issue prompted the National BFF Conservation Center (NBFFCC), in collaboration with reproductive physiologists at NZP-CRC, to conduct semen evaluations and select males on the basis of spermic ejaculates rather than testes size. Prior to breeding encounters in 1998, males were anesthetized for electroejaculation and semen analysis. Males became candidates for breeding only if spermic ejaculates were obtained. Using this new selection strategy, a significantly higher whelping rate (80%; 69 litters from 86 mated females) was achieved in 1998 at the NBFFCC compared to the 'testes size' selection strategy used in 1997 (60% whelping rate; 50 litters from 84 mated females) (Table 4). As a result, the numbers of kits born increased from 190 kits in 1997 to 249 kits in 1998, thus increasing the number of young for reintroduction. Overall, these findings demonstrated that semen collection techniques are beneficial for assessing reproductive potential in male BFFs. Used in combination with natural breeding, this strategy is effective in improving pregnancy rates, increasing the number of young produced and improving reintroduction capabilities.

Using Assisted Breeding to Improve Reproduction in Genetically Valuable 'Non-Breeders'

To establish an AI program for non-breeders, the BFF propagation program at the NZP-CRC changed from a natural breeding program (initiated in 1988) to an assisted breeding program in 1996. The goal of the new program was to: 1) enhance reproductive efficiency in genetically valuable males that failed to sire young; and 2) use AI to produce offspring from non-breeding males thereby increasing the number of kits born. The most genetically valuable, non-proven males were transferred to the facility. Specific non-proven individuals at the NBFFCC also were chosen as candidates for assisted reproduction. Females were monitored for natural estrus as detected by changes in vulvar size and vaginal cytology. Five to seven days after females reached >90% cornified vaginal cells and exhibited maximum vulvar swelling females were given a 90 IU hCG injection to induce ovulation and subsequently anesthetized 12-20 hours later for AI. Under laparoscopic observation, 22 females were inseminated *in utero* with fresh or

frozen-thawed semen (in 18 and 4 females, respectively) from BFF males classified as 'non-breeders'. Pregnancies were achieved in 16 female BFFs, resulting in an overall pregnancy rate of 72.7% (Table 2). Fourteen of the 18 (77.8%) females inseminated with fresh semen became pregnant and produced 44 kits. Two of the four (50%) females inseminated with frozen-thawed semen became pregnant and delivered three kits. One of the cryopreserved semen samples that resulted in a pregnancy was from a male that had died approximately six months before the insemination date, illustrating the value of cryopreserved germ plasm after the death of the male. These assisted breeding techniques continue to be used successfully in BFF recovery to enhance reproductive efficiency in those animals which fail to reproduce naturally.

DEVELOPING A BFF GENOME RESOURCE BANK

The usefulness of any GRB plan ultimately depends on the selection of individuals used to establish the cryobank. To develop a BFF Genome Resource Bank, a systematic sperm cryopreservation strategy was utilized in 1996 to designate male BFFs for sperm banking. Using the GENES pedigree analysis software within the computer program SPARKS, four sperm donor selection strategies described by Johnston and Lacy (1995) were compared: I) All Male Bank; II) Culling Method Bank 1; III) Culling Method Bank 2; and IV) Founder Method Bank. All strategies were based on founder representation and mean kinship (relatedness between individuals in the population) and differed in number of animals selected and percent genetic diversity retained.

The 'Culling Method Bank 1' method was chosen as the optimum strategy for sperm donor selection in BFFs due to retention of maximum genetic diversity using the minimum number of males. The other selection strategies failed to produce most of the gene diversity present in the living male population, presumably because the smaller number of males included in those GRBs provided fewer options for further genetic management. The 'Culling Method Bank 1' method utilizes the mean kinship value of each individual and involves the computerized removal of all females (since only sperm banking is desired) from the 'living' population in the computer database. Then the lowest ranking male with the highest mean kinship value (highly related to the population) is 'culled' from the database. Iterative culling of males from the pool of potential sperm donors is continued until the removal of a further male would cause the gene diversity of the pool to decrease. The males remaining in the population at that point are the candidates selected for the GRB. Interestingly, at that point, there is an increase in gene diversity relative to the actual male population because the males most related to the population have been removed and only genetically valuable individuals remain. The iterative culling of males with the highest mean kinship is important because the mean kinship of the animals remaining in the pool will change as each male is removed from the population.

During the 1996–1998 breeding seasons, the 'Culling Method Bank 1' method was used to select BFF males for inclusion in the GRB. To preserve valuable germ plasm, semen was collected and cryopreserved for long-term storage or future AI procedures. Distribution of the cryopreserved sperm requires direction by authorities with regulatory interests and/or conservation responsibility. The final determination for proposed uses of cryopreserved sperm is the responsibility of the Service. It is important to realize that the GRB is an active repository of

genetic material. Samples are continually being deposited for storage and removed for AI procedures. Furthermore, it is important to store multiple sperm samples from a genetically valuable male.

In 2006, the BFF SSP decided to evaluate the effectiveness of GRB samples that had been stored for up to 20 years. If the samples were still viable, could the use of them effectively restore lost gene diversity? From 2008–2011, semen samples stored in the BFF GRB for 10–20 years were being used for AI (Howard and Wildt 2009; Howard *et al.* 2015). Of the 18 females artificially inseminated with the frozen-thawed GRB semen, five (27.7%) became pregnant producing eight genetically valuable offspring (mean litter size, 1.6 ± 0.4 kits; range, 1-3 kits). Incorporating these offspring into the population improved gene diversity by 0.2% and lowered inbreeding coefficients by nearly 6.0% (Howard *et al.* 2015). However, the pregnancy rate of AI using frozen-thawed semen was overall lower (33%; Howard et al. 1991; 2006; 2015) compared to AIs with fresh semen (59.8%), which was comparable to natural breeding (Howard and Wildt 2009). Litter size was also reduced when frozen-thawed semen was used (~2 kits/litter) compared to AIs using fresh semen (~4 kits/litter; Howard *et al.* 1991; Howard *et al.* 2006; Howard and Wildt 2009; Howard *et al.* 2015). Over the last 27 years, a total 310 (74 wild; 236 captive) semen samples from 225 males have been cryopreserved. The SSP plans on continuing to collect samples from both captive and wild BFFs.

FECAL HORMONE MONITORING

Conventional methods for tracking endocrine status generally rely upon repeated blood sampling, an unrealistic approach for most non-domestic species because of the stresses associated with restraint and/or multiple sedations. Instead, measuring steroid (estradiol, progesterone, and testosterone) metabolites in excreted feces or urine provides a novel strategy to generate longitudinal hormone profiles non-invasively. During the last decade, monitoring excreted reproductive steroid metabolites has proven effective for characterizing seasonal reproductive patterns, estrous cycle length, mechanism of ovulation (spontaneous vs. induced), and duration of pregnancy in a host of non-domestic species.

Knowledge of the reproductive endocrinology of the BFF is limited, and one re-occurring problem is the phenomenon of pseudopregnancy (mating, gross appearance of pregnancy, but no offspring born). Although aspermic ejaculates in young males contributed to the rate of pseudopregnancy in BFFs and whelping rates increased from 60% to 80% by assessing sperm status before mating, this species still experiences an approximate pseudopregnancy rate of 20%. More information on the basic reproductive endocrinology is needed to evaluate possible hormonal causes of this fertility problem. Therefore, reproductive endocrinologists at NZP-CRC conducted a study to validate and use fecal hormonal monitoring in BFFs to: 1) characterize reproductive cycles throughout the year in females and males; 2) compare hormonal patterns during pregnancy and pseudopregnancy; and 3) correlate endocrine data with changes in vaginal cytology and vulval tumescence during the breeding season. Longitudinal profiles of gonadal activity and steroid excretion were generated in male and female BFFs maintained in outdoor enclosures at CRC with exposure to natural fluctuations in photoperiod (Brown et al., 1997). Elevated concentrations of fecal estradiol were associated with an increase in vulvar swelling

and the percentage of superficial cornified epithelial cells in vaginal lavages and subsequent mating activity in April (Fig. 4, Fig. 5). Fecal progestogen concentrations increased about one week after breeding, peaked at midterm and then declined to baseline at parturition (Fig. 4, Fig. 5). Females experiencing pseudopregnancy or death of kits recycled in late June, and several females delivered offspring in early August (Fig. 4). On the basis of days between first mating and parturition, gestation length was 42.6 ± 0.5 days. Assessment of seven pregnancies and nine pseudopregnancies revealed no differences in temporal or quantitative fecal estradiol and progestogen metabolite excretion between these two reproductive states (Fig. 5). Males exhibited seasonal fluctuations in fecal testosterone metabolite excretion with increased concentrations observed several months before females entered estrus (Fig. 6). Fecal androgen concentrations were baseline from July to October, and then increased from November through June. Interestingly, fecal testosterone metabolite in males began to rise as early as November, whereas females exhibited estrus and subsequent breeding in mid- to late April.

This project demonstrated the utility of fecal estradiol, progestogen and androgen metabolite analyses for non-invasively assessing gonadal function in the BFF. Databases of endocrine norms were established, as well as investigating factors influencing reproductive function. By comparing estradiol and progesterone excretory profiles during pregnancy to those during pseudopregnancy, however, the cause of the latter reproductive state was not determined. Pseudopregnancy in BFFs continues to be an obstacle to increasing overall reproductive efficiency in this endangered species.

SEMEN COLLECTION AND PROCESSING

To assess male reproductive status in BFFs, electroejaculation is an effective technique for semen collection. A detailed description of sperm collection and evaluation is presented in **Appendix 1**. In brief, males are anesthetized with a combination of ketamine (37 mg/kg) and diazepam (0.06 mg/kg). A surgical plane of anesthesia is achieved usually within three minutes and is maintained for ~30 minutes. Each male is subjected to ~20 minutes of electrical stimulation using an AC, 60-Hz sine-wave electroejaculator and a 6 mm diameter rectal probe with 3 longitudinal electrodes**.** After insertion of the lubricated probe into the rectum, the penis is everted manually and, if needed, cleaned gently with saline-soaked gauze and then dried. The electrical stimuli are given in a 3-seconds-on and 3-seconds-off pattern with a continuous rise in voltage from zero to the desired peak, then returning to zero. A total of five series of 30, 30, 20, 20 and 20 stimuli are given with a three to four minute rest period between series. During each rest period, the rectal probe is manipulated gently within the rectum. Within series, sets of 10 stimuli each are applied at 2 to 6 volts. Seminal drops (in µls) resulting from each series are collected from the tip of the glans penis by using an automatic pipette with sterile plastic tips warmed to 37°C. The volume of each seminal drop is determined for calculation of ejaculate volume. Each seminal aliquot is transferred immediately into a 1.5 ml plastic, microcentrifuge tube at 37°C containing 100 or 200 µl of either 'PDV-egg yolk diluent' or 'Test Yolk Buffer diluent'. If the sample is to be cryopreserved, the diluent should contain 4% glycerol. If the sample is to be used for insemination with fresh semen, then glycerol is not necessary in the egg yolk diluent. In early studies to determine an optimal cryodiluent, PDV-egg yolk diluent was chosen for its cryoprotective abilities in BFFs (Howard et al., 1991). However, recent studies

demonstrated that the commercially available sperm diluent 'Test Yolk Buffer' (TYB; Irvine Scientific, Santa Ana, CA) modified to contain 4% glycerol was superior to 'PDV' for maintaining sperm motility and acrosomal membranes (see Appendix 1 for composition of modified cryodiluent). For assessment of sperm morphology, a 2 µl aliquot of raw semen is fixed in 50-100 µl of 0.3% glutaraldehyde in phosphate buffered saline. Phase-contrast microscopy (x1000) is used to assess 200 sperm/ejaculate for the incidence of normal and abnormal sperm forms (Fig. 2). An aliquot of fixed sperm also is used to assess sperm acrosomal membranes, classified as one of the four categories: 1) normal apical ridge; 2) damaged apical ridge; 3) missing apical ridge; and 4) loose acrosomal cap (Fig. 3).

At the end of electroejaculation, the sperm suspension is mixed well, and a 3 µl sample is placed on a glass slide under a cover slip and evaluated for sperm percent motility and status (forward progression; scale of 0 to 5, 5 = best). A 5 µl aliquot of diluted semen is used to calculate the sperm concentration/ml of the suspension using a standard hemocytometer method. Appropriate adjustments for the dilution medium are made to determine sperm concentration/ml of ejaculate. Total number of motile sperm/ejaculate is calculated by multiplying the sperm concentration/ml of ejaculate, times the ejaculate volume, times the percent sperm motility value. Each sample is used immediately for insemination, or cryopreserved, if sperm quality is sufficient.

SEMEN CRYOPRESERVATION

A detailed description of the sperm cryopreservation technique is presented in **Appendix 1**. In brief, freshly collected semen is diluted in 100-200 µl 'Test Yolk Buffer' (TYB; Irvine Scientific, Santa Ana, CA) modified to contain 4% glycerol (Appendix 1). The minimum acceptable criteria for freezing a BFF sperm sample are ejaculates containing: a) at least 2 million motile sperm; and b) forward progressive sperm motility ratings of at least 2.5 (scale 0-5, 5=best). The post-thaw viability of ejaculates containing fresh viability ratings less than these probably are inadequate for use in assisted reproduction. However, it may be important to cryopreserve all collected sperm samples from genetically valuable males using present technology. Although this germ plasm may be minimally useful for assisted reproduction, it may have important alternative value in the future. The diluted aliquot is cooled slowly for 30 min at 5°C and then pelleted (~30 µl/pellet) on dry ice for three minutes before plunging into liquid nitrogen. Pellets then are packaged into a cryovial that is labeled with the species, date of collection, location of collection and animal studbook number.

To evaluate post-thaw sperm viability, one pellet from each sample can be thawed and evaluated *in vitro* as described above for freshly collected sperm. In these cases, a pellet is thawed in a sterile 12 x 75 mm glass culture tube containing 70 µl Ham's F10 culture medium in a water bath at 37°C for 20 seconds. Thawed semen suspension is transferred into a 1.5 ml plastic, microcentrifuge tube and centrifuged at 300 g for eight minutes. Supernatant is removed, and sperm pellet is resuspended in fresh Ham's F10 medium. Aliquots are evaluated for sperm motility and forward progressive motility at 0, 30 and 60 minutes post-thaw. Sperm acrosomal integrity is assessed and categorized in one of four morphological classes: sperm with a normal apical ridge, damaged apical ridge, missing apical ridge and loose acrosomal cap (Fig. 3). To

determine an overall sperm viability rating with equal emphasis on both sperm percent motility and progressive motility, a sperm motility index (SMI) is calculated [(sperm progressive motility X 20) + (percent sperm motility), then divided by 2].

INTRAUTERINE ARTIFICIAL INSEMINATION

Due to sexual incompatibility resulting from inappropriate positioning, apathy, or aggression, certain genetic pairings have been unsuccessful. Because a high representation of available genetic material is desired, AI can be used to propagate non-breeding individuals. A detailed description of the laparoscopic intrauterine AI technique developed for BFFs is presented in **Appendix 2.** In brief, females are given an intramuscular injection of 90 IU hCG to induce ovulation 12 to 20 hours prior to laparoscopy. This hormonal regimen is effective in BFFs; however, an interval of more than one year should be allowed between consecutive hCG trials to prevent an antigenic response. For insemination, freshly collected sperm diluted in 'Test Yolk Buffer' or cryopreserved sperm thawed and processed in Ham's F10 medium should exhibit at least 30% motile sperm with a progressive motility rating of 2.5 or greater. The sperm suspension is maintained at 37°C and should be deposited into the female within 30 to 60 minutes after initial dilution or thawing. Each female is anesthetized with a ketamine (25-35 mg/kg) and diazepam (0.12-0.17 mg/kg) combination, surgically prepared, and then subjected to laparoscopy using a rigid laparoscope, 5 mm in diameter. A laparoscopic cannula assembly is inserted through a 1 cm skin incision made ~2 cm cranial to the umbilicus. An abdominal pneumoperitoneum and the use of a 2 mm in diameter ancillary probe (inserted lateral to the midline) are used to observe and manipulate the female reproductive tract.

In BFFs, ovaries are embedded in adipose tissue and are not visible. Each uterine horn is identified and cannulated for direct *in utero* deposition of sperm. A sterile, indwelling catheter (22 g) is inserted through the abdominal wall into the uterine lumen of each respective horn. Upon penetrating the lumen, the stylette is withdrawn from the catheter cannula. Visual movement of the uterine horn with passage of the catheter through the lumen ensures that the catheter tip is positioned into the proximal half of the horn. Diluted semen (50–100 µl) then is deposited in the catheter for intrauterine insemination. Immediately after depositing a second 50-100 µl seminal aliquot in the contralateral horn, the catheter, laparoscope, and ancillary probe are removed and the midline incision site sutured. Anesthetized females are monitored closely until fully recovered and then managed similar to females that bred naturally. Pregnancy and number of kits produced are recorded.

ITEMS FOUND TO BE UNACCEPTABLE OR PROBLEMATIC

ITEM	PROBLEM
hCG	**BFF develop titers so can only be used one**
Non-recommended photoperiod	**Can disrupt repro cycle**

RECOMMENDATIONS

ITEM	RECOMMENDATION
Electroejaculation	**All males before paired every year**
Vaginal cytology	**Pap stain**
Semen Cryopreservation	**Proven and non-proven breeders when possible**
AI	**Using Aggressive and genetically valuable males**
LH	**Use LH on AI females and natural breeding females that aren't ovulating after 2 (? Or 3) pairs**

APPENDIX 1
SEMEN COLLECTION, EVALUATION, AND CRYOPRESERVATION

A. SEMEN COLLECTION AND PROCESSING

PREPARATION OF SEMEN CRYODILUENT: MODIFIED TEST YOLK BUFFER ("TYB")

Various semen cryodiluents have been evaluated for BFF sperm cryosurvival, however, a modified TEST Yolk Buffer containing 4% glycerol currently provides the optimum cryoprotection. The following is a description of the preparation of this commercially available cryodiluent (marketed for human sperm cryopreservation; Irvine Scientific, 2511 Daimler Street, Santa Ana, CA 92705-5588; phone=714-261-7800, 800-437-5706; fax=714-261-6522). Irvine Scientific sells two "TEST Yolk Buffer" products containing a 20% egg yolk solution:

1) "Refrigeration Medium-TEST Yolk Buffer" containing 0% glycerol (catalog#9972)

2) "Freezing Medium-TEST Yolk Buffer" containing 12% glycerol (catalog#9971)

The "Refrigeration Medium-TEST Yolk Buffer" containing no glycerol can be used alone only if the BFF semen is not cryopreserved. For sperm freezing, a 4% glycerol concentration is desired for BFFs, and a combination of both TEST-Yolk Buffer solutions is prepared in sterile glassware. To yield a 4% glycerol concentration, thawed aliquots of "Freezing Medium-TEST Yolk Buffer" containing 12% glycerol are added to thawed aliquots of "Refrigeration Medium-TEST Yolk Buffer" containing 0% glycerol at the following ratio:

ADD: One part Freezing Medium-TEST Yolk Buffer with 12% glycerol

ADD: Two parts Refrigeration Medium-TEST Yolk Buffer with 0% glycerol

The modified TEST cryodiluent with 4% glycerol concentration then is aliquoted into sterile cryovials and frozen. On the day of semen collection, a vial of modified TEST cryodiluent containing 4% glycerol is thawed. Once thawed, 100 µl of cryodiluent is transferred to a 1.5 ml eppendorf vial and maintained at 37°C for semen dilution. Additional eppendorf vials can be filled and maintained at 37°C in case additional semen collection vials are needed.

Rectal Probe Measurements for Black-footed Ferrets (BFFs)

Probe Diameter	0.6 cm
Probe Length	9.0 cm
Electrodes	2 longitudinal electrodes; 1.6 cm long each; spaced 0.5 cm apart

Step-wise Procedure for Electroejaculation and Semen Processing

a. Once the male becomes tractable after inducing anesthesia, place in lateral recumbency and measure the length and width of each testis with laboratory calipers.

b. Prolapse the penis from the preputial sheath and, if necessary, gently clean with damp gauze pad. Gently dry the penis with dry gauze.

c. Lubricate (K-Y lubricant) the rectal probe containing 2 longitudinal electrodes.

d. Insert the probe into the rectum by applying gentle, forward pressure. The sphincter muscles may relax slowly, so the probe should not be inserted quickly or forcefully.

e. Once inserted, position the electrodes immediately cranial to the anal sphincter muscles. Always direct the electrodes ventrally. Apply gentle ventral pressure to the probe during electroejaculation to ensure adequate contact with the rectal lining.

f. Protrude the penis again from the prepuce prior to semen collection. It will probably not be erect at the onset of electroejaculation. Maintain the penis prolapsed during semen collection.

g. Signal to the operator of the electroejaculator unit (60 Hz, AC, sine wave) that you are ready to begin the electrical stimulation. Make sure the rheostat dial on the electroejaculator is turned to 0 before turning the switch to "on".

h. The electrical stimulation will consist of 5 series with rest periods between each series:

Series 1: 2 volts, 10 stimuli; 3 volts, 10 stimuli; 4 volts, 10 stimuli; total of 30 stimuli

Series 2: 3 volts, 10 stimuli; 4 volts, 10 stimuli; 5 volts, 10 stimuli; total of 30 stimuli

Series 3: 4 volts, 10 stimuli; 5 volts, 10 stimuli; total of 20 stimuli

Series 4: 4 volts, 10 stimuli; 5 volts, 10 stimuli; total of 20 stimuli

Series 5: 5 volts, 10 stimuli; 6 volts, 10 stimuli; total of 20 stimuli

i. Series 1 collection should proceed with the following stimulations:

1. increase from 0 volts to 2 volts in 1 second

2. maintain stimulus at 2 volts for 3 seconds

3. abruptly return to 0 volts

4. repeat steps #1, 2 and 3 for a total of 10 electrical stimuli at 2 volts

5. Increase the voltage to 3 volts, and repeat steps #1 through 4 for a total of 10 stimuli at 3 volts

6. increase the voltage to 4 volts, and repeat steps #1 through 4 for a total of 10 stimuli at 4 volts

j. Semen will be ejaculated during the series. The operator of the probe will watch the tip of the penis for semen, which will usually pool near the urethral opening in the groove created by the os penis. When observed, the electroejaculator should be returned to 0 volts until the small volume (several µl) of semen can be obtained. The ejaculate is collected from the penis using a pipette equipped with a sterile pipette tip that has been maintained at 37°C (store tips either in heating block in empty eppendorf vials or in an incubator). The ejaculate volume is recorded by comparison with a similar pipette tip marked with varying µl increments. Semen then is placed directly into the TEST Yolk Buffer with 4% glycerol at 37°C. The operator then will proceed with the series, stopping similarly for any further ejaculates, until the series is complete. The operator indicates when the series is completed. Switch off the electroejaculator unit. Collect any additional semen from the end of the penis with a sterile pipette tip. Add this aliquot to the semen already in the eppendorf vial.

   NOTE: urine contamination of the ejaculate may occur. Urine is less viscous than semen and will have a yellow tinge. If an ejaculate is suspected to contain urine, place it in a separate eppendorf vial also containing TEST Yolk Buffer with 4% glycerol at 37°C. This sample can be evaluated separately. If semen quality is worse than uncontaminated sample, it should not be added to that sample. *Samples contaminated with urine show low (or no) sperm motility and forward progression*. It is helpful to change pipette tips between ejaculations; this will prevent contamination of existing sample and will make it easier to identify urine contamination. The collection media is also yellow making identification of urine difficult. An unused tip can be examined for yellow coloration indicating the presence of urine. If urination occurs, wipe tip of penis with gauze and change pipette tips.

k. Between series, 'rest' the animal (~5 minutes) while the semen sample is being evaluated microscopically. During this time, rectal massage is performed by gently rotating the probe inside the rectum over the underlying accessory sex organs. Often this technique induces further production of ejaculate, which can be added to the collected semen.

l. To assess semen quality, remove 3 µl of the diluted semen sample and transfer to a warm (37°C) slide for sperm evaluation. Evaluate sperm percent motility (0-100%) and sperm forward progressive motility (scale 0-5; 5 = best).

m. For Series 2 collection, repeat steps (e) through (k) using voltage settings of 3, 4, and 5 volts. If uncontaminated with urine, semen produced during Series 2 can be added directly to the

vial containing sperm from Series 1. At the end of the series, again measure sperm percent motility and forward progressive motility.

n. For Series 3 collection, repeat steps (e) through (k) using voltage settings of 4 and 5 volts. Measure sperm percent motility and forward progressive motility.

o. For Series 4 collection, repeat steps (e) through (k) using voltage settings of 4 and 5 volts. Measure sperm percent motility and forward progressive motility.

p. For Series 5 collection, repeat steps (e) through (k) using voltage settings of 5 and 6 volts. Measure sperm percent motility and forward progressive motility.

NOTE: During procedure, collect an aliquot of raw semen off the penis with a sterile pipette tip and place in 100 µl of 0.3% glutaraldehyde (in phosphate buffered saline) for sperm morphology assessment. Do not use a pipette tip that has TEST cryodiluent on it; the egg yolk will 'clump' in the glutaraldehyde preventing visualization of the sperm. Also, use semen that is not contaminated with urine. ***IMPORTANT***: Change pipette tip again before continuing with semen collection; residual fixative in the tip will kill sperm!

q. Combine semen aliquots from all series (if similar quality), and remove an aliquot for assessment of sperm concentration. In addition, assign an overall sperm percent motility and forward progressive motility (0-5) to entire ejaculate. An overall average of the five ratings given in Series 1-5 can be used.

B. SEMEN EVALUATION

Assessment of sperm percent motility

Observe several fields under the microscope and record an average of those sperm that have movement. Include those sperm that have even the slightest movement. Assess sperm motility from 0 to 100%.

Assessment of sperm forward progression status

Observe several fields under a microscope and record an average sperm progression (scale, 0 to 5) using the following guidelines:

0 = no motility or movement

1 = slight side to side movement or quivering with no forward progression

2 = moderate side to side movement with occasional spurts of slow forward progression

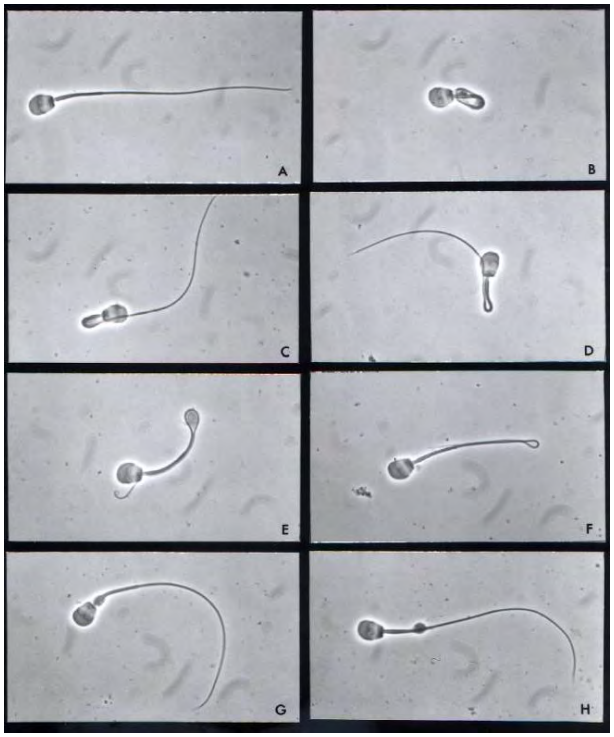
3 = side to side movement with slow forward progression

4 = steady forward progression

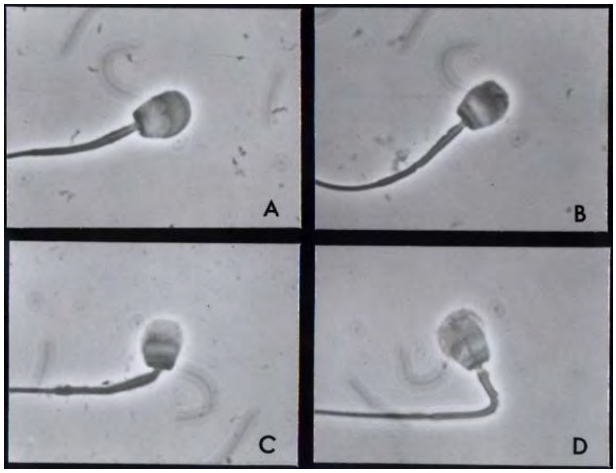
5 = rapid, steady progression

Assessment of sperm morphology

Fix a 1 to 3 µl aliquot of undiluted raw semen in 100 µl of 0.3% glutaraldehyde (in phosphate buffered saline) in a tightly sealed vial (as mentioned in section A-p). Maintain fixed sample refrigerated until analyzed for sperm morphology, categorized as normal sperm or abnormal sperm [abnormal acrosome, coiled flagellum, bent midpiece, bent flagellum, proximal cytoplasmic droplet, or distal cytoplasmic droplet (Fig 2).] The same fixed sperm sample can be used to assess sperm acrosomal morphology, categorized as normal apical ridge, damaged apical ridge, missing apical ridge, or loose acrosomal cap. (See report form at end of chapter)



BFF sperm morphology



BFF sperm acrosomes

Assessment of sperm concentration

a. Mix diluted semen well using pipette.

b. Add 1.99 ml of tap water to a tube. Add 5 µl of well-mixed semen via a pipette.

c. Allow vial to stand at room temperature ~30 minutes to kill sperm.

d. Fill hemocytometer chamber on both sides of unit and allow sperm to settle into one plane (~5 minutes).

e. Count sperm in four corners (16 squares per corner). Use this number and the following equation to calculate sperm concentration/ml of ejaculate:

(total volume of undiluted semen) times ("X"; this is the unknown sperm concentration/ml) = (volume of TEST cryodiluent + volume of undiluted semen) times (number sperm in 4 corners)

Example: (12 µl)(x) = (112 µl)(40); x = 373. The sperm concentration/ml is 373 $x10^6$/ml. The total sperm in the ejaculate is 4.48 $x10^6$. This number is derived by multiplying the concentration/ml (373) by the total volume (ml) of the undiluted ejaculate volume (0.012 ml).

C. SPERM PROCESSING FOR CRYOPRESERVATION

MEDIA PREPARATION FOR SPERM CRYOPRESERVATION (TEST)

No additional media will need to be prepared. The semen will be frozen in the TEST Yolk Buffer with 4% glycerol that it was suspended in at the time of collection.

Pellet method of freezing

a. After evaluating semen sample, place the eppendorf vial containing diluted semen, sterile Pasteur pipettes, and a pipette bulb into a 4°C refrigerator for 20 minutes.

b. For pellet freezing, prepare a flat block of dry ice by making multiple indentations (3 mm deep x 3 mm wide) into the top surface using a 'nail board' or some other suitable device. After the 20 minute cooling interval, thoroughly (but gently) remix the diluted semen sample using a cooled Pasteur pipette, and quickly pipette single ~30 µl drops of the mixture into the individual indentations in the dry ice. Allow the pellets to freeze on the dry ice block for 3 minutes. Invert the ice block over a bucket containing ~6 cm of liquid nitrogen, and let the pellets fall off the dry ice block and sink into the liquid nitrogen.

c. Place an appropriately marked (animal studbook #, date, location) storage cryovial into the liquid nitrogen to freeze (the vial will 'bubble' until reaching the same temperature as the liquid nitrogen).

d. Use a plastic spoon to scoop the pellets into the frozen cryovial. Keep the vial slightly under the level of the liquid nitrogen, and ensure that the pellets always are below the level of the liquid nitrogen while on the spoon during transfer. Once frozen, the pellets should always be kept in liquid nitrogen to prevent thawing. Under the level of liquid nitrogen, fasten the lid on the cryovial containing pellets.

e. Transfer the vial from the bucket into a liquid nitrogen storage tank. These samples must be maintained in liquid nitrogen during transport and permanent storage.

D. SPERM THAWING AND EVALUATION

Media Preparation for Semen Thawing

Prepare Ham's F10 tissue culture medium (GIBCO BRL; catalog #12390-035) for thawing pellets. On the day of thawing, add 5% fetal calf serum (FCS), pyruvate (0.026 mg/ml), glutamine (0.284 mg/ml), penicillin (100 units/ml), and streptomycin sulfate (100 µg/ml) to 95

mls Ham's F10 medium. Filter sterilize using a 0.2 µm filter into a sterile culture tube and maintain at 37°C for thawing semen.

Procedure for Pellet Thawing

Place 70 µl sterilized (filtered) Ham's F10+FCS into a sterile, glass test tube and incubate in a 37°C water bath. Using a plastic spoon, transfer one pellet into the glass tube very quickly. This is done by spooning up a pellet from the fluid (facilitated by angling the bucket), pouring off liquid nitrogen from the spoon and then dropping the pellet into the tube. Immediately, shake the tube in the waterbath for 1 minute to thaw pellet quickly. Remove a 3 µl aliquot and evaluate sperm percent motility and forward progressive motility. Remove ~10 µl and place in vial containing 50 µl 0.3% glutaraldehyde. Evaluation of sperm morphology and acrosomal integrity must be performed quickly to avoid 'clumping' in the sample. Maintain thawed sperm sample at ~37°C, shielded from light, until use.

PELLET THAWING FOR AI

More than 1 pellet will be needed for AI. Each pellet should be thawed separately as indicated above. After all pellets are thawed, the samples can be combined and transferred to eppendorf tubes for washing and centrifugation (300g for 8 minutes at #1 setting). Immediately after centrifugation, supernatant from each vial is removed. Pellets are combined and maintained at 37°C, and total volume is increased to 215 µl with Ham's F10+FCS. For assessment of sperm morphology, remove 10 µl of sperm suspension and place into 50 µl 0.3% glutaraldehyde. For assessment of sperm motility, remove 5 µl for final post-thaw motility evaluation. The remaining 200 µl will be divided into 2 aliquots and deposited into the uterine horn (100 µl/horn).

To calculate the number of motile sperm inseminated, the following equation is used:

[sperm concentration/ml of inseminate] times [total volume of inseminate in mls] times [sperm percent motility] = total motile sperm inseminated

Example: $(74 \times 10^6/ml)$ (0.2 mls) (0.50)=7.4×10^6 total motile sperm inseminated.

BFF EEForm 2016.docx

APPENDIX 2

LAPAROSCOPIC INTRAUTERINE ARTIFICIAL INSEMINATION

ADMINISTRATION OF LH INJECTIONS TO INDUCE OVULATION IN BLACK-FOOTED FERRET (BFF)

LH will induce ovulation if female BFF has mature follicles, so female needs to have >90% cornified vaginal epithelial cells**. hCG is not recommended***

LH for BFF (recommended for BFFs)

Sioux Biochemical

140 19th St. SW

Sioux Center, Iowa 51250

Phone 712-722-4694; Fax 712-722-4649

siouxbio@mtcnet.net

www.siouxbiochemical.com item #925

LUTEINIZING HORMONE (LH) (PORCINE)

LH from Porcine (pig) Pituitary Glands

25 units=50,000 IU/ bottle, Lyophilized,

~$90/bottle, **keep refrigerated**

****DOSE = 1 UNIT = 2,000 IU**

Conversion of Units to International Units

1 bottle of LH = 25 UNITS

1 UNIT = 2,000 IU (International Units)

1 unit = 2,000 IU (International Units)

25 units LH /bottle = 50,000 IU/ bottle

- Dissolve 25 units in 10 ml sterile water for injection= 2.5units/ml,= 5,000 IU/ml
  1 unit = 0.4 ml; 2,000 IU = 0.4ml

- Give BFF female 1 Unit (= 2,000 IU) for ovulation in about 12 hours
- Intramuscular, one time
- Inject female in evening and pair her the next am (per FWS/Paul Marinari)

This is a lot of LH, but titers decline in less than 1 yr. (i.e., can use 2 consecutive years)

Lower dosages even 0.5 unit, (1,000 IU) may work.

Per Randy Meyer at Sioux Biochemical:

OK to dilute aliquots (in sterile water) and store in Ultralow freezer for several years.

****

hCG is NOT recommended due to prolonged titers over years; very antigenic (SIGA) hCG, 2,500 IU/vial. If need to administer: 90 IU IM to female one time to ovulate. hCG will not work on her in following years.

An injection of luteinizing hormone (LH; 1 unit; Sioux Biochemical) should be given the day before AI (10-20 hours before scheduled AI). The syringes containing LH are kept frozen, therefore, one syringe can be thawed about 15-30 min before injecting. Give 1 unit hCG intramuscularly to each female. Syringes usually are prepared to contain more volume than needed, so excess volume can be discarded before injecting the BFF. Be sure to have a good hold on leg while injecting, so animal doesn't jump and get partial injection. Remember that the time between LH and ovulation is ~24-30 hours, and AI should be conducted no later than 20 hours after LH.

Laparoscopy and Intrauterine Insemination

The laparoscopic insemination technique, developed for depositing sperm directly into the uterine horn via a catheter inserted through the abdominal wall, has been developed for BFFs. Anesthetized females are placed in a supine position and tilted head-down at approximately 45° from the vertical. A pneumoperitoneum is created with 5% carbon dioxide or room air instilled through a transabdominally placed 5 mm Verres needle. A 180° laparoscope (usually 5 mm in diameter) inserted through a midline skin incision cranial to the umbilicus is used to view the reproductive tract. In BFFs, ovaries are embedded in adipose tissue and are unable to be visualized laparoscopically. The uterine body can be observed dorsal to the bladder, and uterine horns can be traced from the uterine body. The Verres needle is used to position the uterus for placement of the catheter. The uterine horn is cannulated using a sterile 22 gauge indwelling catheter inserted percutaneously through the ventral abdominal wall into the uterine lumen. The catheter stylette is removed, and the sperm sample is deposited into the catheter hub. A 1cc syringe then is attached to the catheter and a small amount (~ 0.3cc) of air is used for

delivery of sperm suspension out of the catheter and into the uterine lumen. The entire procedure is repeated on the contralateral uterine horn.

Sperm processing for AI

To withdraw sample from an eppendorf tube, use a pipette tip to remove semen contents. Sperm suspension then is deposited into the catheter, which is inserted into the uterine lumen. Semen is expelled from the catheter using a 1cc syringe filled with air (~0.3 ml) attached to the catheter for displacement of semen out of catheter and into uterus. Repeat procedure on other uterine horn using semen in second aliquot.

The following information is recorded for the AI procedure: 1) date and time of LH; 2) date and time of AI; 3) name of sperm donor; 4) date of semen collection and cryopreservation; 5) number of pellets thawed for AI; 6) total volume inseminated; 7) percent sperm motility and forward progression (0-5) of inseminate; and 8) sperm concentration/ml of inseminate. The total number of motile sperm inseminated is calculated by multiplying the [sperm concentration/ml of inseminate] times [total volume of inseminate in mls] times [sperm percent motility].

Example: $(74 \times 10^6/ml)$ (0.2 mls) (0.50)=7.4×10^6 total motile sperm inseminated.

BFF Semen Media Recipes

BFF 0.3%glut 400mOsm Sperm Fix

22.5ml deionized water to 2.5ml PBS (10x) add ~1.04g sucrose to make it 400mOsm

pH to 7.4

add 0.3ml Glut (25%) to 24.7ml of the PBS 400mOsm you just made

Filter and make 100ul aliquots

TEST 8%

2 bottles (5ml each) of 12%

1 bottle (5ml) of 0%

TEST 4%

1 bottle (5ml) of 12%

2 bottles (5ml each) of 0%

Unopette dilution for Sperm Counts

Put 1.99 ml of water (any type) into cryovial add 5 ul of extended semen

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Table 1. Pregnancy rates after non-surgical vaginal insemination versus laparoscopic intrauterine insemination in the domestic ferret.

	Nonsurgical vaginal insemination	Laparoscopic intrauterine insemination
Number of females inseminated	10	24
Number of pregnant females	0	17
Pregnancy rate	0%	70.8%

(Data from Wildt et al., 1989, J. Reprod. Fertil.)

Table 2. Ferrets produced by laparoscopic intrauterine artificial insemination using fresh or frozen-thawed semen.

	Domestic ferret *(Mustela putorius furo)*	Siberian polecat *(Mustela eversmanni)*	Black-footed ferret *(Mustela nigripes)*
Number of females inseminated	34	7	22
Number of pregnant females	24	6	16
Pregnancy rate	70.6%	85.7%	72.7%
Number of kits produced	116	32	47
Mean number of kits/litter	4.9 ± 0.6	5.5 ± 1.2	2.9 ± 0.4

(Data from Wildt et al., 1989, J. Reprod. Fertil.; Howard et al., 1991, J. Reprod. Fertil; Howard et al., 1996, J. Androl.; Howard, 1998, Zoo Wild Anim. Med.)

Table 3. Semen traits in Siberian polecats and BFF before and after cryopreservation using PDV cryodiluent and pellet freezing.

	Siberian polecats (n = 8 males)	Black-footed ferrets (n = 9 males)
PRE-FREEZE		
Sperm motility (%)	80.6 ± 2.9	69.4 ± 4.9
Sperm progression (0-5)	3.0 ± 0.2	2.9 ± 0.3
Sperm motility index	72.5 ± 2.4	63.6 ± 4.4
Normal sperm (%)	74.5 ± 2.6[a]	50.1 ± 5.1[b]
Abnormal sperm (%)		
Abnormal acrosome	3.2 ± 0.7[a]	17.9 ± 4.3[b]
Coiled flagellum	0.4 ± 0.2[a]	2.6 ± 0.7[b]
Bent midpiece	7.3 ± 0.8[a]	15.2 ±1.3[b]
Bent flagellum	9.7 ± 1.1	11.0 ± 3.0
Proximal droplet	4.4 ± 1.2[a]	1.1 ± 0.3[b]
Distal droplet	0.5 ± 0.2	2.1 ± 1.2
Acrosomal morphology (%)		
Normal apical ridge	96.8 ± 1.0	82.1 ± 4.3
Damaged apical ridge	1.8 ± 0.6	15.0 ± 3.9
Missing apical ridge	1.0 ± 0.2	2.1 ± 0.6
Loose acrosomal cap	0.4 ± 0.2	0.8 ± 0.3

POST-THAW

Sperm motility (%)	68.8 ± 2.5[a]	39.0 ± 5.6[b]
Sperm progression (0-5)	3.3 ± 0.1[a]	2.6 ± 0.2[b]
Sperm motility index	67.5 ± 2.3[a]	40.6 ± 5.2[b]
Acrosomal morphology (%)		
Normal apical ridge	61.3 ± 2.7[a]	34.9 ± 4.2[b]
Damaged apical ridge	18.9 ± 1.9	19.1 ± 2.5
Missing apical ridge	8.9 ± 1.5[a]	24.1 ± 2.0[b]
Loose acrosomal cap	10.9 ± 1.8[a]	21.9 ± 2.5[b]

Values (mean ± SEM) with different superscripts are different ($P < 0.05$).

(Data from Howard et al., 1996, J. Androl.)

Table 4. Influence of male selection strategy on whelping in 1997 and 1998 at the U.S. Fish and Wildlife Service's (Service's) National Black-Footed Ferret Conservation Center (NBFFCC).

	1997	1998
Criteria for male selection	Testes size	Spermic ejaculate
Number of females	84	86
Number of litters	50	69
Whelping rate	60%	80%
Number of kits born	190	249

WHELPING AND KIT CARE

QUICK FACTS

- **MINIMIZE NOISE AND DISTURBANCE DURING BREEDING/WHELPING TO REDUCE CANNIBALIZATION**
- **INCREASE FEMALE RATIONS ACCORDING TO SCHEDULE**
- **TRY TO DISTURB DAM AS LITTLE AS POSSIBLE DURING THE FIRST FEW DAYS FOLLOWING BIRTH**
- **BEGIN TO INTRODUCE SOLID FOOD TO KITS AT 25–30 DAYS OF AGE**
- **KITS MAY BENEFIT FROM SUPPLEMENTAL FEEDING IF LITTER IS FAILING**
- **BFF DAMS ARE SURPRISNGLY ACCEPTANT OF FOSTERED KITS AND THIS STRATEGY CAN BE USED FOR CROSS-FOSTERING FAILING KITS OR MIXING GROUPS FOR MANAGEMENT PURPOSES**
- **INTRODUCE WHOLE CARCASS TO KITS AT 30 DAYS OF AGE**
- **INTRODUCE KITS TO LIVE PREY AT 50–60 DAYS OF AGE**
- **BE SURE DAM HAS ACCESS TO KITS THE FIRST FEW TIMES THEY ARE OFFERED LIVE PREY SO THE KITS CAN LEARN FROM HER HOW TO CATCH AND KILL PREY**
- **DAM AND KITS MAY BE SEPARATED FOR MANAGEMENT PURPOSES AT 90 DAYS OF AGE**
- **KITS ARE 90% GROWN AND SOCIALIZED AT 120 DAYS OF AGE AND MAY BE HOUSED SEPARATELY**

WHELPING AND KIT CARE

INTRODUCTION

The techniques offered here are based upon several facilities' protocols. Each facility may find it necessary to deviate slightly to accommodate individual needs, address specific problems, and meet facility protocols. The National Black-footed Ferret Conservation Center (NBFFCC) policies are included at the end of the chapter in **Addendum A**.

WHELPING PROTOCOL OF LACTATING FEMALES (DAMS) AND KITS

Several preparations should occur prior to a black-footed ferret (BFF) dam whelping to reduce the amount of stress the female experiences after the birth of her kits. The first, most important consideration involves **reducing the amount of noise and activity in the vicinity of pregnant dams**. Excessive noise and activity are commonly believed to have an influence on the high rate of cannibalism of BFF neonates. Other preparations include the following activities:

- ✓ Flag enclosures containing pregnant females with their due date and, after whelping, with the number of kits
- ✓ Attach a camera to the lower nest box, if available; remove if dam seems overly stressed
- ✓ Close off access to the upper nest box
- ✓ May remove any "furniture," tubing, etc. to discourage whelping anywhere other than nest box
- ✓ Increase the amount of bedding material on the den, or nesting, side of the box by approximately one third
- ✓ Quietly clean the dam's nest box and enclosure one day prior to her due date
- ✓ Be sure all day old food is removed from enclosure daily
- ✓ Tours are discouraged during breeding and whelping months
- ✓ Due dates for females should be clearly posted for all attendants
- ✓ Discontinue offering enrichment items to dams that may end up in nest box and interfere with kits immediately prior to whelping

Begin looking for kits on the camera monitor, or listen for "squeaks" coming from nest box, two days prior to the expected whelping date. If no kits are observed, continue with normal husbandry practices, but check monitor or listen for kits before opening the lower nest box. Once kits are observed on the monitor or heard in the nest box, the whelping protocol applies. The **whelping protocol** consists of an alteration in the normal feeding and cleaning practices as outlined below.

Food Increases

Females need increasing amounts of food during gestation and especially during lactation after whelping. Gradually increase the food amounts to minimize the chance of digestive upset, diarrhea, and poor eating. Most facilities increase the dam's food by 25% every 2 weeks until she

reaches diet amounts 1.5–2 times her normal diet. If a gestational female is consistently eating all of her rations at a particular level, she can be adjusted up to double ration of Toronto Small Carnivore diet (TC). On the due date, the dam's ration may be cut back to half ration, around 50–-60 grams (g) (1.75–2 ounces (oz)) TC, as the dam usually doesn't eat on day of whelping.

Whelping Day to Fourth Day Post Whelp

Listen or check camera to see if there are any kits. If not, clean enclosure first thing. If kits are evident, discontinue cleaning of the dam's enclosure. Depending on her temperament, you may need to discontinue cleaning of all adjacent enclosures as well. Remove any leftover food from enclosure surface. Continue to offer the female 1.5–2 times her normal diet ration daily. There should always be food left over to indicate that the dam is satiated. Increase the amount of food offered as the kits get older. Place food and water close to the tunnel entrance, as the dam may not want to leave the tunnel, and this practice encourages her to stay close to her kits. Monitor the kits closely for the first few days. **Weak, small, quiet, and lethargic kits may indicate kit health problems, or an agalactic (non-lactating) dam. If the dam is not producing enough milk, cross fostering may be necessary.**

Second To Fifth Day Post Whelp

Let the dam's behavior guide you as to whether you can clean the adjacent nest boxes and enclosures. Abandon cleaning if the female appears to become agitated by this activity.

Fifth Day Post Whelp

On the fifth day, clean the dam's nest box and enclosure, as well as the nest boxes and enclosures of adjacent animals. When cleaning the dam's nest box, try to get her into the tunnel or to the enclosure surface prior to locking her away from her kits. This can be accomplished by cleaning the enclosure surface first. She may become agitated and scratch at the slider anyway, but continue cleaning quietly. Get an accurate count and assessment of the kits at this time. Minimal disturbance is ideal, but it may be necessary to carefully move the kits to clean around them, or to look for a "lost litter mate." Dead kits will often be buried in the nesting material; therefore, always check bedding if a kit is missing. If the dam becomes upset and grabs or carries kits into the tunnel, abort cleaning and try again the next day. The dam's nest box may be cleaned on an every other day routine until kits are 3–4 weeks old, then institute daily cleaning. It is more important to clean daily when kits are older (3 weeks), but stress reduction is key when kits are very young. Many facilities use disposable gloves and change them between each litter. Always use separate cleaning utensils for each litter.

KIT CARE PROTOCOL

Kit care is the seasonal activity of weaning kits from their lactating mothers. Many kits continue to suckle up to 90 days of age, or longer. However, most lactating females are unable to produce sufficient quantities of milk to feed their kits after the kits reach 30–40 days of age. Therefore, it is necessary to provide the kits with additional food sources at this time. Most facilities start offering either Toronto Carnivore diet or rat halves in the nest box at 25–30 days of age. Closely monitor the kits' growth and dam's behavior to determine if further intervention is needed. A visual assessment of growth, color, and activity every few days is usually sufficient to determine the overall health of the kits (see FCC Pinwheel Chart **Addendum B**).

If the dam appears to be spending a lot of time away from her kits (on the other side of the nest box or on the enclosure surface), this may indicate that she is either agalactic or uninterested in feeding them. The dam's inattentiveness may be due to environmental factors such as excessive heat in the nest box. If the kits are less than 21 days of age, cross fostering should be considered. If the kits are older, initiate kit care with multiple feedings of a slurry consisting of Toronto Carnivore, water, and Nutrical®.

Check kits to determine gender **at 7-10 days of age**.

At **30 days of age**, kits should be eating some of offered diet, rat pieces, or cut open hamsters on their own. Once carcass diet is being fed, TC diet ration can be cut back if not being totally consumed. Be sure all leftover food is removed daily during cleaning (LZG offers ½ large rat per kit AND dam per day. Large rat is 150–160 g (5–6 oz); all rats are cut in half). Kits may be weighed and offered prophylactic ponazuril. Confirm sex identification at this time.

At **50–60 days of age**, kits are considered "weaned," and are generally consuming solid food well. Feed kits live hamsters or mice at this time, along with a comparable increase in the regular diet. **Note: be sure that the dam has access to the kits the first few times they are introduced to live prey, so that they can learn how to catch and kill prey.** Provide water ad-libitum. Kits will begin to venture to the surface, so provide increased enrichment items to promote exploration and to provide hiding places. Clean tunnels and connectors on a regular basis, as kits often urinate, defecate, or cache food in tunnels.

Since all animals are potential release candidates, when available feed prairie dog at a rate of 50 grams (1.75 oz) per animal (kits **AND** dam) three times per week to insure imprinting on this natural food source. When available, NBFFCC may supply frozen processed prairie dog to SSP captive-breeding facilities, contingent on the facilities' regulations and restrictions (with regards to the change in prairie dog quarantine procedures). Fifty grams (1.75 oz) of prairie dog is considered a half-serving size, so a half-serving size of TC diet should also be offered at this time. Also offer live hamsters or mice at least once a week (preferably not on the same day that prairie dog is offered) to encourage natural predatory behavior. Provide live prey at the rate of one prey item per kit per week if possible.

If not already doing so, offer prophylactic ponazuril to all kits.

At **60 days of age** kits can begin the vaccination schedule (see Veterinary Protocols). Kits should also be scheduled for transponder placement and overall health assessment.

At **90 days of age**, kits may be fed individual rations, and it is no longer necessary to increase food amounts. It is possible to separate kits from dam at this time if needed for management purposes.

At **120 days** of age kits are now considered sub adults. They are completely weaned and sufficiently socialized so that if enclosure space is available and circumstances warrant it, kits may be separated from their dam. BFFs reach 90% of their adult weight by 120 days of age.

CROSS FOSTERING

Cross Fostering Kits Becomes Necessary Under Certain Circumstances:

- Agalactic dams
- Very large litters
- Runt of litter not competing well with littermates
- Single kit litter (if not doing well/not growing)
- Surviving kits of maternal neglect or cannibalism

Trap a suspected agalactic dam only after she is locked out of the nest box containing the kits. Once dam is in a restraint cage, manipulate mammary glands to ascertain whether or not she is lactating. If she is not, plan to cross foster her kits. When choosing a litter to receive the cross fostered kit, take into account the dam's demeanor. Additionally, all kits should be comparable in size, as this is more important than age in sibling competition. Lastly, all cross fostered kits should be placed with a small to moderately sized litter.

To introduce a new kit to a litter, lock dam out of nest box, preferably at same time as doing kit care. Gently "mix" kits in hands, distributing keeper's scent on all kits. Then "mix" kits in bedding material for approximately 5 minutes before allowing dam access to kits. Closely monitor dam for hostility or rejection. Attempt to cross foster a kit to a litter of the opposite sex. Mark all kits for identification, either by shaving patches of fur, or with Nyanzol-D® dye if kits

are older. Be vigilant in keeping track of all cross fostered kits for genetic purposes. If kits are older, it may be possible to separate the two largest kits and maintain them in a separate enclosure. Keep detailed records of all cross fostering activity.

HAND RAISING

If problems arise within a litter, it is best to try and cross foster kits so they can be dam reared. However, on rare occasions, it may become necessary to attempt hand rearing BFF kits. Realize that due to the size and helpless nature of BFF kits, hand rearing is not even feasible before the kits are at least 21 days old. A detailed hand raising agenda appears at the end of this section in **Addendum C.**

[Note: the gruel formulas in the Louisville paper used the old Standard Diet. If attempted again, Toronto Small Carnivore Diet would be substituted. Phoenix Zoo protocols are also included.]

RECORD KEEPING

As described in the **Indoor Facility Management** section, maintain detailed, accurate records of all kit care activity, including kit identifications, weights, types of food offered, amount of food consumed, enrichments provided, medications given, etc. Examples of data sheets appear in the **Indoor Facility Management Addendum E.**

ENRICHMENTS

As kits begin to explore tunnels and caging, provide a variety of enrichment items to stimulate kits mentally and physically. For types of enrichment, see **Enrichment** chapter of this manual.

ADDENDUM A

PRE-WHELPING/WHELPING MANAGEMENT (FCC 2016)

No pregnant female should be given Albon® or metronidazole without approval from veterinarian!!!

It's typical, prior to whelping, that females do not eat all of their food. So this alone shouldn't be a cause of concern.

Make sure to check tunnels of females that are being fed increased food every day. They sometimes cache the food in the tunnel or elbow. It is imperative that any day-old food be removed at room or flag cleaning. Females that are receiving more than a three-quarter scoop of Toronto diet will be marked with a colored (orange) flag.

All activities in rooms should be done in the most efficient way possible and noise and talking should be kept to a minimum. Make sure to be careful when pushing carts around so as not to bump cages or boxes. Tours are usually discouraged during breeding and whelping months.

Due dates of females will be placed on the erase board across from surgery room as well as posted on the door next to surgery room and on the cage index cards.

Food Increases

Females need increasing amounts of food during gestation and especially during lactation after whelping. The idea is to gradually increase the food amounts. Increases that are too rapid can result in digestive upset, diarrhea, and poor eating. The idea is to have the female getting double rations by the time she whelps. If a gestational female is consistently eating all of her rations at a particular level she can be adjusted up based on consultation with vet and supervisor, but no gestational female will ever be given more than 2 scoops of TC or 160 grams (g) (6 ounces (oz)) of whole carcass. The feeding schedule for each female will be on the whelping cage card. The schedule below will apply in most cases.

- A normal female ration is a three-quarter scoop of TC or 60–80 g (2–3 oz) of whole carcass for special diets.
- 14 days after the first day of pairing, increase rations to 1 flat scoop or 80 g (3 oz) whole carcass.
- 21 days after the first day of pairing, increase rations to 1¼ scoops or 100 g (3.5 oz) whole carcass.
- 28 days after the first day of pairing, increase rations to 1.5 scoops or 120 g (4 oz) whole carcass.

Cage Preparations 5 days before a female is due:

- Remove orange flag and replace with a lime green flag.
- Close off access to the upper nest box using a slider (clean the nest box after locking it off).
- Remove all plastic tubes and caps from cage floor, remove suspended tunnels. Alpha-dri® in corners, Nylabones® and water dishes are okay.
- Highlight the box on the Pregnant Female Feeding Schedule on the door next to surgery after cage prep is done and mark a • next to her name on the white board in the hallway.
- Female will be taken off the rat feeding schedule and will only receive Toronto until her litter is ready for hamsters at Day 35 or she is declared Did Not Whelp (DNW).
- Whole carcass only females will receive smaller pieces of meat that they are likely to entirely consume (i.e. avoid tail and head pieces, give smaller hamsters (40–50 g/1–2 oz)
- Three days prior to due date increase bedding material on nesting side of box by one-third. If there is no apparent nesting side, increase it on both sides.

The day before the female is due:

- Do a full clean on her cage even though her room might not be scheduled.
- Turn over the yellow index card to read "half-scoop" and due date.
- Highlight the box on the Pregnant Female Feeding Schedule on the door next to surgery after cage prep is done.
- Feed half-scoop of food (or half-rations rat/hamster) as they typically don't eat prior to whelping.
- Continue feeding half-scoop until it is confirmed female has whelped **and** eaten half-scoop (usually done during cleaning) or female is considered as DNW (1 week past due date).
    - If female is enthusiastically taking all her food down after her due date but hasn't reached the day 4 clean yet (or hasn't whelped but is inside the 3-day typical whelping window), her ration may be increased if deemed necessary by the breeding manager/veterinarian.

On the due date and for all expectant females:

- First thing in the morning (before anything else!), one person should very quietly listen for squeals from the lower nest box. Do not open next box if you hear squeals, do not clean cage if you hear squeals! Definitely remove any leftover food from cage surface but do not disturb anything else!
- Only check number of kits if the female is not in the nest box; i.e. when up for food or during scheduled cleaning days. Females have whelped over the course of two days before.
- If you hear kits, place RED flag on cage and record whelp date on breeding summary, kit tally, erase board and tell coworkers. Data sheets are on wall near Room 3.
- When checking kits, do not close the slider! Be as quick and non-disruptive as possible!
- If no kits, clean cage first thing.

After females give birth:

- Their boxes will be cleaned on day 4 (kit age) before being incorporated back into the regular cleaning schedule (every other day). DOB is day 0. Cleaning information will be posted on the Kit Important Dates sheet on the door next to surgery. Kit dates file should be updated as necessary to keep up with whelping, and it is easiest to have this done prior to making color wheels for litters. Check tunnels on cleaning days.
- After day 10, place GREEN flag on cage to signify the all clear that litter is in normal cleaning rotation. Check tunnels every day.
- Amount of food will increase based on consumption by dam and litter. Food will only be increased in half- scoop increments! 1 scoop = 1 flat scoop
- Some females may cache large amounts of the offered Toronto Diet before their kits are eating solids. If a dam starts to lose weight and becomes emaciated while nursing, offering **pre-killed** whole carcass diet may tempt her into eating. Be sure to offer these food items on the cage surface or the side of the nesting chamber away from the kits.
- A kit progression chart (color wheel) will be placed on the clipboard attached to each female's cage.
- Place a circle on the white board. Kit tally will go in this circle when opportunity arises to count litter.

If a female does not whelp:

- Give females 1 week to whelp (based on the first possible due date).
- If they do not whelp, remove slider from upper box, remove due date card. Return toys and caps to cage.

Recycling Females:

- If a female does not whelp, it is possible she may come back in to estrus and can be "recycled".
- Recycling will only occur if there are release sites available for excess kits.
- Recycling will not take place after SSP determined "drop dead" date (usually July 4).
- The Vet or supervisor will determine whether this is appropriate and necessary.
- Once the female is classified as DNW, she will be put back into the weekly vaginal wash schedule. If she is ≥90% keratinized cells she will be bred immediately.

Cleaning Litters:

- **Put on a fresh pair of disposable gloves.**
- Clean cage surface. Try to get female to come up.
- If female is up, open nest box quickly and close slider IF ALL KITS ARE FULLY IN BOX. Dam needs to be locked out of nest box during cleaning.
- **NEVER CLOSE A SLIDER WITHOUT VISUALLY OBSERVING ALL KITS ARE CLEAR.**
- Check tunnel for leftovers. Dams can be aggressive, keep hands well away from openings of tunnel to prevent bites or use a tunnel cap/ball/plug.

- Change out water or fill water bottle if more than half empty.
- Kit side of nest box can be left un-cleaned until Alpha dri® starts to look dirty (yellow) or if dam defecates on that side. The dam usually meticulously cleans up after young kits.
- If you need to clean the kit side, clean opposite side first and replace all Alpha-dri®. Carefully move kits and place on clean Alpha-dri®. Clean nest side as normal. Replace all Alpha-dri® and carve out a depression. Carefully replace kits when done. **If you've never done this before, consult more experienced personnel *prior* to doing this.**
- If dam takes any kits up tunnel, immediately stop, close box (with inserts in place), and leave female alone. Return to finish cleaning after 20–30 minutes, and again try to lock her out of box with all kits in box.
- If you have any problems, consult a more experienced co-worker for help.
- Generally, new employees/volunteers will not be allowed to clean kits until they have significant experience.

Sexing kits

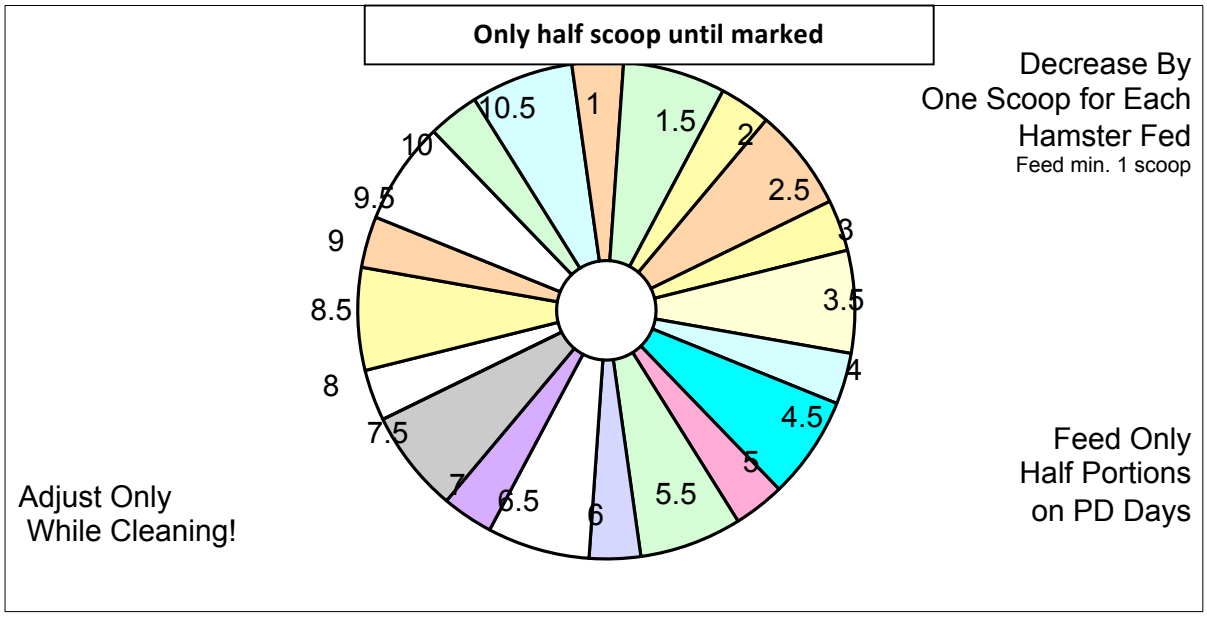
- The first or second time the litter is cleaned (Day 4 or Day 6); kits should be sexed and counted.
- Males will have a larger anogential distance than females. The penis sheath will appear like a small dot on the midline within 1 cm (0.4 in) of the anus in the anterior (toward the head) direction.
- Females will have two small openings very close together at the base of the tail.
- Indicate the number of kits and ratio (i.e. 7 (♂, ♀)) at the top right corner of the color wheel and on the important kit date sheet next to surgery door.
- The number of kits needs to be written on the white board in the hallway and on the kit tally sheet located outside Room 3.

Addendum B

Pinwheel Feeding Chart

Dam: June (SB 6070) **DOB: 8/5/2011**

Sire: UNK **Litter Size/Ratio: 1 (1.0)**



Day 0: Kits born (Red Flag)

Day 4 (clean): **August 9**

Day 6 (clean): **August 11**

Day 8 (clean): **August 13**

Day 10(regular cleaning begins; Green Flag): **August 15**

Day 30 (begin feeding food in box & ***DAILY*** cleaning –Orange Flag): **September 4**

Day 35 (begin feeding fresh killed hamsters/cut open-only one until kits are eating: **September 9**

Check when kits observed eating hamsters ____ ⇒ Begin live hamster schedule below

Day 50 (Begin feeding Prairie Dog – 50g per kit – White Flag): **September 24**

Day 90 (Cease Prairie Dog feeding unless preconditioning kits): **November 3**

	Sunday	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**
Day 36-49							
Day 50-90							

ADDENDUM C

HAND RAISING INFORMATION

LOUISVILLE ZOO 1992

SUMMARY OF HAND RAISING BLACK-FOOTED FERRETS AT THE LOUISVILLE ZOO

Following the sudden death of a Black-footed ferret dam, Louisville Zoo staff members quickly devised a protocol for hand raising the 5 (1.4) orphaned kits. Not all techniques employed by the staff, when faced with hand raising the 20 day old kits, would be practiced in any future endeavors. Staff recommendations for Black-footed ferret hand raising protocol appear at the end of this paper.

FEEDING

The first night, staff members used a curve tip syringe to deliver formula. For all other feedings, staff administered formula via Pet-Nip Nursers®.

The initial formula consisted of undiluted KMR® liquid. Staff members eventually added Poly-Vi-Sol® vitamins, distilled water, and Lactaid® to the formula. To minimize any possible problems, staff added vitamins slowly, beginning with one drop per 12 ounces of formula, when the kits were 24 days old. Because the kits experienced gastrointestinal problems, staff members began diluting KMR® with distilled water (28 days of age,) and later, supplemented the formula with Lactaid® (40 days old.) When the kits reached 30 days of age, the staff added a small amount of Standard Diet® to the diluted KMR® formula, using the following recipe:

to 12 ounce can of KMR®, added 60 milliliters distilled water,
1 drop vitamins, and 1 tablespoon SD; mixture shaken, then
strained through cheese cloth before feeding.

The following day, staff members added a noon gruel feeding, consisting of 50 grams SD and 120 grams by weight of undiluted KMR®. Gruel amounts fed were subsequently increased, depending on the amount of gruel eaten and the weight gain of the kits. The kits received the gruel feedings in a flat ceramic bowl, which was placed in a large, heavy-duty plastic box that had an attached heat lamp. Due to the kits' exuberant style of eating, the staff found it necessary to virtually bathe each kit after every feeding. To accomplish this, staff members held a kit under the heat lamp while cleaning it with a warm wet towel, and then dried it with another towel, while still under the heat lamp, before placing the kit back in the incubator.

Feeding Schedule:

kit age	feeding schedule
28 days	5 milliliters KMR® and 1 milliliter distilled water, 8 times daily.
30 days	KMR®/SD formula, 8 times daily.
31 days	KMR®/SD formula 7 times daily; one gruel feeding at noon.
33 days	KMR®/SD formula 5 times daily; 2 gruel feedings, one at noon and one in the evening.
37 days	gruel fed 3 times daily; formula fed 4 times daily.

39 days	gruel fed 5 times daily; formula fed once daily.
45 days	offered a bowl of water to kits after each feeding.
46 days	gruel fed 5 times daily; no formula feedings.
52 days	distilled water added to gruel recipe.
	example: 100 grams SD, 25 grams by weight KMR®, and 25 grams by weight distilled water.
65 days	kits weaned; fed SD twice daily

INCUBATOR/HOUSING

Initially, staff members set the incubator temperature at 90° F., then gradually decreased the temperature as the kits grew. For increased humidity, the staff kept distilled water in the water receptacle at all times. The kits were housed in an altered rodent cage placed inside the incubator. Once the kits grew, the staff allowed them to have the run of the incubator, as long as someone was in the building. Staff members enhanced the incubator with Tuffy® toys, french drain elbows, and other furniture.

When the kits were 52 days old, staff members moved the kits to an enclosure in the "clean room."

WEIGHTS

Staff members weighed the kits daily, at noon, until the age of 66 days. After that, they weighed the kits twice weekly. MedARKS weight reports are attached.

RECOMMENDATIONS

The biggest problem encountered by the staff during their hand raising efforts was diarrhea and excessive intestinal gas. The male kit's gastrointestinal problems became so severe that staff members had to reinstate feeding of KMR® only, for a few days. The staff then slowly introduced him to solid food by adding small amounts of strained chicken baby food to the formula. Once he ate the KMR® and baby food well and his stools improved, staff members added small amounts of SD to his diet. The male kit's problems began at age 40 days; by age 52 days, he was on the same gruel diet as the other kits. A female kit died at age 36 days, after experiencing bloat for 24 hours, and subcutaneous emphysema just prior to her death. A necropsy revealed Proteus sp. and E. coli, cultured from the lung, liver, spleen, and intestine, the only significant findings on histopathology.

Staff members think that several changes in the original formula strategy would keep diarrhea and other intestinal upsets to a minimum.

1) Dilute KMR® with distilled water from day 1.
2) Add Lactaid® to KMR® from day 1.
3) Use strained chicken baby food as the first step in kit weaning, rather than SD.*

*The staff recommends this as good protocol for weaning mother-raised kits as well.

CONCLUSION

All those involved in hand raising the Black-footed ferret kits found it to be a very satisfying, and very frustrating, learning experience. For more detailed information, please direct any questions or requests for data to any of the following Louisville Zoo staff members:

Cindy Morton, Keeper III, Infant Care Coordinator
Virginia R. Crossett, Veterinary Technician
Guy Graves, Conservation Center Keeper
Joanne Luyster, Conservation Center Keeper
Roy B. Burns, DVM, Staff Veterinarian
Kim Oakes, Animal Health Center Keeper

[1] Standard Diet fed at the Louisville Zoo:
6 parts mink chow (Path Valley Farms 60/40 Pellets[R])
4 parts ground rabbit
1/4 part blood meal
1/4 part Bioliver[2]
6 parts water

Co-authored by Virginia Crossett, RVT and Joanne Luyster Keeper II

PHOENIX ZOO HAND-RAISING PROTOCOL -2016

If kits must be separated from their dam and cannot be fostered to another dam with similar aged kits, then hand rearing may be the only option.

Equipment needed:

- Incubator or suitable small enclosure that can be maintained at a constant temperature and humidity. This is to be roughly the size of a nest box.
- Bedding
- Hand towels (take care that these have no frayed edges that might get caught)
- Small animal nursing bottles. Those designed for kittens are fine.

Food:

- KMR® (liquid, kitten milk replacement)
- Carnivore Care®, powder available through ACC
- Alternative to Carnivore Care®: Science Diet® Feline AD canned food. This is a prescription only diet that may be found at big box pet stores, but requires a prescription from a Veterinarian.
- After 30 days old: meat such as Toronto diet
- After 35 days old: thawed frozen rats, gutted.

Since the kits must be kept warm, and there is no dam to provide heat, keep the incubator between 29–32° Celsius (C) (85–90°Fahrenheit (F)) for the first few days and provide not only bedding, but a small towel for the kits to nestle under. If they are too warm, they will wiggle out from under the towel and the temperature may be reduced. If the kits are cool to the touch when handled, raise the temperature a few degrees. Multiple kits will snuggle tightly together and help to keep each other warm. A single kit may need a warming pad–just ensure that the pad is well insulated.

Very young kits will take small amounts of food at a time, 1-3 milliliters (ml), and must be fed frequently–every 1–2 hours. Kits older than 2 or 3 weeks may have the time between feedings increased to 4–5 hours. Increase time between feedings gradually as long as kits are gaining weight.

When very young, kits will need to be encouraged to defecate and urinate after feeding. Using a paper towel dampened with warm water, gently massage the anal area of each kit after feeding. This will induce defecation and urination. Clean the kit with a warm, damp towel and dry thoroughly. Once kits are urinating and defecating on their own, this step may be discontinued. (About 2–3 weeks).

KIT DEVELOPMENT

• WEIGHT	7 - 10 GMS. AT BIRTH FEMALES - 95% OF WEIGHT AT 15 WEEKS MALES - 95% OF WEIGHT AT 18 WEEKS
• EYES	OPEN @ 35 DAYS
• EARS	OPEN @ 33 - 35 DAYS
• PELAGE	MASK, FEET, TAIL MARKINGS EVIDENT 16-18 DAYS MARKINGS PROMINENT, COAT THICK, 32- 35 DAYS
• TEETH	DECIDUOUS ERUPT FROM 16 DAYS TO 7 WEEKS PERMANENT ERUPT FROM 8 WEEKS TO 12 WEEKS
• WEANING	USUALLY BY 42 DAYS
• MOTOR ACTIVITIES	CRAWL SHORTLY AFTER BIRTH WALK @ 35 DAYS ON CAGE SURFACE @ 7 WEEKS 12 - 16 WEEKS EXTENSIVE PLAY
• VOCALIZATIONS	CHEEPING AT BIRTH BARKING, HISSING BY 35 DAYS
• PLAY ACTIVITIES	WANDERING AND EXPLORING OBJECT PLAY PLAY DIGGING SOCIAL PLAY
• CRITICAL PERIODS	1 - 5 DAYS OF AGE WEANING, AGE 28 - 40 DAYS
• CONCERNS	COCCIDIA, CRYPTOSPORIDIOSIS

BLACK-FOOTED FERRET KIT DEVELOPMENT

DISCUSSION

After 42–43 days' gestation, black-footed ferret (BFF) kits are born helpless, their eyes closed and ears folded. Silky fine white fur covers their long, thin, pink bodies and short legs. Their footpads and snouts are naked, although the muzzle is darker than the rest of the skin. Relatively active, the neonates move by using their forelimbs. Unable to regulate their body temperature, they cuddle with the dam and siblings, often appearing as a squirming mass (Vargas 1994).

DEVELOPMENTAL LANDMARKS

BFF kits grow rapidly. The kit development table presents a gauge for developmental landmarks. Several of these landmarks are discussed below [information from Vargas 1994, 1996]:

WEIGHT At birth BFF kits weigh 7–10 grams (g) (0.35 ounces (oz)) (Hillman and Carpenter). Females reach 50% of their body weight a week before males. Females reach 95% of their adult weight at 15 weeks, while males reach 95% of their adult weight at 18 weeks. The size of the litter and sex ratio may affect kit size and subsequent weight gains. **Addendum A** shows predicted growth curves for males and females.

EYES Eyes open around 35 days, with ranges from 30–41 days. Initially upon opening, eyes are a deep marine blue, but turn to brown, their permanent color, within a few days.

EARS Ears start to open at 33 days, and exhibit the adult flat, upright position in 2–3 days.

TEETH ERUPTION Deciduous teeth begin erupting at 16 days and are completely in at 7 weeks.

2 (incisors 3/3; canines 1/1; premolars 3/3) = 28

First to erupt are canines and shearing premolars, then second upper incisors; other incisors appear last, at 6–7 weeks. Kits possess deciduous shearing teeth at 4–5 weeks, and make a rapid transition from milk to solid food. Permanent teeth begin to erupt at 8 weeks and are completely in at 12 weeks. Adult dentition consists of 8 pairs of teeth in the upper jaw, and 9 pairs of teeth in the lower jaw.

2 (incisors 3/3; canines 1/1; premolars 3/3; molars 1/2) = 34

Permanent canines erupt before deciduous teeth are lost; therefore, kits maintain a double set of some teeth for up to three weeks.

PELAGE CHANGES Feet and tail markings are faintly visible at 2–3 days. Hair quickly thickens and darkens. Face mask is evident at 16–18 days. Mask and markings are prominent by 32–35 days. By this time coat is full and guard hairs are apparent. There is no difference in pelage change between males and females.

WEANING Most kits start solid food by 42 days, depending on the dam's temperament and milk production and are fully weaned at 90 days.

BEHAVIOR Kits are able to emit a cheeping sound at birth. By 35 days, vocalizations include hissing and barking. Young kits move by crawling. By 4 weeks, kits wrestle with littermates and play-bite. After their eyes open, they walk more readily, and play begins in earnest. Kits whose eyes have not yet opened are often at the mercy of littermates whose eyes are already open.

PLAY ACTIVITIES Kits appear on the cage surface around 7 weeks of age. Their time on the surface increases as the kits mature. Kits are most active during the day, after cleaning, and at twilight and early evening. At 12–16 weeks of age, kit activities include wandering and exploring, object play with food bowls, tunnels, and toys, play digging, and social play. Most nest box social play consists of biting and wrestling, while chase and escape games constitute most surface social play. This social play simulates prey killing and predator avoidance. (See **Enrichment** Chapter of this Manual)

REFERENCES

Hillman, C.N., and J.W. Carpenter. 1983. Breeding biology and behavior of captive black-footed ferrets. International Zoo Yearbook 23: 186 - 191.

Vargas, A. 1994. Ontogeny of the endangered black-footed ferret (*Mustela nigripes*) and effects of rearing conditions on predatory behavior and post release survival. Dissertation, University of Wyoming.

Vargas, A. and S.H. Anderson. 1996. Growth and physical development of captive-raised black-footed ferrets (*Mustela nigripes*). American Midland Naturalist 135: 43-52.

Addendum A

Table 1

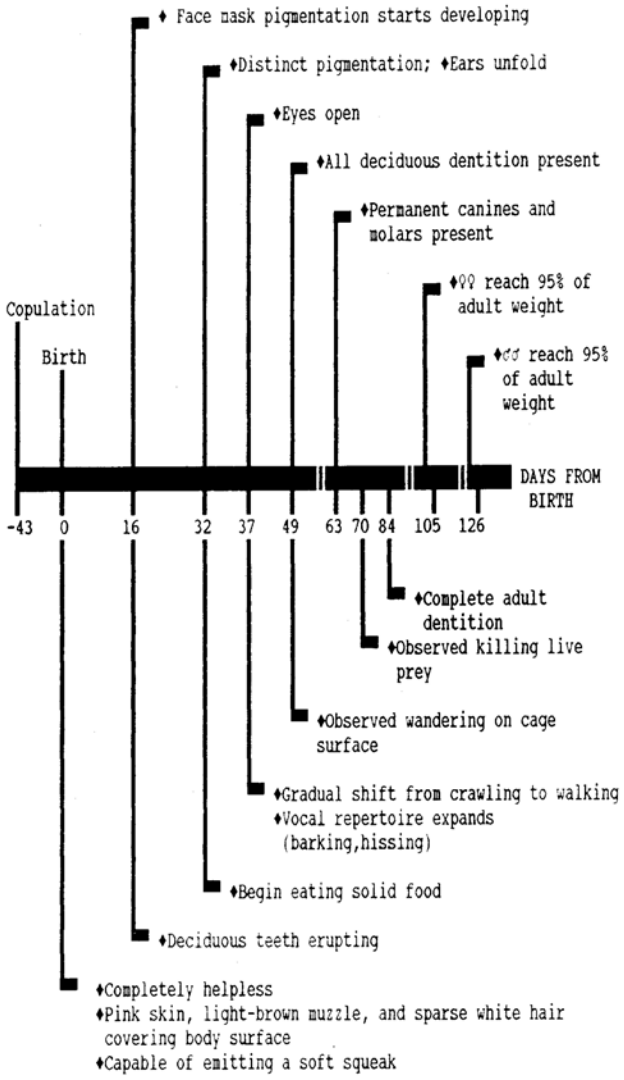


FIG. 2 -- Developmental landmarks for juvenile captive-raised black-footed ferrets. Postnatal days at which the various morphological and behavioral characteristics appear - approximate.

from Vargas 1996 American Midland Naturalist

BLACK-FOOTED FERRET VETERINARY CARE

QUICK FACTS

- **ANY BFF THAT LEAVES ALL OR PART OF A MEAL WARRANTS VETERINARY INVESTIGATION AND DIAGNOSTICS FOR THE CAUSE**
- **DO NOT GIVE VACCINE BOOSTERS AFTER 1 YEAR OF AGE DUE TO THE RISK OF RENAL AMYLOIDOSIS**
- **DIGESTIVE UPSET IS THE MOST COMMON ILLNESS, OFTEN DUE TO COCCIDIA, CLOSTRIDIUM OR CRYPTOSPORIDIUM.**
- **ONLY USE APPROVED VACCINES. DO NOT USE MODIFIED LIVE CANINE DISTEMPER VACCINES**
- **BFFS DO NOT TOLERATE MANUAL RESTRAINT OR OVER-HANDLING**
- **RENAL FAILURE IS COMMON IN OLDER ANIMALS**

BLACK-FOOTED FERRET VETERINARY CARE

INTRODUCTION

Black-footed ferrets (BFFs) are remarkably stoic animals. This is one reason why the veterinary care program for captive BFFs emphasizes preventive medicine. Diagnostic, prophylactic, therapeutic, and anesthetic techniques are described below as well as common diseases and disorders. Biological reference values and necropsy information are also included. FCC protocols are listed under **Addendum F.**

**Note: The Research Section included in the BFF Husbandry Manual contains an extensive bibliography pertinent to this chapter.

PREVENTATIVE MEDICINE

Quarantine

All BFFs in the SSP breeding program are considered in permanent quarantine. This type of protective environment provides a barrier between the BFFs and potential pathogens in an attempt to prevent an outbreak of canine distemper, human influenza, plague or other serious diseases infectious to BFFs. Many captive breeding centers help maintain a barrier environment by operating as 'shower-in' facilities with staff wearing separate clean clothing, shoes, gloves and facemasks. (Some facilities use only clothing changes, masks, foot dips, and disposable gloves or hand -washing.)

Exposure Quarantine:

Any BFFs exposed or potentially exposed to any known contagious pathogens, (e.g. when in transit or unusual exposure) should have a 30 day quarantine from other BFFs. Refer to facility protocols.

Annual Exams

Conduct annual health examinations during the pre-breeding season (usually October – December.) Exams should consist of:

Minimally

- ☑ visual exam
- ☑ oral exam (may do awake in handling cage with cotton tipped swabs)
- ☑ weight
- ☑ check AVID® transponder, replace if needed
- ☑ fecal exams

Ideally also include:

- ☑ brief Isoflurane anesthesia for thorough physical exam
- ☑ CBC/Chemistry Panel
- ☑ teeth cleaning/root canals as needed

Fecal Screening

- ☑ At least twice a year, preferably monthly; both direct and float exams; evaluate for clostridia.
- ☑ Include acid fast or PCR for cryptosporidia periodically and/or when indicated *[**Note**: cryptosporidia have been identified in normal looking feces of kits with no clinical signs other than failure to gain weight.]*

Pre-Shipment Exam Protocol (see Transport Protocol chapter)

- ☑ Visual exam, weight, oral exam (check for chipped, broken teeth)
- ☑ Check AVID® transponder
- ☑ Fecal exam - if parasites found, treat, recheck and clear before shipping.
- ☑ Ponazuril: oral: 30mg/kg 1 day pre-ship, during ship, 1 day post ship
- ☑ Current (2 doses) on CDV Merial Purevax® vaccine (none given to adults)
- ☑ Current on rabies vaccine, ImRab®

Vaccinations

Canine Distemper:

BFFs should NOT be vaccinated with modified live product. The only SSP approved CDV vaccine is *Merial's recombinant Purevax® Ferret Distemper*.

Vaccination Schedule:

- Kits 1st dose: 1ml, SQ @ 8-10 weeks
  2nd dose: 1ml, SQ @ 10-14 weeks
- Adults As most animals have high, protective titers from the initial series, **adult vaccination is NOT recommended.**

Rabies ImRab® Killed Virus vaccine: *1ml, IM, q 3 years*

Recommended for all BFFs. Given to kits prior to pre-conditioning

Use Of Sulfa Drugs

Any drugs in the sulfa family, including **Albon®** and **SMZ/TMP**, may prevent uterine implantation of the embryo. The most crucial time to avoid these medications is during the two weeks prior to breeding and the two weeks immediately after breeding. **Any female that has vulvar swelling or is pregnant should not receive any sulfa based medications.** There is

some evidence that sulfa drugs may also impair sperm development. The SSP has stopped using sulfa drugs as an anti-coccidial, using ponazuril instead. See **Addendum B.**

DISEASE

BFFs are unfortunately very stoic animals. They are able to endure severe dental, gingival disease, neoplasia, enteritis, pyothorax, and pyometra without showing obvious clinical signs until near death. Caretakers need to be especially vigilant and sensitive to changes in an animal's appetite, stool, and general attitude. Veterinarians are encouraged to be proactive and aggressive in therapy if they are to help BFFs that are clinically ill. In general, **a BFF that skips a single meal is reason for concern and initial diagnostics** (e.g. fecal exam).

Dr. Suzanne Kennedy-Stoskopf documented that BFFs lack interleukin-6 activity as compared to domestic ferrets. This deficiency may predispose them to infectious agents that require a strong humeral response or neutrophil mobilization.

Parasitism

Coccidiosis: Coccidia is the most common, and sometimes fatal, intestinal parasite of BFFs, (*Eimeria ictidea, E. furonis*). Shedding of oocysts, however, is variable. A sample can be negative one day to 50,000 oocysts/g the next. Clinical signs include mucousy, loose stools, partial to total anorexia, and lethargy. However, sudden death can occur without premonitory signs. Clinical disease and shedding of oocysts is often associated with stressful events. On histopathological exam, the organism can be seen to invade nearly all intestinal epithelial cells. Any abnormal stool should be collected for examination. Since acute death is a possibility; if fecal exam results are not available within a few hours of collection, a clinically ill animal should be prophylactically treated for coccidia while awaiting definitive fecal exam results.

If the BFF is not eating the oral suspension of ponazuril (*Marquis®)* should be instilled in the animal's mouth. Secondary sepsis can occur due to the compromised intestinal lining. Penicillin may be helpful in preventing fatal infections.

Prevention is key with use of ponazuril, reduction of stress and thorough removal of feces from environment.

Coccidia treatment:

Ponazuril (*Marquis*®) 30-50mg/kg PO once, may repeat weekly as needed.

May use toltrazuril with similar dosing protocol.

Ponazuril Guidelines & Dosing

- For coccidiosis- prophylaxis and treatment
- Ponazuril, a coccidia-cide comes in an equine paste called "*Marquis*®"
- To make a 30 mg/ml solution:

- Mix 1 part ponazuril to 4 parts tap water (e.g. 10ml paste to 40ml water)
- Shake well before each use
- Store at room temperature, date and discard after 30 days

- For clinical cases:
  - Based on PK studies, we recommend a dose 30 to 50 mg/kg once/week as indicated.
  - If large numbers of oocytes are found on the fecal exam it is best to treat daily with ponazuril 30-50 mg/kg for 3-7 days until clinical signs have ceased or until the numbers of oocytes shed in feces is greatly reduced
  - For 50 mg/kg dosage mix 1 part ponazuril with 2 parts tap water
  - Shake well before each use
  - Store at room temperature, date and discard after 30 days
  - Also recommended is a penicillin product to cover increased intestinal bacterial risk.

- For prophylaxis:
  - Transfers, anesthesia, EEJ, - give 30mg/kg one day prior to scheduled procedure. Give immediately in case of unrelated illness/injury or unusual stress.

Disinfection:
Coccidial oocysts are extremely resistant to common disinfectants like bleach or quaternary ammonium products. *Oo-cide*®, a product made by Antec International, has been shown to reduce environmental contamination; however it requires removal of animals and special protective gear prior to application. Therefore, thorough removal of stools during routine cleaning is critical.

Cryptosporidiosis: This zoonotic intestinal parasite is less commonly diagnosed than coccidia and has lower associated mortality. It is, however, more difficult to diagnose and effectively treat. Clinical signs include a persistent mucousy, loose stool, and poor growth or loss of condition. Shed oocysts are infectious to other animals, and difficult to kill. Acid fast stains of fresh feces must be used to find the tiny oocysts. BFFs shed a large number of oocysts compared to reptiles. Acid fast staining is used to identify oocysts. (see **Addendum C.)**

Resolution of clinical symptoms and reduced shedding of oocysts has been seen with the use of **azithromycin @ 40mg/kg PO SID x 21 days**. For severe cases, treatment can be up to 30 days. **Paromomycin may also be effective @ 165mg/kg PO BID x 5 days**. Recheck seven days after treatment ends. **Nitazoxanide at 25-30 mg BID for 14 days may also be tried, but has not proven to be effective**. Currently, there is no therapy that will completely eliminate the parasite. Under stressful conditions (shipment, breeding, whelping), BFFs may again shed infectious oocysts and show signs of illness.

A solution of 3% hydrogen peroxide for 10-15 minutes is recommended for disinfection.

Common disinfectants against Cryptosporidium species - *O'Donoghue, 1995*

Agent	Application
Ammonia 5%	for 20 min.
Chlorine dioxide 0.4 parts per million	for 15 min.
Hydrogen peroxide 3%	for 10 min.
Ozone 1.1 parts per million	for 5 min.

Toxoplasmosis: A documented outbreak of *T. gondii* in a captive BFF colony occurred at Louisville Zoo in 1992 (Burns, Am. Assoc. ZooVet. Proc. 1993). Clinical signs included partial to total anorexia, lethargy, corneal edema, ataxia, and death. Predominant histopathological findings were hepatitis, splenitis, pneumonitis, myocarditis, nephritis, and nonsuppurative encephalitis. Rabbit used in preparing the captive diet (60/40) was the most probable source of the infection. The initial treatment at Louisville Zoo was TMS @ 30 mg/kg PO BID x 5 days, then sulfadimethoxine @ 30 mg/kg PO BID x 10 days. **The infection is life long as *T.gondii* organisms encyst in tissues. The BFFs that survived the initial infection clinically reactivated at times of stress or as the animals aged. When treating clinical cases of reactivation, LZG used clindamycin @ 12.5-25mg/kg, PO or IM, BID x 14 days (Greene, Infectious Diseases of the Dog and Cat). Months to years later, all BFFs that were present as adults at LZG during the epizootic acquired encephalitis due to chronic toxoplasmosis.**

Giardiasis: *Giardia sp*. has been documented in BFFs in outdoor pens and was associated with diarrhea. Treatment consisted of metronidazole @ 30 mg/kg, PO SID x 5 days **or** paromomycin @ 165 mg/kg PO, BID x 5 days.

Heartworm: If exposed to mosquitoes in a heartworm prevalent area (e.g. Front Royal, VA) treat BFFs prophylactically with **ivermectin** monthly at **0.2mg/kg**. [SCBI prepares a **1mg/ml** solution and doses orally at 0.2 ml for males, and 0.15 ml for females after confirmation that filarids aren't already present as adults.]

External Parasites: Ticks and fleas occur on outdoor pen-housed and free-ranging BFFs. To treat, use a 0.1% pyrethrin, Frontline® topical (kitten dose), or 5% carbaryl powder labeled for use in kittens. Ivermectin may also be helpful.

Dr. Beth Williams identified a demodex type mite on a few BFFs with pruritus and reddened skin. However, no treatment has been used for this parasite.

Viral

Canine Distemper Virus (CDV): Exquisitely sensitive to this virus, BFFs exposed to CDV experience 100% mortality. Signs include oculonasal discharge, dermatitis, pruritus, hyperkeratotic footpads, and neurologic signs. Some modified live virus vaccines, even of chick embryo origin, can cause fatal distemper in this species. **Merial PureVax® Ferret Distemper is the only safe CDV vaccine; see above section under Preventative Medicine.**

If signs of distemper are suspected **immediately isolate the animal** from other BFFs. Veterinary staff should consult with USFWS.

Influenza: Domestic ferrets are used as a model for the human influenza virus since they display similar signs of lethargy, anorexia, oculonasal discharge and diarrhea. BFFs are also susceptible to influenza infection from human caretakers. Toronto Zoo confirmed a human influenza outbreak in BFFs, including kits, in 1997. Morbidity was higher in kits than adults, but all survived with supportive care. This is the primary reason for wearing facemasks when near BFFs.

Bacterial

Plague (*Yersinia pestis*): BFFs are also exquisitely sensitive to plague. A single fleabite dose of *Y. pestis* is usually fatal. Exposure from ingesting a plague-infected carcass is just as deadly. Signs seen are acute mortality with or without rectal hemorrhage. *Yersinia pestis* from flea-bite is not likely to be encountered in the captive indoor environment. It is possible in outdoor pen enclosures and in free-ranging animals. Also, despite the effective quarantine for prairie dogs fed to BFFs, mistakes can occur that could expose indoor animals to plague. If plague is suspected, notify USFWS for direction. Plague is a zoonotic disease and appropriate precautions should be taken. Pyrmethrin or carbaryl dusting for flea control in and around outdoor pens in plague endemic areas is justified.

Dr. Tonie Rocke at National Wildlife Health Center modified an F1-V protein vaccine from the US Army. Injectable vaccine trials in BFFs were successful, but availability of the vaccine is limited, The Service gives the vaccine to animals planned for release and going into the pre-conditioning pens. Deltamethrin dust is used in plague-threatened reintroduction sites and a promising oral vaccine for prairie dogs is currently undergoing field trials.

Clostridium: *Clostridium perfringens* is a common cause of gastrointestinal upset. It may present as a loss of appetite or loose (often mucoid or bloody) stool. It can also present as a sudden death due to enterotoxemia. It can easily be identified on a direct fecal smear, stained with Diff Quik® type stain. An over abundance of large rods will be present with many sporulating rods. The presence of spore forming rods is diagnostic enough to begin treatment with oral amoxicillin. Be sure to include a direct smear in your routine fecal evaluations.

Pneumonia and Pyothorax: This not uncommon and usually fatal disease complex may be present even though a BFF shows no evidence of respiratory illness. Symptoms may include lethargy and anorexia. BFFs may also present with concomitant coccidiosis. Radiographs, blood work, and thoracocentesis (chest tap) are diagnostic. Aggressive antibiotic and supportive therapy with the possibility of a chest tube may save the animal. Aspiration or severe dental disease may be responsible for some of the cases. The SSP recommends a dust-free bedding, such as Alpha-dri®.

Gingivitis: Older animals (>3 years old) commonly have some degree of gingivitis, ranging from mild, associated with dental calculi, to severe, with apical abscessation and tooth loss. Often animals will continue to eat even with severe disease. Alpha-dri® paper chip bedding has not been associated with tooth problems. The soft diet traditionally fed to BFFs may contribute to gingival disease. Whole carcass feeding at least 2x/week is currently recommended.

Conjunctivitis: Years ago, several institutions had kits with purulent conjunctivitis prior to their eyelids opening. Culture, antibiotic treatment, and drainage helped alleviate symptoms. Other kits had no discharge, conjunctivitis or any sign of infection, but had spontaneous ulcers or rupture of the cornea in a descemetocele after the eyelids naturally separated. If there is a descemetocele, a temporary tarsorraphy and antibiotics are indicated. These ocular problems may be related to a congenital infection from the dam's birth canal, high ammonia vapors, or a genetic predisposition. Preventative management includes added ventilation of nest boxes via computer fans and or prophylactic pre and peri-natal treatment of dams with amoxicillin.

ADDITONAL MEDICAL CONCERNS

Neoplasia

Older BFFs (more than 4 years) have a high frequency of malignant neoplasia, but the types seen are very different from domestic ferret tumors. Biliary cystadenoma/carcinomas, renal tubular neoplasms and apocrine gland neoplasm are the most common. Other common malignant tumors include squamous cell carcinoma and adrenal carcinoma. BFFs have a high frequency (>50%) of multiple neoplasms (see **Addendum D**).

Renal

As BFFs age, many experience polycystic kidney disease. Symptoms are polydipsia and polyuria. Often this disease progresses to neoplasia. Additionally, some post-mortem exams have revealed an incidental finding of a unilateral kidney, apparently linked to the “Dean” genetic lineage.

Amyloidosis

Renal and other areas of amyloidosis have become more common, even in younger animals, and are being studied by our SSP® pathologist Mike Garner of NorthWest Zoo Path. See Post Mortem section for address)

Other

BFFs may incur various other conditions such as seasonal /humoral alopecia, dermatitis, pruritus, vaginitis, bite wounds, cataracts, heart murmurs, corneal ulcers, cryptorchidism and hernias.

REFERENCE VALUES

		ADULTS	90 DAY KIT
Weights:	Male	850-1200 g	850 g
	Female	650-850 g	650 g

(See growth curve for kits in Kit Development Section)

Adult Pulse Rate: 247 BPM (calculated) for 900g BFF at rest.
180-255 BPM under isoflurane anesthesia
75-150 BPM under medetomidine/ ketamine anesthesia

Temperature: 99-102ºF; variable under medetomidine/ ketamine, may be as high as 104°F in excited animals

Adult Respiration: 20-40 breaths per minute

Adult Dental formula: 2 x (incisors: 3/3, canines:1/1, premolars: 3/3, molars: 1/2) = 34

Clinical Pathology: reference values compiled by MedArks, 1998 (**Addendum E**.)

VETERINARY PROCEDURES

Venipuncture

Brief anesthesia is required, preferably isoflurane. The *jugular vein* is ideal for most blood collection needs, but requires practice to perform safely and effectively. The cervical skin is thick and tough and the vein can be "weasel-y" if not held off firmly. Use a ¾ inch, 20 or 22 gauge needle on a 3cc syringe and hold the entire neck and vein between forefinger and thumb. Cranial vena cava puncture is also an option for the experienced.

An alternate site is the *cephalic vein* in the forearm. If a small tourniquet is applied, this vessel may be entered with a 25-gauge needle and blood collected in capillary tubes from the needle hub. Squeezing the paw increases the flow. Another alternate site is the *caudal vein* on the ventral side of the proximal tail using a 25-gauge needle as above. The cranial vena cava may be used by an experienced veterinarian.

Most hematology, chemistry, and serology tests can be run from **3 ml of blood**. An absolute maximum of 17 ml/kg can be taken, if necessary, e.g. for transfusions. In the event that maximum blood volume is withdrawn, subcutaneous or IV fluids and supportive care should be given and the patient monitored closely.

Transponder ID

The BFF SSP uses the AVID® transponder chip identification system. All animals should have a functioning microchip implanted SQ over the interscapular area. After a surgical prep of the site, the chip is implanted, and the needle hole is closed with a fast-acting tissue glue. Reintroduction sites may request a second chip be placed. The Service provides AVID® transponder chips for all SSP captive-breeding facilities.

RESTRAINT AND ANESTHESIA

Restraint

BFFs can be superficially examined and non-invasive procedures (e.g. reproductive assessments) carried out in a small, covered wire-mesh handling cage. By briefly restraining the tail through the mesh or opening the cage and gently pressing the BFF to the back of the cage with a glove or towel, SQ and IM injections can be given relatively easily. BFFs should never be manually restrained unless they are young kits. Attempted manual restraint of an adult BFF can cause severe stress, leading to hyperthermia, possibly to the point of death.

Anesthesia

The preferred anesthetic is isoflurane gas. A small, ferret-sized anesthetic chamber can be easily built of Plexiglas® or Lexan®. Once induced, most procedures can be carried out with a facemask. For oral or prolonged procedures a 2.5 to 3.5 mm cuffed endotracheal tube is required. Pulse oximetry can be monitored via the ear, foot, base of tail, fold of skin, or, with the appropriate probe, via the esophagus or rectum. Heart rates may be in excess of 255 bpm. Non-medicated ophthalmic ointment or saline eye drops may be instilled while BFF is anesthetized. MidMark produces/sells a small (5.25 liter) induction chamber with scavenge port. (800-MIDMARK; www.midmark.com)

If gas anesthesia is not available or practical, as in some field sites, injectable ketamine, medetomidine/ketamine mix, or ketamine/valium mix is useful. (Ketamine/valium mix is used for electro-ejaculations. See Semen Collection protocols Addendum D in Reproduction chapter.) Ketamine alone may be adequate for short procedures in captive animals where full muscle relaxation and quick recovery is not important, though seizure have been seen. Telazol at 8-12mg/kg has also been successful though it has a longer recovery and is rarely used. For muscle relaxation and a quick recovery medetomidine combined with ketamine has been used successfully. Heart rates and pulse oximeter oxygen saturation readings are usually quite low with medetomidine; therefore supplemental oxygen should be administered. Thermoregulation is also impaired so external heat or cold should be applied depending on the BFF's temperature and ambient conditions. At least 20 minutes after medetomidine/ketamine administration the **medetomidine can be antagonized by atipamezole @ 0.45 mg/kg IM**. Recovery is usually seen in three to seven minutes.

Dosages:	**Diazepam alone**	**0.2mg IM for adult BFF**
	Ketamine/diazepam	**20-25 mg/kg ketamine, 0.1 mg/kg diazepam**
	Ketamine alone	**20-25 mg/kg IM**
	Ketamine/ medetomidine	**3mg/kg ketamine, .075 mg/kg medetomidine**
	Atipamezole	**0.45 mg/kg IM**

POST-MORTEM PROCEDURES

Refrigerate, **do not freeze**, BFF carcasses until all exams are complete. A prompt, thorough and complete necropsy should be performed by a veterinarian on any BFF that dies.

Histopathology tissue samples of all organ systems should be preserved in 10% buffered formalin at a ratio of 1 part tissue to 10 parts formalin. Preserved tissues can be sent to the SSP Pathology Advisor, Dr Mike Garner at Northwest ZooPath, or to any veterinary histopathologist. If sending to your own pathologist, please obtain a second set of tissues to be kept for the SSP or request the tissues and slides back from your pathologist after examination.

Neonates

- ✂ weight and sex
- ✂ include umbilical stump
- ✂ examine for malformations
- ✂ determine if breathing occurred; do the lungs float or sink in formalin?
- ✂ determine nursing activity by looking for milk curd or milk stool in the GIT
- ✂ assess hydration (tissue moistness)
- ✂ culture appropriate tissues for bacteria
- ✂ If appropriate, examine the dam for mammary development, agalactia or mastitis

Reproductive tissue

Remove entire female tract (ovaries, uterus and vagina). Fix whole tracts in large volume, (10:1 formalin to tissue) of 10% buffered formalin.

Post Mortem Check List

☑ Email or fax a completed copy of the BFF **Mortality Form** (**Addendum A**) by next business day to USFWS at the NBFFCC. [fax # 970-897-2732] and to the Studbook Keeper, Paul Marinari.

☑ You may use any pathology service; however, Dr. Mike Garner at NorthWest ZooPath is the SSP's official pathology advisor.

☑ Send a copy of final histopathology report to:

- NorthWest ZooPath, 645 W. Main St., Monroe, WA, 96272
  360-794-0630 zoopath@aol.com

☑ After necropsy, the carcass should be frozen, labeled with studbook # and date of death and sent by overnight currier to USFWS at: US Fish and Wildlife Service
National BFF Conservation Center
19180 I-25 Access Road
Carr, CO 80612

Please alert USFWS staff of shipment: Phone: 970-897-2730 or email: either robyn_bortner@fws.gov or mary_wright@fws.gov

Pursuant to permits issued by the Service, all BFFs and any of their parts belong to the Service. Disposal of animals and/or by-products must be approved by and at the discretion of the Service.

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ADDENDUM A

Black-Footed Ferret Mortality Report
Facility Name/Release Site Date BFF Studbook #
Facility/ISIS ID Name/other ID Male☐ Female☐UNK☐
Date of Birth {If Kit: Dam SB# Sire SB# }
AVID Transponder # front: #back
Release ☐ Death☐ Euthanasia ☐ Presumed death ☐ Escape☐ Date :
Comments

Was the carcass retrieved? Yes☐No☐ If yes, fill in information below:
Submitted for necropsy by Date
Gross exam performed by Date
Histopathology performed by Date
List any cooperating labs:

Copy of pathology results sent to NBFFCC? Yes ☐ No ☐ Date Initial
Describe history prior to death, (clinical signs, antemortem test results, diet changes, treatment, circumstances of death, etc.) Last Recorded Weight: Post-mortem weight:

Medical record attached? Yes ☐ No, Will be sent ☐
Location of carcass Date
Location of carcass Date

- Mail **FROZEN** carcass in double zip-loc bags labeled with studbook # and date of death by **overnight** delivery to: USFWS, 19180 I-25 Access Rd, Carr, CO 80612 (970)897-2730
  Date sent:

- **Within 24 hrs of death**: Mail/ Fax/ OR Email a Copy of this report to:
  - USFWS-Nat'l BFF Conservation Center, PO Box 190, Wellington, CO 80549
  - FAX# 970-897-2732
  - email to robyn_bortner@fws.gov and marinarip@si.edu

- Original Mortality Form should be kept on file by the submitting agency or facility.

Addendum B

Sulfas and Reproduction

Sybille experienced an unusually high incidence of non-pregnancies or pseudopregnancies from 5-8 through 5-21. 23 females were due to whelp during this 13 day interval. Only 2 live kits were produced from these 23 females. 5 females whelped a total of six kits. 4 females had one dead kit each, and 1 female had 2 live kits on 5-14. A retrospective study of this phenomenon led to a possible correlation between Sulfas in the ration and this extremely low fertility rate.

If this consecutive string of 23 females, hereby dubbed the "Sulfa 23" are compared to the rest of the female population at Sybille the following is noted:

	Sulfa 23	Other Sybille 50
Whelping rate	22%	76%
Kits born per female	.26	2.64
Kits surviving per female	.04	1.88

Extrapolating back to the breeding dates of the "Sulfa 23" yields breeding dates of 3-27 to 4-8.

Sybille nutrition records show that on 4-7 Sulfadimethoxine (Albon, Roche Labs) was added to the 60/40 regular Sybille diet. This was done to treat all adults for coccidiosis due to 2 recent acute coccidiosis deaths among the adults. 625 ml of Albon (50mg/ml) was added to 160 lbs of 60/40 rations. These rations were fed for the next 6 consecutive days. From 4-10 until 5-7, no significant decrease in fertility was seen between 1993 and previous years. On 5-8 production at Sybille essentially ceased for 13 consecutive days with 2 live kits from 23 females.

It was suspected that there could be a correlation between Sulfas and the infertility. Sulfa's are contraindicated in the last trimester of pregnancy and during lactation in humans, due to the ability of the sulfa drugs to readily pass the placenta and to be excreted in the milk, causing kernicterus in infants (PDR 1994). However any references to problems associated with these sulfas and early pregnancy are not easily found.

A brief review of the pharmacology of Sulfas:
-interfers with folic acid synthesis by competition with PABA
-diffuses well after aborption (Neu 1979)
-crosses cell membranes readily, distributed after absorption to body water, high levels in most body fluids (PHM 520)
-classified by long and short acting

Sulfadimethoxine is one of the long acting sulfas, readily absorbed from the GI tract maintaining high serum levels due to

Addendum C

ACID FAST STAIN FOR *CRYPTOSPORIDIUM* FECAL SMEARS

Mycobacterium and some fungal/yeast spores are also stained by this technique, but are morphometrically different from *Cryptosporidium*. This stain is sometimes referred to as the ZIEHL-NIELSEN stain. The protocol that follows is described for use in staining jars, however, it could also be done on a staining rack but stains should be collected and properly disposed.

1. Smear specimen on slide
2. Allow to dry
3. Dip slide in Carbol-Fuchsin and leave for about 4 min.
4. Dip repeatedly in Acid-Alcohol until red color bleeds out of smear
5. Dip in water
6. Dip in Methylene blue (~5 dips)
7. Dip in water
8. Allow to dry
9. View with 40X objective lens, look for red cysts about 4-6 um in diameter. The smear can also be viewed with 100X and oil when questionable red objects are seen using 40X.

Solutions used in our lab
Carbol-Fuchsin ZN, TB from Fisher Scientific
Methylene Blue TB (stains used for Mycobacterium work well), Fisher Scientific
Acid Alcohol= 95% Ethanol, 5% concentrated HCl

Addendum D

Table 4.5 Prevalence of biliary cysts and neoplasms at death and relative frequencies of neoplasms for black-footed ferrets in the study group.

Conditions	No. of cases	Prevalence [a] (%)	Frequencies [b] (%)
Biliary cyst	121	65.76	-
Total neoplasms	185	55.43	100
Total epithelial neoplasms	171	47.28	92.43
Renal tubular neoplasm	38	20.65	20.54
Total biliary neoplasm	37	20.11	20
Cystadenoma	14	7.61	7.57
Cystadenocarcinoma	23	12.5	12.43
Total apocrine neoplasms	71	28.26	38.38
Total sweat gland neoplasms	36	19.57	19.46
Adenoma	13	7.07	7.03
Adenocarcinoma	23	12.5	12.43
Total mammary gland neoplasms	13	7.07	7.03
Adenoma	3	1.63	1.62
Adenocarcinoma	10	5.43	5.41
Adenocarcinoma - apocrine gland, anal sacs	16	8.7	8.65
Total preputial gland neoplasms	6	3.26	3.24
Adenoma	3	1.63	1.62
Adenocarcinoma	3	1.63	1.62
Sebaceous gland adenoma	5	2.72	2.7
Total squamous cell carcinomas	8	4.35	4.32
Oral squamous cell carcinoma	7	3.8	3.78
Cutaneous squamous cell carcinoma	1	0.54	0.54
Carcinoma of the anal sac	2	1.09	1.08
Epidermal cyst	2	1.09	1.08
Basal cell tumour	2	0.54	1.08
Nasal carcinoma	2	1.09	1.08
Transitional cell carcinoma	1	0.54	0.54
Undetermined epithelial neoplasm	3	1.63	1.62

Normal Blood Values Addendum E

TEST	BFF CALCULATED RANGE	BFF ACTUAL RANGE	DOMESTIC ABAXIS & ISIS RANGE
WBC	2.07-6.05	1.79-13.05	2.0-10.0
LYM	1.01-2.93	0.64-5.04	0.4-6.5
MON	0.03-0.33	0.02-0.56	0.1-0.7
GRA	0.64-3.18	0.67-7.87	0.8-4.5
LY%	35.5-62.5	23.5-69.3	27-80
MO%	1.1-8.3	0.8-11.9	0-10
GR%	34.8-57.8	19.7-64.6	16-70
RBC	10.09-11.39	9.47-11.91	7.8-13
HGB	17.4-19.8	14.6-20.2	12.4-18.7
HCT	40.71-53.07	37.22-52.33	36-56
MCV	42-47	32-47	40-48
MCH	16.2-18.4	12.6-19.1	13.5-16.5
MCHC	37.6-40	37.3-42.5	32.1-35.5
RDWc	15.7-22.6	15.7-31.5	//
PLT	501-747	360-850	96-776
PCT	0.38-0.64	0.26-0.80	//
MPV	7.4-8.8	6.2-9.5	//
PDWc	34.5-39.3	30.5-41.2	//
ALB	2.9-3.9	2.1-4.1	3.2-4.2
ALP	38-61	29-81	1-215
ALT	84-163	74-213	63-205
AMY	9.0-16.0	5.0-19.0	15-47
TBILI	0.2-0.3	0.2-0.5	0.2-0.6
BUN	13-28	11.0-47.0	15-33
CA++	9.0-10.2	7.7-10.6	7.8-10.0
PHOS	5.0-7.0	4.0-8.0	3.5-7.1
CRE	0.4-0.6	0.2-0.8	0.4-1.0
GLU	137-180	116-208	114-147
NA+	149-155	148-161	134-164
K+	4.2-5.2	4.1-5.7	4.0-5.2
TP	5.2-6.0	4.9-6.4	5.9-7.1
GLOB	1.6-2.8	1.4-3.7	2.2-3.4

Addendum F

USFWS NBFFCC Annual Physical Exam Protocol

Updated 2014 by Mary Wright DVM

All black-footed ferrets (BFFs) in the facility should have a complete physical exam each year. This is to screen for problems and changes that may not be noted with routine observation during husbandry operation.

Physical exams for adults are to be done in the non-breeding season. Generally this can be done December through mid- February. All annual physical exams are done with the BFF under general anesthesia (see anesthesia protocol) to allow complete relaxation.

BFFs less than one year of age are not included as they had a complete physical within the last 10 months, during their kit preventative care protocol.

Physical exam includes:

- Check vaccination record to be sure current and complete, review file for prior notations
- Gross external exam (skin, limbs, wounds, masses)
- Scan for AVID® transponder, verify matches identity of BFF, confirm transponder working
- Palpate abdomen, special attention to palpation of kidneys to record shape and size
- Testicular palpation or note vulvar swelling if present
- Auscultation of heart, lungs sounds are difficult to hear
- Check ears
- Oral/dental exam, complete dental record, scale teeth if needed and record such, perform extractions or plan if needed
- Note any congenital abnormalities if not previously noted
- Collect blood for blood chemistry and CBC on a case by case basis, generally BFFs greater than 4 yrs. of age should be evaluated

Keep anesthesia time to a minimum and record anesthesia time from beginning of induction until removed from Isoflurane gas.

BFF Dental Chart.pdf

KIT PREVENTATIVE CARE SCHEDULE: "KIT PROCESSING"

USFWS NBFFCC updated 2014

Mary Wright DVM

30 Days of Age

Kits are given ponazuril 50 mg/kg (0.1 cc/100 mg of body weight) once by mouth. The Dam is locked out of the nest box and each kit is gently handled for a brief exam (external exam for defects, weigh, confirm sex and auscultate the heart) and given ponazuril orally calculated for the kit's weight. Findings are reported in the dam's medical record.

50-60 Days of Age

Each kit is examined under Isoflurane anesthesia (see anesthesia protocol) for a complete physical. A medical record is begun for each kit by studbook number. Multiple vaccines **are** given at the same time but administered at separate locations on the BFF's body. At this time no complications have been noted with this protocol.

Each kit is given the first vaccine dose for the following:

- Merial's *Purevax*® Ferret Distemper (CDV) vaccine, 1 cc, SQ, left shoulder.
- Plague (F1V) vaccine, **0.5 cc**, given SQ on the right shoulder.
- A dose of penicillin G, 60,000 mg/kg, is given SQ, right hind.

Each kit is micro-chipped with an AVID® transponder via syringe SQ implantation between the shoulder blades. This is done while the kit is under anesthesia. One drop of tissue adhesive is placed to close the injection site. The transponder number is recorded both in the BFFs medical record and the Transponder Log. Kits planned for travel and release in Canada receive a second transponder (under anesthesia) over the rump, after they complete preconditioning.

Once the kit has recovered from anesthesia a prophylactic dose of ponazuril 50 mg/kg (0.1 cc/100mg of weight) is given by mouth before the kit is returned to the nest box.

70 Days of Age

From historical data in our facility, it was found we have an increased incidence of coccidial diarrhea develop in kits after 70 days of age. This is believed to be due to crowding and adolescent stress as the litters mature since in the wild at this age they would begin to separate from the common nest chamber.

In order to prevent this from occurring it has been found to be beneficial to dose the litters and dams with ponazuril at 50 mg/kg added to their food pieces every 7 or 14 days. Realizing we cannot assure whether each kit ingested an adequate dose we have elected to treat the litters in the OD pens once a week and the indoor litters only every other week. The indoor

litters can be monitored more closely for signs of infection, and this is less labor for the staff as we know the plasma level maintains for at least 10 days.

90 Days of Age (2-6 weeks post first vaccines)

Each kit is caught up into a catch cage to be given booster vaccines and a rabies vaccine. These are given without anesthesia as no detailed physical exam is needed. All 3 vaccines are administered at the same time but in separate locations on the ferret's body as listed below.

- Booster of Merial's *Purevax*® Ferret Distemper (CDV) vaccine, 1 cc, is given SQ.
- Booster of Plague (F1V) vaccine, **0.25 cc**, is given SQ.
- Rabies vaccine, Imrab3® by Merial, 1 cc, is given SQ.

The kit is manually, but gently, squeezed in the catch cage with a gloved hand and injected SQ in 3 separate locations, one for each vaccine. It is difficult to select a certain location for each due to their wiggly nature, but an attempt should be made to follow the pattern of CDV left shoulder, F1V right shoulder and Rabies right hip.

Incoming transfers

BFFs arrive into this facility from SSP partner facilities. They may arrive to be added to our breeding population or for preconditioning prior to release. These may be kits or adults. All animals to be released must go through, and pass, preconditioning at this facility before release.

Vaccination status of all transfers must be checked shortly after arrival. All transfers should be current on CDV and rabies. Kits may not have received both doses of CDV or a rabies vaccine due to age. These can be administered on a case by case basis after arrival. Medical records should be provided by the facility of origin and should be reviewed carefully. A medical record must be prepared or located here (folders for medical records for adults held at other facilities are kept on file here) to transfer the information into and to keep on hand as for any other BFF within the facility. All additional vaccines given here must be entered into the new record. Any adult record should be checked carefully for prior vaccines given, especially F1V.

All BFFs transferred into this facility will be vaccinated for rabies if not already done so prior to arrival. All BFFS transferred into the facility for preconditioning or breeding will be vaccinated for F1V. F1V vaccinations are only administered in this facility or in the field for wild populations. All kits transferred here will need F1V vaccinations.

Vaccination Records

All vaccinations are recorded in the individual BFF's medical record. In addition, all vaccinations should be recorded on the Vaccination Log by date administered.

Ponazuril Use and Dose

Plasma serum levels for ponazuril in BFFs were studied in 2013. Plasma levels achieved therapeutic levels quickly and were found to be maintained for 10 days after a single dose of 50

mg/kg given orally. A dose of 30 mg/kg was also found to be adequate if assured the BFF ingested it, thus a 3 day regimen of 30 mg/kg is used if applied onto food. With these results in mind a prophylactic dose following a "stressful event" of 50 mg/kg orally is used.

Ponazuril is supplied by Bayer, under the trade name of Marquis® for equine use. Marquis® is a 15% ponazuril paste formulation. We use a 1:2 dilution to produce a 50 mg/ml solution. Typically 10 ml of Marquis® paste is mixed with 20 ml of tap water to produce our 1:2 dilution, 50 mg/ml solution. It must be mixed well before each use. A 100 mg/ml solution can be produced by making a 2:1 dilution, 20 ml of ponazuril to 10 ml of water. This concentration reduces the volume needed to be administered and is useful when applied to food since it is thicker in consistency.

PROTOCOL FOR GROUND AND AIR TRANSPORT OF BLACK-FOOTED FERRETS

PRESHIPMENT PREPARATIONS

Animals

- Conduct a visual physical examination of black-footed ferrets (BFFs) within 2 days pre-shipment to ascertain if healthy. Do not ship symptomatic animals (e.g., weight loss, diarrhea). BFF(s) may be shipped once treated and back to normal.
- BFF(s) should be current on distemper vaccinations, following Species Survival Plan (SSP) guidelines. BFFs destined for Canada, as well as a number of states, require a rabies vaccination. Check with BFF SSP Veterinary advisor for current state/country requirements.
- If possible, collect a fecal for analysis. Each facility may have specific pre-ship requirements. Once transfer recommendations are approved, facilities should be in communication for pre-ship requirements.
- Verify BFF's AVID® transponder number day of shipment.
- Prophylactically treat BFFs) with ponazuril day before, day of and day after shipment.

Paperwork

- Inform the receiving institution if the animal is on medication. Send remaining doses if possible.
- Check with receiving institution to determine if they require specific testing, etc. pre-shipment.
- Make certain all permits are in order:
    - *State permits
    - *Certificate of Veterinary Inspection (International health certificate if applicable)
    - *CITES permits, NAFTA, commercial invoice, air bill, and other required documents for international shipments
- Complete APHIS form 7020.
- Compile husbandry, diet, and medical records, if appropriate.
- Check airline for additional shipping document requirements (domestic and international).
- All required paperwork should accompany BFFs. (Individual zoos may have other specific paperwork that needs completed for registrars, managers, etc.)

Transport Vehicle

- Inspect vehicle to make sure it is mechanically sound, including air conditioning and ventilation.

- Thoroughly clean (and disinfect if necessary) vehicle.
- If it is going to be hot, shield BFFs from direct sunlight.
- Keep radio noise down to reasonable levels.
- Avoid excessive heat and ensure vehicle is not parked next to running vehicles to reduce likelihood of CO poisoning.
- Compile a list of supplies- handling cage and gloves, flashlight, bottled water, contact numbers.

Shipping Container - *Ground Transport*

- Small to medium pet kennels work best for a variety of transportation modes. Some facilities use a Plexiglas® door covering (with ventilation holes drilled into it) attached to the inside of the kennel door to keep BFFs from chewing on wire and breaking teeth. (See photo **Addendum**)
- Equip kennel with short piece of 10 centimeter (cm) (4 inch (in)) diameter corrugated plastic tubing and clean shredded paper (make sure all staples are removed).
- In excessively hot weather, transporter may wish to elevate kennels from vehicle floor to increase ventilation around crates and prevents overheating.
- Be sure heating/air conditioning is working properly to keep BFFs within acceptable temperature parameters (10^{o}Celsius (C) (50^{o}Fahrenheit (F)) to 23.8^{o}C (75^{o}F), although heat should be avoided.
- Label kennel with BFF's stud book number, transponder number, ISIS number, and sex, as well as LIVE ANIMAL signs. (Use a black Sharpie® marker on a piece of silver duct tape to label kennels; tags attached with string may come loose and get lost.)
- -Provide each animal with one food item, cut open along midline, dosed with .5 ml ponazuril.
- If transporting for release, provide one 80–100 gram (3–4 ounces (oz)) piece of prairie dog per BFF stored in Styrofoam® container with ice packs to be left at BFF's release site.

Air Shipments

- Attempt to arrange for direct flight. This may make it necessary for the sending and/or receiving institutions to drive to another city, if a non-stop flight is not available to or from local airport.
- Several days prior to shipment, call the airlines to make reservation and provide them with:
    - name, address, telephone number, name of contact person of sending institution.
    - name, address, telephone number, name of contact person of receiving institution.
    - dimensions and weight of shipping containers (including weight of BFF).

- information regarding special circumstances, i.e. endangered species with low disease resistance, and request that BFFs be kept away from other animals, especially dogs, during shipment. Also advise them that the sending institution will provide food in the kennel to minimize disturbance during transport.

Note: **New regulations specify that airport personnel may request that air kennels be opened for inspection. A letter in hand from the U.S. Fish and Wildlife Service (Service) and Canadian officials may reduce the likelihood of this. You may use reusable wire ties to facilitate inspection.**

- Obtain information from airlines, such as cost estimation, for Air Bill.
- Airline will provide labels to attach on outside of each kennel. To facilitate efficient counter time at the airline, obtain labels and fill them out prior to shipping if possible.
- Inform receiving institution of shipping cost and other pertinent information (Air Bill, flight information, etc.). (It is customary for the receiving institution to pay for air shipment).

Shipping Container - *Air Transport*

- Thoroughly clean and disinfect kennel.
- Kennel should be adequately ventilated. Either use smaller kennel or construct wire insert enclosure to fit inside transport kennel; wire mesh enclosure should be slightly smaller than transport kennel. [Note: two BFF can fit within a 53 centimeter (cm) x 41 cm x 41 cm (21 inches (in) x16 in x16 in) kennel by utilizing two wire inserts; provide a hinged top to use as a door.] (See **Addendum**)
- Place fresh Alpha dri® in bottom of kennel.
- - Place single or double wire inserts in bottom of kennel that is only slightly larger than wire insert(s). **If using two wire inserts be sure to place cardboard between inserts!**
- Cut a piece of cardboard to fit inside of kennel door opening. Label this cardboard with BFF(s) stud book number(s), sex, and transponder number(s).
- -Add "LIVE ANIMALS," Up arrows, and "in Case of Emergency" instruction stickers on kennel (see photos in **Addendum**).
- On day before shipment gather two-sectioned plastic food dishes, one for each wire insert; fill one side half way with water and place in freezer.
- Attach envelope containing permits and records to top of kennel. Paperwork must be accessible to airline personnel and customs agents. Label envelope with "original shipping documents and permits" along with name of contact person, receiving institution, and telephone number. ***In the case of an international shipment make sure there are two copies of CITES permits and associated documents in the shipping envelope!*** It may be helpful to place a third set plus medical records in a separate envelope attached to side of kennel.

DAY OF SHIPMENT

When shipping BFFs, two people should accompany all ground transports if possible. Ideally, at least one person should be an experienced BFF handler. If neither person has worked with BFFs, transporting personnel should participate in a short training session before the shipment to familiarize themselves with the idiosyncrasies of BFFs.

- Shipments should be postponed for severely inclement weather.
- During hot summer months, shipments should be scheduled for early or late in the day; for all day trips, consider traveling at night.

Ground Transport

- Stock transport vehicle with extra food and water in the event of a problem with the transport vehicle, or unexpected delays due to road conditions or traffic.
- - Carry a length of 10 cm (4 in) diameter plastic corrugated tubing, handling/restraint cage, net, gloves, and transponder reader (if available) in case a BFF escapes from its shipping container during transport.
- Take a flashlight to aid in checking BFFs.
- - Place BFF in kennel that is pre-stocked with a killed hamster, rat, or piece of prairie dog with prophylactic dose of .5 ml ponazuril, (use frozen hamster, rat or prairie dog pieces or trips over eight hours), a short piece of 10 cm (4 in) diameter plastic corrugated tubing, loosely packed shredded paper, and Alpha-dri® bedding.
- Adult BFFs should be shipped individually. If space limitations are a factor, two sibling kits or a dam and her kit may be shipped in the same kennel, provided they are compatible. In this case, provide one piece of carcass (hamsters, rats, or pieces of prairie dog) and one piece of 10 cm (4 in) diameter corrugated plastic tubing per BFF.
- It may be necessary to cable tie kennel doors in place.
- Arrange kennels in transport vehicle for best access to ventilation system with adequate air flow around all boxes.
- Do not place kennels face to face to avoid injuries.
- BFF thermal range is 10–24°C (50–75^{o} F). Make certain that BFFs are not left exposed to the weather if the temperature is above or below this thermal range.
- Check BFFs periodically during trip.
- It is best to use drive-through restaurants when transporting BFFs. Stop vehicle only long enough for refueling and bathroom breaks. If two people are transporting BFFs, always have one person attending the vehicle. When refueling, try to avoid areas where vehicles are idling nearby.

- On extended trips, provide additional food daily. Offer water if deemed necessary.
- If kennels are removed from vehicle and placed indoors overnight, ensure that kennel doors are not placed face to face (door to door) to prevent injury. Do not place kennels on top of each other.

Air Transport

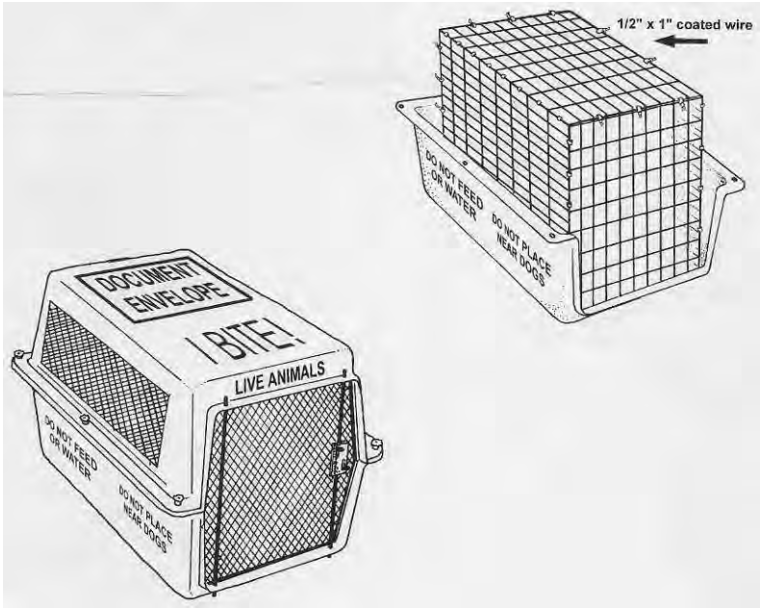
- Although many airlines limit shipment of animals during hot or cold weather, DO NOT SHIP BFFs if the temperature is above 32.2° C (90° F) or below 0° C (32° F) at time of transport.
- BFF thermal range is 10° C (50° F) to 23.8° C (75° F). Make certain that BFFs are not left exposed to the weather if the temperature is above or below this thermal range.
- Stock transport vehicle with extra food and water in the event of a problem en route with the transport vehicle, unexpected road conditions, or a delay in scheduled flight.
- Carry a length of 10 cm (4 in) diameter corrugated plastic tubing, restraint/handling cage, net, gloves, and transponder reader (if available) in case a BFF escapes from its shipping container during transport. Include a flashlight to aid in checking on BFFs.
- Before loading BFFs, call airline and confirm flight is still scheduled and there are no delays.

Final Prep of Kennels Before Placing BFFs Inside Kennels for Air Transport

- Gather the two-sectioned plastic food trays from freezer (frozen water will that and provide water source for trip) and wire tie one tray per wire insert about ½ way up one side of insert.
- Stock wire inserts with a piece of hamster or rat dosed with .5ml ponazuril. (See above for wire insert setup).
- All BFFs must be shipped individually, one animal per wire insert.
- Place BFFs in wire enclosure insert and secure top of insert with two twist ties.
- **If sending two BFFs in one kennel, be sure a piece of cardboard separates the two wire enclosure inserts!**
- Place labeled cardboard in front of forward most wire enclosure insert inside kennel frame before putting top on kennel.
- Attach top of kennel.
- Wire kennel door closed. Reusable ties work best and are often required by the airline in case of inspection or emergency access is required.
- Make sure paperwork is securely attached to outside of kennel.
- Position kennel in transport vehicle so that it has access to ventilation system. Place additional kennels so there is adequate air flow around all boxes.
- Check BFFs during any refueling or rest breaks as applicable on drive to airport.

- Allow plenty of time for trip. Most airlines require that animals be checked in ***at least*** two hours before the plane's departure time.
- After arrival at airport, check in with airlines and complete any paperwork.
- Stay with kennels until they are loaded on plane or taken to airline holding area.
- DO NOT LEAVE AIRPORT until verifying that the BFFs were loaded on the plane and that the plane is in the air.
- Inform the receiving institution that BFFs are on their way, and provide pertinent information such as flight number, arrival time, and Air Bill number. Track flight on-line and ensure that individuals are notified once animals arrive at receiving institution.

ADDENDUM

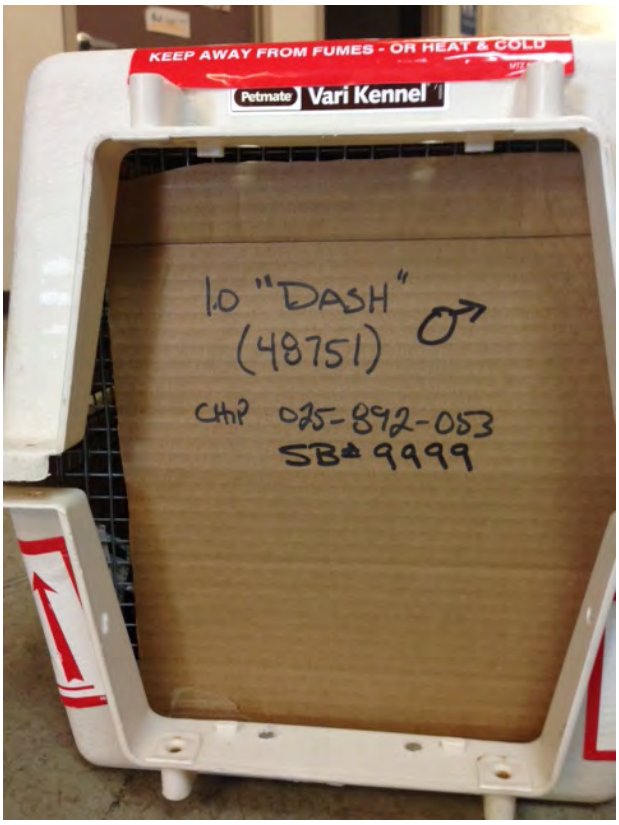


Upper figure shows single wire enclosure insert

Kennel door with Plexiglas® insert with vent holes, attached with cable ties

Double wire enclosure insert arrangement; cage top secured with wire ties in two locations

Double wire insert with cardboard separation



Cardboard insert with BFF information

Double holes drilled in kennel for twist ties

Kennel with labels

REINTRODUCTION OF CAPTIVE-REARED BLACK-FOOTED FERRETS

INTRODUCTION

The black-footed ferret (BFF) has existed in captivity since 1985, when the last known wild population became extinct (Williams et al. 1988) and 24 free-ranging animals were brought into captivity (Thorne et al. 1988). Captive propagation has been very successful and, presently, there are six facilities involved in a Species Survival Plan (SSP) breeding program. The U.S. Fish & Wildlife Service (Service) National Black-footed Ferret Conservation Center (NBFFCC) in Colorado, maintains two-thirds of the captive population. The remaining captive animals are distributed among the following North American zoos: Cheyenne Mountain Zoo, Colorado; Louisville Zoological Garden, Kentucky; Smithsonian Conservation Biology Institute, Virginia; Phoenix Zoo, Arizona; and Metro Toronto Zoo, Canada. The BFF SSP reached its genetic and demographic goals in 1996, and now manages 280 (105.175) breeders (animals of 1–3 years of age). Every year, the SSP program retains the most genetically valuable young of the year, (85–100 kits) to maintain the core population. BFFs in excess of SSP needs are available for reintroduction, for appropriate research, and for educational exhibit.

SITE SELECTION AND ALLOCATION OF BFFS FOR REINTRODUCTION

Biological habitat requirements for BFFs have been documented (Hillman et al. 1979, Forrest et al. 1985, Miller 1988, Biggins et al. 1993, 2006). Biggins et al. (1993, 2006) established a working definition of what constitutes a prairie dog (*Cynomys spp.*) complex suitable for BFF reintroduction (including distance between colonies, colonies separated by geographic barriers, etc.). Potential reintroduction site managers assess prairie dog habitat quality using Biggins et al. (1993, 2006) BFF family ratings. However, there is great variation in the application of this model, which makes it difficult to compare data across sites and the Service has recommended standardizing measurements of prairie dog densities and resultant BFF family ratings across the program. Prairie dog colonies should be mapped and qualitative assessments of prairie dog activity levels recorded (e.g., McDonald et al. 2011).

Reintroduction programs must comply with pertinent legislation and regulations, (Beck et al. 1994). In particular, the reintroduction of BFFs may involve "federal actions" requiring compliance with the Endangered Species Act (ESA) and the National Environmental Policy Act (NEPA). Compliance with NEPA may be accomplished under a relatively simple Categorical Exclusion process, or, depending on the nature of the proposed reintroduction project, may necessitate preparation of an Environmental Assessment (EA) or Environmental Impact Statement (EIS). The presence of reintroduced populations of endangered species is often viewed as a detriment to the management and economic capabilities of affected private interests. Section 10 of the ESA provides a number of tools, including the issuance of enhancement of survival permits and the designation of reintroduced populations as "nonessential" and "experimental," which allow less restrictive levels of protection. The use of any Section 10 tool requires coordination with the appropriate Service Ecological Services Field Office in the affected state, as well as coordination with NBFFCC staff. To date, 28 sites have fulfilled all necessary Federal requirements (have

adequate habitat and have been granted approval of an experimental rule or issuance of an enhancement of survival permit) to receive BFFs for release. Release sites in Canada and Mexico have similar requirements, and coordination with the proper authorities is required prior to the planning of reintroduction efforts.

In 1996, the Service, in concert with members of the Black-footed Ferret Recovery Implementation Team (BFFRIT), developed the "Black-footed Ferret Allocation Guidelines," with a standardized proposal format and annual reporting procedures. A request for proposals, which includes these guidelines, is distributed every February to interested parties. Proposals must include information on specific project background and justification, involved agencies/parties, habitat conditions, BFF population information/reintroduction procedures, predator management, disease monitoring and management, contingency plan, preconditioning, veterinary and husbandry support, and program information contributions. The Service transmits allocation proposals to select BFFRIT members and requests comments. All comments are considered and proposal merit is scored through an "allocation matrix" and is ranked based on overall contribution to BFF recovery efforts. A preliminary allocation of BFFs is forwarded to reintroduction proponents in early May. Final allocation decisions are transmitted by August, following site visits and resolution of outstanding issues that originated during the proposal review process.

PREPARATION OF BFFS FOR RELEASE

BFFs considered for release have either been raised in indoor enclosures (with an approximate surface area of 1.5 meter $(m)^2$ 5 feet (ft) supported on 1 m (3 ft) legs) and exposed to prairie dogs and their burrows in outdoor naturalistic pens for varying periods of time, or captured and translocated from extant wild populations. Almost all breeding facilities maintain their BFFs in indoor enclosures. NBFFCC also manages 48 on-site preconditioning pens, as well as six "retired" pens at F.E. Warren Air Force Base, Wyoming, which may be used again in the future. The Smithsonian Conservation Biology Institute also maintains their BFFs in outdoor pens (approximate surface area = 12 m^2 (39 ft^2). All preconditioning pens are designed to establish and maintain an intricate prairie dog burrow system conducive to BFF imprinting.

Data indicate that BFFs preconditioned in outdoor pens enjoy a significant survival advantage after release (Biggins et al. 1993 and 1998, Vargas 1994). Data from 1991–1996 showed that survival rates were highest for BFFs reared from early developmental stages (< 60 days of age) in preconditioning pens (30-day and 6-month post-release survival was 30% and 20%, respectively). Short- and long-term survival was 11% and 2%, respectively, for BFFs raised in indoor enclosures (Biggins et al. 1998). BFFs transferred to preconditioning pens at 90 days of age showed intermediate levels of survival (Biggins et al. 1998). Telemetry information indicated that preconditioned BFFs showed adaptive responses to the new environment by remaining closer to release areas and making quicker surface movements, behaviors that likely decrease the chance of encountering predators (Miller et al. 1992; Biggins et al. 1993). BFFs that were not preconditioned tended to travel further and often were detected in substandard habitat (Biggins et al. 1993).

Based on several years of data, the Service and the BFFRIT determined that all BFFs targeted for reintroduction should be preconditioned prior to release. This recovery approach has been in effect since 1997. As many kits as possible are preconditioned at 60 days or earlier; BFFs that cannot be placed in pens at such an early age are preconditioned at 90 days of age. Various explanations exist for the higher weaning and survival success for BFFs maintained in preconditioning pens. Access to a large naturalistic area has potential for sharpening motor coordination and physical fitness (Vargas 1994). In addition, animals raised outdoors are exposed to oscillations in climatic conditions and to native parasites such as fleas and ticks. Therefore, the physiological fine-tuning (e.g., development of specific resistances and physical endurance) after release is less extreme for pen-raised BFFs than for kits raised in a benign enclosure setting.

Simulated prairie dog colonies may also help prevent BFFs from learning unnatural behaviors. Prairie dog burrows provide a living area with reduced noise and a cooler, more humid, and relatively stable climate, a climate that is radically different from a nest box environment (Biggins et al. 1998). Burrows also provide high quality escape cover and allow BFFs to make individual choices regarding exposure to aboveground stimuli (compared to disturbances of animals in nest boxes during routine cleaning and feeding procedures). Moreover, familiarity with burrows likely sharpens prey-searching abilities and increases escape reactions to threatening stimuli (Biggins et al. 1998).

Large pens may enhance breeding by stimulating more exercise and reducing crowding. Crowding is an aberrant social situation for a relatively solitary carnivore like the BFF, and could induce adverse behavioral and physiological changes. Animals held in indoor enclosures in close proximity to one another are undoubtedly exposed to an abundance of olfactory and auditory stimuli, even when visual barriers are provided between enclosures. In addition, the level of care necessary for BFFs in indoor enclosures is higher than that required for animals in outdoor pens, and increases the likelihood that the animals will become habituated to humans. There is, however, a greater risk of BFF mortality in outdoor pens than in an indoor enclosure environment, something that must be considered when determining where to house the animals.

RELEASE OF BFFS

During the act of a BFF release there are some specific steps that can be taken by field staff to minimize stress on the animals during a very stressful time in their life. Many sites will have pre-determined release sites/burrows where local biologists wish to have the animals released. In the case that specific locations have not been determined, transport BFFs in their carrying kennels directly to an active (physical signs of digging and/or scat) prairie dog burrow. Spread the BFFs as evenly as possible throughout the densest parts of the prairie dog colony, with distances of at least 200 m (656 ft) between releases (especially for females). Family groups, however, can be released together.

A short (10–15 cm/4–6 in) piece of 10 cm (4 in) diameter corrugated black plastic tubing can be placed in the burrow to help the BFF recognize the burrow as a place of refuge. Place the carrying kennel on the ground pointed toward the burrow and open the door to allow the animal

to leave the kennel on its own. If the BFF does not leave the kennel willingly after 5 minutes or more, it can be encouraged by removing the corrugated tubing that is in the kennel (and probably contains the BFF) and placed directly up against the short piece of corrugated plastic tubing placed in the prairie dog burrow. Tools such as tongs and/or animal handling gloves can be used to safely remove the corrugated plastic tube from the carrying kennel. If the animal still refuses to leave the corrugated plastic tube, the releaser(s) should leave the site as is and come back later to retrieve the corrugated tube(s) and any bedding material that may have fallen out of the carrying kennel during release. The animal should never be physically forced to leave its tube as this causes severe stress and can cause disorientation and injury. If BFF food has been brought to the site, or is leftover in the carrying kennel, it should be placed at the entrance of the burrow.

Photographs may be taken during the release process, but it should absolutely not interfere with the animal's safety, natural behavior, or well-being. In some cases a BFF will not enter a prairie dog burrow and instead will stay above ground and sometimes run. In this case the releaser(s) should attempt to encourage the animal to enter a burrow either by "herding" the animal and/or by placing additional corrugated plastic tubing in nearby prairie dog burrows. If the animal continues to run and not go underground, it is best to leave the animal alone at this point, as additional human interaction will most likely only cause more stress on the animal and still not accomplish the desired effect. When leaving the site, be mindful that many of the BFFs may be above ground attempting to find suitable burrows, so be careful while driving in the area.

POST-RELEASE MONITORING

Marking individuals is essential for the determination of post-release survival. Post-release monitoring efforts include tracking animal movements, analyzing habitat use, determining physical condition, disease presence and reproductive status, identifying causes of mortality, and evaluating behavioral traits that enhance survival (Temple 1978, Berg 1982, Jeffries et al. 1986, Kleiman et al. 1986, Phillips 1990b, Cade 1990, Beck et al. 1991, Miller et al. 1992, Kleiman et al. 1994). Tracking of individual BFFs through the use of radio telemetry has been a used and recommended monitoring technique (Berg 1982, Jeffries et al. 1986, Stanley Price 1989, Phillips 1990, Beck et al. 1991, Biggins et al. 1993), although its use is limited by high cost and other factors (Berg 1982, Wiley et al. 1992, Biggins et al. 2006). Radio telemetry proved effective in determining BFF habitat use, animal movements and dispersal, causes of mortality, and behaviors of released BFFs (Service 1992, Biggins et al. 1993, 1998). In addition, this monitoring technique permits tracking and retrieval of BFFs from areas where the animals' survival would be seriously compromised (Service 1992; Biggins et al. 1993).

Attaching radio collars to BFFs is an art that requires training and experience. One frequent problem is that collars can be placed too loosely around the animal's neck and are shed soon after release. Occasionally collars were attached too tightly and affected BFFs developed neck abscesses and abrasions. Other forms of monitoring include snow-tracking, capture of animals, use of passive transponder technology, and systematic or opportunistic observations. Wildlife camera traps can also be used to help determine presence of BFFs.

To facilitate individual identification, all reintroduced BFFs are marked with a subcutaneous passive transponder, or PIT tag. Ring-shaped transponder readers placed over prairie dog burrow openings have proven effective in post-release monitoring efforts of individual BFFs (Stoneberg 1996). Spotlighting and snow-tracking surveys have also been effective in determining the presence of free-ranging BFFs at different times of the year (Hnilica and Luce 1992). Although observation surveys are valuable and necessary, they are not adequate in determining individual survival rates and only provide minimum estimates of BFF presence. Capturing animals helps to assess physical condition, administer vaccines, detect pathogens, and monitor reproduction (if successful breeders are trapped). Intensive post-release monitoring is critical during initial release efforts at each site so that optimal reintroduction strategies can be developed.

PAST AND ONGOING REINTRODUCTION EFFORTS

The first BFF reintroduction effort was attempted in 1991 at Shirley Basin, Wyoming. Since then, BFFs have been released at 27 additional sites in Arizona, Colorado, Kansas, Montana, New Mexico, South Dakota, Utah, Wyoming, Mexico, and Canada (Service 2013). Most years, allocation requests exceed the number of kits that will likely be produced in captivity. Site proposals are reviewed and ranked by members of the BFFRIT Conservation Subcommittee. The Service takes into account all received comments and provides a preliminary BFF allocation by mid-May of each year. Allocation decisions are based on the biological suitability of proposed reintroduction sites and on the technical merit, scientific significance, and management foundation of reintroduction projects.

RECOVERY PROGRAM DIRECTION

The current direction for BFF recovery is outlined in the recently revised Black-footed Ferret Recovery Plan (Service 2013). While BFF recovery is beset with many challenges, sylvatic plague, a zoonotic disease caused by the bacterium *Yersinia pestis* and introduced into the U.S. in the late 1800's, is the greatest biological challenge to BFF recovery. In addition, prairie dog habitat is currently insufficient to meet recovery objectives for establishing wild BFF populations. If BFF recovery is to succeed, incentives for the conservation of prairie dog habitat coupled with effective plague management and the availability of regulatory assurances for private landowners are urgently needed. Implementation of these goals could help safeguard many other species that could soon need federal protection if prairie dog habitat is not secured.

The BFF recovery program uses scientific research as a tool to direct recovery efforts. To date, experimental work has demonstrated that the early preconditioning of captive-reared BFFs is critical for survival after release (Biggins et al. 1998; Vargas 1994). The combination of increased productivity and increased post-release survival of pen-born BFFs will hopefully lead to a more expedient approach to overall species recovery. Therefore, the Service encourages and promotes preconditioning and pen-breeding/whelping techniques as a state-of-the-art approach to BFF recovery. As sylvatic plague management becomes more effective and wild BFF populations increase, translocations from established populations will likely prove to be a more effective means of establishing wild populations.

Post-release survival is greatest for BFFs released in black-tailed prairie dog complexes (Biggins et al. 1998), possibly due to higher prey densities in black-tailed prairie dog towns. Additional reintroduction sites are being evaluated and preliminary inquiries are being made to raise interest in BFF recovery by states/sites with high habitat quality. Allocation priority will be given to sites with large prairie dog complexes where plague management activities are being implemented; in addition, the program will continue to support disease research, specifically focused on the development of plague vaccines for use in the field.

BFF recovery depends on the establishment of effective conservation measures for prairie dogs across North America. Successful re-establishment of BFFs at reintroduction sites will help

increase public awareness of the importance preserving prairie dog habitat. Other species that depend on prairie dog habitat will also benefit from efforts to recover this endangered carnivore (Miller et al. 1994).

Kit in preconditioning pen NBFFCC

Kit at release site Conata Basin, SD

Complete information on BFF reintroduction efforts and procedures is available in the **Black-footed Ferret Field Operations Manual.**

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EDUCATION

Each Species Survival Plan (SSP) Program coordinates the individual activities of participating member institutions including conservation, research, husbandry, management, and educational initiatives from the zoo perspective. The Association of Zoos and Aquariums (AZA) encourages all SSPs to include an educational component as part of their program. The AZA defines educational components as programming and interpretation for targeted audiences such as school groups, teachers and families, and other community stakeholders. Examples of educational programming include providing consistent conservation messages and raising public awareness about, and actions towards, conservation concerns.

Although the Black-footed Ferret (BFF) SSP does not currently have an education liaison, prior education initiatives include:

- The BFF is one of North America's most endangered mammals, primarily due to the disease plague, loss of prairie habitat and associated prairie dogs.
- Immediate recovery efforts are needed to save the BFF and the prairie for future generations.
- Self-sustaining BFF populations are indicators of healthy prairie ecosystems.
- BFF recovery is compatible with most other land use activities.

Institutions involved in BFF recovery engage in various educational initiatives such as commemorating the anniversary of the rediscovery of BFFs, celebrating Endangered Species Day, conducting outreaches, giving paper and poster presentations, and participating in media events that champion the BFF.

BFF 30th Anniversary Celebration

Poster presentation at ZACC conference

Educational materials are available at www.blackfootedferret.org and www.prairiewildlife.org as well as through websites of institutions involved in BFF recovery. A number of institutions exhibit BFFs in conjunction with the Service. The current list of exhibiting institutions is included in the **Exhibit Chapter** of the manual.

U.S. FISH AND WILDLIFE SERVICE
REQUIREMENTS FOR EXHIBITING LIVE BLACK-FOOTED FERRETS
ATTACHMENT TO U.S. FISH AND WILDLIFE SERVICE LETTER OF AUTHORIZATION

Revised 5/1/15

EXHIBITING FACILITY

1. Black-footed ferrets (BFFs) are on loan to the above display facility and remain the property of the U.S. Fish and Wildlife Service (Service). If the display facility should no longer wish to maintain the BFF(s) or if the Service requires the BFF(s) at a future date, they may be recalled. The Service requires that all display facilities wishing to exhibit BFFs adhere to the following requirements.
2. Any display facility wishing to publicly exhibit BFFs must receive written authorization from the Service. It is also advised that any AZA institution complete MOP for Educational Exhibit (**Addendum B**).
3. The display facility is responsible for obtaining all Federal, State and local permits.
4. All BFFs are permanently identified with AVID transponder implants and these must not be removed under any circumstances.
5. All BFFs available for exhibit are no longer part of the Species Survival Plan (SSP) Management. These **BFFs may not be bred for any purposes**, thus display BFFs are neutered prior to being transported to any display facility.
6. The display facility must provide to the Service an annual report due by December 31st of each year. See attached BFF Live Display Annual Report Template. (**Addendum A**)
7. The display facility must report the death of any BFF to the USFWS National Black-Footed Ferret Conservation Center (NBFFCC) within 24 hours via phone (Kimberly Fraser: 970-897-2730 x238), FAX (970-897-2732) or e-mail Kimberly_fraser@fws.gov. (**Addendum D)**
8. Only if advised by the Service, a necropsy should be completed by the display facility or a qualified associate with care taken to preserve the skeleton and skin for future mounts and other display uses. For necropsy approval, contact Dr. Mary Wright at (970) 897-2370 x233, cell# (970) 231-9722. Necropsy results should be sent to NBFFCC upon receipt. The BFF carcass must be returned to USFWS NBFFCC. The sender should notify Kimberly Fraser prior to returning the carcass. The carcass should be frozen, placed in double Ziploc bags and packed in sufficient ice or dry ice, and sent overnight via FedEx or UPS. A mortality report must be included with the carcass.

Please send the carcass to: National Black-footed Ferret Conservation Center
19180 I-25 Access Rd.
Carr, CO 80612

HUSBANDRY

9. The display exhibit and any associated windows should be cleaned at least once daily. If the BFFs have access to the off exhibit holding cage after hours, the primary exhibit may stay cleaner. Use a bactericidal and virucidal disinfectant such as Virosan® or chlorhexadine for cleaning the display exhibit and off exhibit holding.
10. The bedding/display substrate for the den boxes and off exhibit holding cage, can be a variety of materials. These include: Alpha-Dri® or Cellu-Dri®, CareFresh® Ultra, or Eco-Bedding®. Wood shavings are discouraged due to problems with pieces getting lodged between BFF teeth and gums causing suppurative gingivitis.
11. BFF feeding can occur on exhibit or off exhibit. Feeding may temporarily enhance BFF activity if done while on exhibit.
12. BFFs being transferred from the display exhibit to the off exhibit holding cage can be challenging. Some BFFs like to bite shift doors, which can fracture teeth. BFFs are very fast, so tails, feet, and heads may get caught in shift doors. At any time, when working with BFFs, the caretaker's patience is key.
13. BFFS DO NOT TOLERATE HAND RESTRAINT. They will stress quickly and overheat. Handling should be done with a restraint cage made of vinyl-coated wire, long, cylindrical, or "ferret shaped" with a secure latch or a section of 10 cm (4 in) corrugated plastic tubing with securable slider doors. If a BFF escapes, a good way to catch it is to open its den box and place a piece of tubing on the floor. A BFF readily seeks out dark spaces such as black tubing. BFFS WILL BITE. It is advised caretakers should wear a pair of thick gloves (welding gloves suggested), during restraint or capture for protection against biting. Restraint cages are used when examining, treating or weighing BFFs. **BFFs are not to be hand restrained/touched except when anesthetized by a licensed veterinarian.** There must be a solid (glass or Plexiglas) barrier between BFFs and visitors at all times.

DIET

14. BFFs are strict carnivores and must be fed accordingly. BFFs should be offered a maintenance diet of a high quality commercial raw meat base small carnivore diet (Toronto/Milliken or Nebraska brands). A whole carcass (mice, hamster, or rat portions) supplemented to their diet will help prevent gingival disease which is common in older animals. The amount of diet will vary depending on the animal's weight and condition, generally 60–80 grams (g) (2–3 ounces (oz)) fed per day. All BFFs are fed every day and there is NO fasting day. The BFF's weight is recorded monthly. A BFF's weight naturally fluctuates during the year, and it can either gain weight or lose weight when eating the same amount of food. Normal weight for BFF adult males is 850–1000 g (30–35 oz), and adult females 650–850 g.
15. The diet can be fed a.m. or p.m. In general, a BFF that skips a single meal is reason for concern and initiating diagnostics. Any concerns contact Dr. Mary Wright, (970) 897-2370 x233, cell# (970) 231-9722.
16. BFFs should have water ad lib.

VETERNARY CARE

17. The facility must have a licensed veterinarian on staff or have 24-hour access to a veterinarian. Please note than not all veterinarians that treat dogs and cats will treat BFFs or wildlife. Be sure to establish contact with a veterinarian prior to exhibiting BFFs. It is recommended for smaller display facilities to have a secondary vet to contact, in case the primary vet is not available for emergencies.
18. BFFs are very stoic animals. They are able to endure severe dental and gingival disease, neoplasia (cancer), enteritis (intestinal inflammation), and kidney disease without showing clinical signs until they are near death. Caretakers must be especially vigilant and sensitive to changes in an animal's appetite, stool, and general attitude. Veterinarians are encouraged to be proactive and aggressive in therapy if they are to help BFFs that are clinically ill. Any concerns contact Dr. Mary Wright.
19. BFFs may have compromised immune systems compared to domestic ferrets. This deficiency predisposes them to infectious agents. During the daily husbandry routine, if a caretaker is ill (a cold, upper respiratory, etc.), it is recommended a mask be worn to protect the BFF from exposure.
20. BFFs used for display are non-breeding animals and tend to be older. Because of this, they often experience health problems associated with advanced age, especially kidney disease and cancer.
21. **DO NOT VACCINATE DISPLAY BFFS. BFFs sent out for display will be current on their vaccinations (for Rabies with ImRab® vaccine and Purevax® Ferret Distemper by Merial) and do not require boosters. BFFs hold titers well and may have adverse, even fatal reactions to boosters.**
    Consult with the Kimberly Fraser (970-8970-2730 ext. 238) or Dr. Mary Wright (970-897-2730 ext. 233, cell# 970-231-9722) before considering any vaccine for BFFs or if you have any questions.
22. **Venipuncture**: If brief anesthesia is required for any procedure, isoflurane is recommended and preferable. The jugular vein is ideal for most blood collection needs, but requires practice to perform safely and effectively. The cervical skin is thick and tough and the vein can be "weasel-y" if not held off firmly. Use a ¾", 22 gauge needle on a 3cc syringe and hold the entire neck and vein between forefinger and thumb. Cranial vena cava puncture is also an option.
23. **Disease Concerns**
    The most common symptoms of illness seen in BFFs are:
    - Diarrhea
    - Anorexia
    - Lethargy
    - Sneezing
    - Swollen jaw
    - Distended abdomen

    The most common veterinary concerns are:
    - Diarrhea/Enteritis: Causes include, Clostridia Enteritis, Coccidiosis, and Cryptosporidiosis

- Dental Problems: Fractured canines, Wood chips lodged between teeth or across palate, Abscessed teeth and gingivitis
- Hepatic adenocarcinoma (liver cancer) and other neoplasias

Other important health concerns are:

- Canine distemper is 100% fatal. DO NOT vaccinate with modified live vaccine!
- Influenza (flu)—wear mask and wash hands frequently or re-assign the caretaker; this includes H1N1 and Avian Influenza!
- Plague
- Pneumonia
- Pyothorax
- Giardiasis
- Toxoplasmosis
- External parasites
- Heartworm

24. **Identifying the cause of diarrhea:** A fecal smear (Diff-Quik®), float, and acid fast staining should distinguish the cause. The recommended diarrhea treatments are listed below.
    - **Clostridial/Bacterial Overgrowth**

      A shift in the bacterial population in the BFF gut can occur for numerous reasons including stress, concurrent disease or change of food. Greater than normal numbers of large rods in a fecal smear can be an indication along with spore forming large rods. This will usually resolve with Amoxidrops® given at 0.5 ml by mouth for 3 days.
    - **Coccidia**

      Coccidiosis is a common cause of BFF diarrhea, but often found in normal stools as well. It can cause sudden death, especially in young animals. A fecal analysis positive for Coccidia and/or clinical signs, (lethargy, inappetance, diarrhea), indicate the need for treatment. It will often cause clinical disease during stressful events including travel for display purposes, anesthesia, or shipping.
        - **Treatment: Ponazuril Guidelines & Dose**
            - For coccidiosis- prophylaxis and treatment
            - Ponazuril, a coccidia-cide comes as an equine paste called "Marquis" by Bayer.
                - You will need to dilute paste
                - Be sure to shake well before use
                - Store at room temperature
                - Dosage is 30–50 mg/kg orally. Daily x 3 days or longer until fecal is negative for oocysts.
                - Since they can get secondary GI bacterial overgrowth penicillin may be helpful in clinical cases.
                - Maintain hydration with SQ fluids as indicated.
            - For prophylaxis
                - Shipping or travel for display: give 30mg/kg one day prior to transfer

- It is also indicated at other stressful times such as illness or anesthesia.

- **Cryptosporidia**

  Cryptosporidia (crypto) causes mucousy, possibly greenish, stools, moderate morbidity, and low mortality. It may be zoonotic. Recheck BFF 7 days after crypto treatment ends. Crypto is difficult to eradicate.

  - **Crypto treatment**

    Azithromycin @ 40mg/kg PO SID x 21 days, extend if severe cases. Or may be pulse dosed as needed for 10 days to reduce clinical signs and discomfort in chronic cases. Currently, there is no therapy that will completely eliminate crypto. Under stressful conditions (shipment, breeding, whelping), BFFs may again shed infectious oocysts and show signs of illness.

25. **BFF Reference Values**

Adult heart rate:	220–250 bpm awake
	180–255 bpm under Isoflurane
	5–150 bpm under medetomidine/ketamine
Temperature:	99–102° F
Respiration rate:	20–70 bbm
Adult dental formula:	2x (incisors 3/3, canines 1/1, premolars 3/3, molars ½) =34
Weights:	Adult male 850–1000 g (30–35 oz)
	Adult female 650–850 g (23–30 oz)

TEST	BFF CALCULATED RANGE	BFF ACTUAL RANGE	DOMESTIC ABAXIS & ISIS RANGE
WBC	2.07-6.05	1.79-13.05	2.0-10.0
LYM	1.01-2.93	0.64-5.04	0.4-6.5
MON	0.03-0.33	0.02-0.56	0.1-0.7
GRA	0.64-3.18	0.67-7.87	0.8-4.5
LY%	35.5-62.5	23.5-69.3	27-80
MO%	1.1-8.3	0.8-11.9	0-10
GR%	34.8-57.8	19.7-64.6	16-70
RBC	10.09-11.39	9.47-11.91	7.8-13
HGB	17.4-19.8	14.6-20.2	12.4-18.7
HCT	40.71-53.07	37.22-52.33	36-56
MCV	42-47	32-47	40-48
MCH	16.2-18.4	12.6-19.1	13.5-16.5
MCHC	37.6-40	37.3-42.5	32.1-35.5
RDWc	15.7-22.6	15.7-31.5	//
PLT	501-747	360-850	96-776
PCT	0.38-0.64	0.26-0.80	//
MPV	7.4-8.8	6.2-9.5	//
PDWc	34.5-39.3	30.5-41.2	//
ALB	2.9-3.9	2.1-4.1	3.2-4.2
ALP	38-61	29-81	1-215
ALT	84-163	74-213	63-205
AMY	9.0-16.0	5.0-19.0	15-47
TBILI	0.2-0.3	0.2-0.5	0.2-0.6
BUN	13-28	11.0-47.0	15-33
CA++	9.0-10.2	7.7-10.6	7.8-10.0
PHOS	5.0-7.0	4.0-8.0	3.5-7.1
CRE	0.4-0.6	0.2-0.8	0.4-1.0
GLU	137-180	116-208	114-147
NA+	149-155	148-161	134-164
K+	4.2-5.2	4.1-5.7	4.0-5.2
TP	5.2-6.0	4.9-6.4	5.9-7.1
GLOB	1.6-2.8	1.4-3.7	2.2-3.4

VETERNARIAN CONTACT

For further information on any of these conditions contact the staff veterinarian at the National Black-footed Ferret Conservation Center (NBFFCC), Dr. Mary Wright at (970) 897-2370, cell# (970) 231-9722, and email: mary_wright@fws.gov.

26. **Disposition:** All BFF transports are directed by the USFWS.
    After reporting a BFF death within 24 hours (Kimberly Fraser: 970-897-2730 x238), the facility should contact Dr. Mary Wright at (970) 897-2370 x233, cell# (970) 231-9722, to consult if a necropsy should be performed and sent for histopathology exams. If a necropsy is preformed, copies of the report should be provided to NBFFCC. A Disposition Form (**Addendum D**) should also be faxed within three days of the death. If possible during the necropsy, please request that care is taken to preserve the skeleton and skin for future mounts and other display uses.
    Disposition of dead specimens must be coordinated with the Service. Contact Kimberly Fraser.

ENRICHMENT

27. BFFs should be provided with enrichment both on and off exhibit. Behavioral enrichment is an integral part of exhibiting BFFs. Placing a BFF on public display may stress the animal. Enrichment may reduce BFF stress. Many types of enrichment are available and practical for use with animals on display.
28. **On-exhibit enrichment suggestions:**
    - Tunnels
    - Cage props- sage brush/ tumbleweed for climbing or hiding
    - Rocks-of a size to be moved around but not ingested
    - Snake sheds
    - Dirt/other substrate for digging
    - cattle skull
    - prairie dog skulls
    - Scents of other BFFs or rodents
    - Food items:
        - mice or hamsters
        - live crickets
        - cat or mink chow
        - large raw bones

    Off-exhibit enrichment considerations:
    - Large hollow balls
    - Shredded white paper (no staples)
    - Chew toys (monitor closely!)
    - Tunnels
    - Paper bags- empty or filled with crinkled paper strips
    - Colanders- plastic ones with holes cut in the bottom
    - Cardboard boxes with holes cut in them
    - Milk crates
    - Small rodents

EXHIBIT DESIGN AND EDUCATIONAL MESSAGING

29. BFF exhibits should be constructed to mimic their natural, prairie dog burrow habitat. Exhibits depicting other scenes or otherwise inaccurate backgrounds do not further the goal of conserving prairie dogs and therefore BFF habitat.
30. BFF exhibits should be naturalistic, including "underground" tunnel systems. Displays should be a minimum of 0.7 m² 6 ft² and provide the BFF a place to hide while on display. Minimum space requirements for the exhibit and holding areas are based on Federal welfare guidelines for domestic ferrets and protocols established by the BFF AZA SSP for breeding BFFs. Since BFFs are nocturnal, institutions may wish to utilize reverse light cycles in an effort to promote BFF activity during peak visitor hours. Facilities should employ a sliding door system to prevent escapes and maintain catch tubes or boxes to facilitate re-capture. An off-display holding cage should also be provided.
    CAUTION: TEMPERATURES IN THE EXHIBIT OR HOLDING AREAS SHOULD BE BETWEEN 13–24°C (55–75 °F). BFFS CAN NOT TOLERATE HIGH HEAT AND EXTREMES OF TEMPERATURE CAN BE LETHALTO ALL BFFS.
31. A naturalistic exhibit may employ fiberglass, poly-resin molds or sealed concrete with mounds mimicking a prairie dog burrow opening. The mold should fit snugly to the walls of the exhibit and corners must be rounded to prevent chewing. You may consider painting the back of the exhibit, or other additions, to resemble a prairie landscape. Add vegetation (grass, sage brush) and cage props such as prairie dog skulls (but not logs or large branches) to enhance the display.
32. "Underground" tunnels, viewed through cut-aways in small windows, enable visitors to see the BFFs in their simulated underground dens. These can be illuminated using fiber optics, or subdued lighting from above, to aid viewing in the tunnels or den. Using reversed light and feeding cycles may increase activity while animals are on exhibit. A water dish or bottle located within the exhibit should be naturalistic and well anchored to prevent spilling or removal by the BFF. Place food dishes in either the on-exhibit (hidden) or off-exhibit areas. Make sure the exhibit is easy to clean, disinfect and service. Ideally, den boxes should be removable to facilitate cleaning and disinfecting. Devise a tunnel system with sliders for shifting BFFs into the off exhibit holding area that also provides easy access for placing restraint cages or BFF boxes for capturing animals. Be sure all tunnel connections are secure as BFFs are escape artists.
33. If authorized, each participating display facility may maintain two or more animals, separately, for display, thus allowing for rotation or back- up for an exhibit. BFFs are solitary animals and should be exhibited as such. While some BFFs may tolerate each other, cohabitation is unduly stressful to the animals and conveys an inaccurate message about the BFF's natural history. Arrangements that display two animals without physical contact or that alternate the display of multiple BFFs are acceptable.
34. BFFs may be used in mobile display projects but may not be loaned out to third parties. A representative of the permitted institution must be present at any outreach event. Specific guidelines will be drafted for any facility interested in presenting a mobile display. Contact Kimberly Fraser **(970-8970-2730 ext. 238)**.

35. An educational message must be presented with the BFFs and facility include the following minimal information: common and scientific names, historic and current distributions, endangered status (threats, conservation needs), relationship to the prairie dog, brief history of recovery, and the difference from domestic ferrets. NBFFCC will review this material for accuracy. Contact Kimberly Fraser **(970-8970-2730 ext. 238)**.
36. Public education is the primary objective for live BFF exhibits. Exhibits must present a strong prairie conservation message, including details of habitat loss, relationship with prairie dogs, other prairie dog associate species, and the status of BFF reintroduction efforts. The USFWS can provide an endangered species conservation message. Contact Kimberly Fraser **(970-8970-2730 ext. 238)**.

For further information contact:

Kimberly Fraser
National Black-footed Ferret Conservation Center
PO Box 190
Wellington, CO 80549
970-897-2730 x 238
970-897-2732 (FAX)
Kimberly_fraser@fws.gov

ADDENDUM A

ANNUAL LIVE BLACK-FOOTED FERRET REPORT

Name of Facility

Date

General Information

Name of Facility: **Address :**	**Date of Acquisition**	**Acquired from (Name of Breeding Facility) and Any History (if Known):**
	Date of Disposition	

Species: Black-footed Ferret *(Mustela nigripes)* **Status:** Live animal display and education use	**Name:** **Nicknames:**

Studbook #:	**Gender:**	**Age:**

Animal Care Information

Diet:

Water:

Enrichment:

Habitat care (include average & maximum room/display temperature):

Weight Record:	**General Health and Other Comments:**

Habitat, Education, and Outreach Information	
Annual Days on Display: **Number of Outreach Programs:** **Yearly Facility Visitor Attendance Total:** **School Program Attendance Totals:**	**Habitat Description**:
Brief Description of BFF Outreach Programs/Activities:	**Brief Description of Other Prairie Conservation Programs:**

Brief Description of Special Events:

ADDENDUM B

Association of Zoos and Aquariums (AZA) SPECIES SURVIVAL PLAN® (SSP)

MEMORANDUM OF PARTICIPATION FOR
BLACK-FOOTED FERRET EDUCATIONAL EXHIBITION LOAN
Mustela nigripes

The _____
(Institutional Name) will participate in the Black-Footed Ferret SSP's® educational exhibition program. This commitment will automatically be renewed on an annual basis until the SSP Chair and AZA Director of Animal Conservation are informed that your institution is withdrawing from the program. Your institution reserves the right to withdraw from the program at any time.
The commitment is to participate in the educational exhibition of black-footed ferrets under the guidance of the Species Chair and SSP Management Group. Participating institutions will manage their animals in accordance with the recommendations of the Species Chair and Management Group, including the established written protocol for black-footed ferret display and disposition. Participants in this program agree not to breed animals that have been designated for exhibition only. In addition, institutional representatives from participants in the educational exhibition program cannot be nominated to serve on the SSP Management Group.
I have read and agree to abide by the provisions of this memorandum:

Endorsement of institution's Chief Executive Officer

Name Title Date

_____

Institutional Program Representative:

Name Title Date

_____

Please return a signed copy to both:

Candice Dorsey, PhD
AZA Director of Animal Conservation
AZA Executive Office
8403 Colesville Rd, Suite 710
Silver Spring, MD 20910
Fx: 301-562-0888
cdorsey@aza.org

Guy Graves
Black-Footed Ferret SSP Coordinator
Louisville Zoological Garden
P.O. Box 37250
Louisville, KY 40233-7250
Fx: 502-459-2196
guy.graves@louisvilleky.gov

Addendum C

Live BFF Display Facilities Contact List

2016 (Updated 8/8/2016)

Facility	Contact	Name SB#	Name SB#	Name SB#	LOA or Permit Comments
National Black-footed Ferret Conservation Center Pete Gober PO Box 190 Wellington, CO 80549	Kimberly Fraser (970) 897-2730 kimberly_fraser@fws.gov	Two Bit #6690	Rebus #6715		2014 LOA- #6690 2015 LOA- #6715
Species Survival Plan Facilities					
Cheyenne Mountain Zoo Jeff Baughman 4250 Cheyenne Mtn Zoo Rd Colorado Springs, CO 80906-5755	Jeff Baughman 719-633-9925 ext. 120 jbaughman@cmzoo.org	Dillinger # 6535			FWS Permit 2012-2016 2015 LOA- #6535
Louisville Zoological Garden Guy Graves 1100 Trevilian Pkwy Louisville, KY 40213	Guy Graves (502) 238-5360 FAX 459-2196 guy.graves@louisvilleky.gov	Rowdy #6082			FWS Permit 2012-2016
Smithsonian Conservation Biology Institute (SCBI) Steve Monfort 1500 Remount Rd. Front Royal, VA 22630	Warren Lynch (540) 635-6575 FAX 635-6551 Lynchw@si.edu	Capone #6536			FWS Permit 2012-2016
Phoenix Zoo Bradley Poynter 455 North Galvin parkway Phoenix, AZ 85008	Sharon Biggs (602) 273-1341ext 7209 sbiggs@thephxzoo.com				FWS Permit 2013-2018
Toronto Zoo Maria Franke 361A Old Finch Ave. Scarborough, Ontario M1B 5K7 Canada	Maria Franke 416-392-5967 fax - 416-392-4979 mfranke@torontozoo.ca	Gibbs #7187			FWS Permit 2012-2016
Other Display Partners					
Abilene Zoo William Gersonde	William Gersonde (325) 676-6590	Romano			2013 LOA-

2070 Zoo Lane Abilene, TX 79602	FAX 325-676-6084 bill.gersonde@abilenetx.com	#6344			#6344
Amarillo Zoo Rhonda Votino N.E. 24th & Dumas Hwy Amarillo, Texas 79105-1971	Rhonda Votino (806) 381-7911 FAX 806-381-7901 Rhonda.votino@amarillo.gov	Chardee MacDennis #7183			2015 LOA-#7183
Dakota Zoo Terry Lincoln 711 Sertoma Rd Bismarck, ND 58502	Terry Lincoln (701) 223-7543 FAX 258-8350 director@dakotazoo.org	Coppelli #7076	Nervous #7149		2015 LOA-#7076 2015 LOA-#7149
Elmwood Park Zoo Dave Wood PO Box 348 Norristown, PA 19404	Dave Wood (610) 277-3825 x 239 FAX 292-0332 Dwood@elmwoodparkzoo.org			Scooter #7035	2015 LOA-#7035
El Paso Zoo John Kiseda 4001 E. Paisano El Paso, Texas 79905	John Kiseda (915)521-1860 FAX 915-521-1857 KisedaJJ@elpasotexas.gov	Bayou #6869	Santorini #7077	Ian #6860	Amaz aska #7390 2015 LOA-#6869 2016 LOAs #7077, 6860,7390
Fort Collins Museum of Discovery Cheryl Donaldson 408 Mason Street Fort Collins, Colorado 80524	Cheryl Donaldson (970) 416-2709 cdonaldson@fcmod.org	Two Bit #6690	Rebus #6715		2014 LOA-#6690 2015 LOA-#6715
Hutchinson Zoo Kiley Buggeln 6 Emerson Loop E Hutchinson, KS 67501	Kiley Buggeln (620) 694-2653 kileyb@hutchgov.com				
Lee Richardson Zoo Kristi Newland 312 E. Finnup Dr. Garden City, KS 67846	Kristi Newland 620-276-1227 direct 620-276-1250 Kristi.newland@gardencityks.us	Fazool #6996			2015 LOA-#6996
Milford Nature Center Pat Silovsky 3415 Hatchery Drive Junction City, Kansas 66441	Pat Silovsky (785)238-5323 Pat.silovsky@ksoutdoors.com	Sinclair #7093			2015 LOA-#7093
National Zoological Park, Dennis Kelly 3001 Connecticut Ave, NW Washington, DC 20008	Steve Sarro (202) 633-3242 FAX 673-4607 sarros@si.edu	Dusty #6693	Digger #7197		2014 LOA-#6693 2016 LOA-#7197
Navajo Nation Zoo David Mikesic PO Box 1329	David Mikesic (928)871-6573 FAX 928-871-6644				

Window Rock, Arizona86515	dmikesic@navajozoo.org				
Northeastern Wisconsin Zoo Carmen Murach 4418 Reforestation Rd Green Bay, WI 54313	Carmen Murach (920) 662-2403 FAX (920) 434-4162 Murach_CD@co.brown.wi.us	Lydia #6750	Muad' Dib #7068		2013 LOA-#6750 2015 LOA-#7068
Prairie Park Nature Center Marty Birrell 2730 Harper Street Lawrence, Kansas 66044	Marty Birrell (785) 832-7980 mbirrell@lawrenceks.org	Gyrfalcon #6777			2015 LOA #6777
Rocky Mountain Arsenal Wildlife Refuge David Lucas 6550 Gateway Road Commerce City, Colorado 80022	David Lucas (770) 329-1685 David_lucas@fws.gov	Louise #6774	Chigger #7399		2015 LOA #6774 2016 LOA #7399
Saskatoon Forestry Farm Park & Zoo John Moran 1903 Forest Dr. N Saskatoon, Saskatchewan S7S 169 Canada	John Moran (306) 975-3385 FAX 975-3326 John.Moran@saskatoon.ca	Pippa #7415			
Topeka Zoological Park Dennis Dinwiddie 635 SW Gage Blvd Topeka, Kansas 66606	Dennis Dinwiddie (785) 368-9134 ddinwiddle@topeka.org	Cleopatra #6740			2014 LOA-#6740
Zoo America Katie Govern North American Wildlife Park 100 W. Hershey Park Dr. Hershey, PA 17033	Katie Govern (717)534-3864 KAGovern@hersheypa.com	Orianthi #6912	Elson #6980		2013 LOA-#6912 2015 LOA-#6980

Any corrections please send to Kimberly Fraser, kimberly_fraser@fws.gov**.**

Addendum D

BFF Disposition Blank 2013 (2).pdf

BFF Research Guidelines

All research projects pertaining to black-footed ferret (BFF) recovery, including captive breeding, preconditioning, and evaluation of reintroduced populations, are reviewed by a research committee assembled by the U.S. Fish and Wildlife Service's (Service's) BFF Recovery Program. This committee typically consists of the following BFF Recovery Program employees:

- Field Biologist and Chair (John Hughes at present)
- Captive Propagation Manager (Robyn Bortner at present)
- Deputy Recovery Coordinator (Julie Lyke at present)

In addition to these individuals, the chair of the research committee can appoint 1–2 subject matter experts that have experience in the research topic under consideration. All research proposals are reviewed in a timely manner, and a decision to approve or disapprove each project is provided to the applicant in writing by the research committee chair. For questions about this process, please contact John Hughes at john_hughes@fws.gov or (970) 305-1158.

A Summary of Black-Footed Ferret Published Research Projects and Graduate Theses

CATEGORIES:

Behaviors and Development

Reproduction

Genetics

Diseases

Nutrition/Food Requirements

Field Biology

Habitat

Immobilization and Marking Techniques

Reintroduction

Other

CAPTIVE BEHAVIORS and DEVELOPMENT

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U.S. Fish & Wildlife Service

Annual Report Form for
Black-footed Ferret Captive Breeding Recovery Permits

Any facility permitted by the Service to house and propagate endangered black-footed ferrets under a native endangered species recovery permit is required to submit an annual report to the Service to remain in compliance. Please refer to the terms of your permit for more information.

Please provide the following information; if a section is inapplicable, please state 'n/a' or '0'. Attach sheets as necessary or submit in a comprehensive report. Please note if a section is already included in the SSP Annual Report Form.

Please submit reports by January 31 to the following offices, either electronically or mailed:

Black-footed Ferret Recovery Coordinator: pete_gober@fws.gov
Black-footed Ferret Breeding Manager: robyn_bortner@fws.gov
National Black-footed Ferret Center
P.O. Box 190
Wellington, CO 80549

Mountain-Prairie Region 6 Office: permitsR6ES@fws.gov
Recovery Permit Coordinator, Ecological Services
P.O. Box 25486, Denver Federal Center
Denver, CO 80225

A) The AZA SSP Annual Report Form, and the following information if it is not already included:

B) A detailed breeding summary of all animals in the institution's current population of black-footed ferrets, including kits produced and inventory changes;

C) Summary of research proposals and results and their importance with regards to recovery of the species;

D) Current protocols in place at the institution including husbandry, propagation, lighting, veterinary care, and any others pertinent to the program;

E) Quantification of take for the species, including numbers of individuals incidentally killed (including dates, locations, and circumstances of lethal take), and an estimate of the numbers of individuals otherwise harmed or harassed;

F) Quantification of take for other listed species not authorized under this permit, including numbers of individuals incidentally killed (including dates, locations, and circumstances of lethal take), and an estimate of the numbers of individuals otherwise harmed or harassed;

G) Discovery information and documentation for any potential criminal activities that was reported to the Service's Office of Law Enforcement (OLE);

H) The disposition of any injured or dead individuals;

I) Repositories where the specimens were sent, including salvaged specimens that were found malformed, abnormal, injured, sick, or dead, and any issued diagnostic or examination reports from a repository;

J) Reports or other documents that include information gathered under the authority of this permit; and

K) Planned future activities if authorized under this permit.

Black-footed Ferret Animal Care Manual Acknowledgements

We thank all who gave their time and energy, past and present, to the compilation of this manual.
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Photographs are courtesy of current and past staff members of BFF Recovery institutions and partner organizations.

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