Clouded leopard (*Neofelis nebulosa*)

Husbandry Guidelines

(originally published 2000)

Disclaimer: The information provided here is a guideline and is a result of the collective experiences of a number of clouded leopard managers. Scientific studies conducted since publication of this original document and other management practices developed since may alter these guidelines. The Clouded leopard SSP does not support ownership of clouded leopards by those individuals/facilities not qualified to care for them. The SSP strongly discourages ownership of clouded leopards as "pets".

Introduction

The Clouded leopard (*Neofelis nebulosa*) is a medium-sized cat found in the jungle and forested regions of Malaysia and the countries of Southeast Asia. First mentioned by Sir Stamford Raffles in the Descriptive Catalogue of a collection made in Sumatra it was given the scientific name of *Felis nebulosa* by Griffith in 1821. Known common names in range countries include *harimau-dahan* (branch or tree tiger) or mint leopard by the Chinese due to its markings being similar to mint leaves. Regardless of its name one thing is readily accepted-this cat is arguably the most beautifully marked felid and perhaps the most challenging to study.

Little to nothing is known of its habits in the wild. This has compounded captive management efforts as this species' behavior is most unique. This document is designed to serve as a "jumping off point" by offering guidelines for current captive management practices and, perhaps more importantly, to encourage intensive study of this species in captivity and in the wild. Information included in this document has been drawn from the experiences of clouded leopard managers as well as published works.

Natural History

Range

Historically the clouded leopard was found from Nepal to Indochina, Indonesia, southern China and Taiwan. Population numbers are thought to be lower outside protected areas and are probably healthiest in Borneo because of the absence of tigers and leopards. Surveys in Borneo suggest a density of one individual per 4 square kilometers. Four sub-species are recognized:

Neofelis nebulosa brachyurus: Taiwan. Probably extinct in the wild.

Neofelis nebulosa diardi: Sumatra, Borneo and Java

Neofelis nebulosa macrosceloides: Nepal to Burma

Neofelis nebulosa nebulosa: Southern China to East Burma

Habitat

Once thought to be highly arboreal, recent evidence indicates the clouded leopard may spend considerable amount of time on the ground. Its arboreal talents are numerous—it can hang upside down from branches by its hind feet, climb on horizontal branches with its back to the ground and run headfirst down tree trunks. These talents are most likely utilized to move through the trees and for hunting. Clouded leopards are most closely associated with primary evergreen tropical rainforest but do utilize other types of habitat such as secondary and logged forest, dry tropical forest, mangrove swamps, grassland and scrub. Sightings at elevations as high as 1,450 meters in the Himalayans have been reported. They are primarily nocturnal.

Diet

Birds, primates (proboscis monkey, pig-tail and other macaques and gibbons), muntjac and wild pigs. Clouded leopards are thought to stalk from the ground and ambush from the trees.

Social Organization

Due to their highly secretive nature virtually nothing is known of the clouded leopard’s habits in the wild. Knowledge of its social behavior is based on observations of this species in zoological institutions. In captivity they are typically housed with the same mate for life once they are paired. Mated pairs are most successful when animals are introduced by one year of age.

Conservation Status

The cat is listed as Vulnerable in the IUCN Red Data Book, as Appendix I species under CITES and Endangered under the United States Endangered Species Act. The species is estimated to be in decline based upon the decreasing number of sightings of live clouded leopards by resident peoples within its range.

Physical Description

The clouded leopard is sometimes described as bridging the gap between large and small cats. In general the clouded leopard is short in stature with short legs and a long body. The head is large in proportion to the rest of its body and the tail is nearly as long as the body. Body length (head, body and tail) averages 1.3-2 meters. Body weights range from 11 to 20 kilograms for adults. Shoulder height is 50-55 centimeters. Males tend to be larger than females.

Clouded leopards have an ossified hyoid that allows them to purr but not roar. They also can make a very distinctive chuffing sound as well as other vocalizations. When threatened this cat can make a sound similar to a roar that is unnerving to the unfamiliar. The clouded leopard is capable of climbing down vertical branches with ease and frequently hangs from its rear legs.

The head and neck of the clouded leopard is patterned variably but general characteristics do occur. There are rows of fine spots (some running together) on top of the head. Patterns of smaller dark spots on the sides of the head lead to stripes predominating on the cheeks. Eyes are large with vertical apertures. Short rounded ears are black on the backside with lighter colored central patches. The most prominent feature of the clouded leopard head is the unusually large canines. These teeth can be 3.8-4.5 cm long and have a very sharp back edge. The canines in the clouded leopard are the largest in relation to body size of any extant feline and it is the closest living relative to *Smilodon tatalis.*

The body pattern is one of elliptically shaped black edged spots with the insides of the spots generally darker in color than the background pelt color. Pelt color is widely variable from ochre to tawny brown to silvery gray. Melanistic as well as pale white individuals are reported in the literature. Two longitudinal black stripes run along the back. The underside of the belly is whitish but marked with large black spots. Legs are covered with rings of black spots. Feet are finely spotted with hair growing between the balls of the feet. Soles of the feet are generally dark colored. Differing sub-species do display differences in color patterns. *Neofelis nebulosa nebulosa* has a more yellowish coat and the spots are more elongated across the body. *Neofelis nebulosa macrosceloides* is darker with a grayer coat and larger blotches. *Neofelis nebulosa diardi* is darker with smaller, sometimes rosette-like blotches. The tail is encircled with black rings with longitudinal lines on the upper half of the tail. The fur on the tail is quite thick.

Captive Management

Individual Identification Methods

All clouded leopards should be permanently identified via transponder or tattoo. For consistency purposes transponders should be used as recommended by the AZA Veterinary Advisors group. Tattoos should be on the inner right thigh and include the animal's International Studbook number. Individuals can be identified via pelage pattern, however, this should not be the only method utilized.

Restraint Methods

Generally adult clouded leopards are too large to restrain manually. Some institutions have had success using nets and specially designed squeeze chutes/boxes (see resting box illustration at end of this section). Managers must be aware of the secretive and easily stressed nature of this species when selecting restraint methods. Remote capture equipment, particularly blow pipes are acceptable. Higher powered equipment such as CO2 can cause serious injury and should be avoided, particularly at close range.

Crating and Transport Procedures

Ideally clouded leopards should be "crate trained" before relocation. This involves gradually acclimating the animal to the transport container over a period of time. For shipment purposes all transport crates should meet IATA (International Air Transport Association) guidelines. For international transport a licensed broker should be used. Proper permits should be obtained with flexibility in timing to allow for dealing with logistical problems. Crate sizes vary depending upon the size of the individual but generally a minimum size of 36 inches long by 24 inches wide by 26 inches tall is recommended for adults. As Clouded leopards are intolerant of cold temperatures air shipments during winter months should be avoided. It is essential that all openings in the crates be covered with burlap or shade cloth to allow the animal privacy.

Diets

Clouded leopards are maintained on a variety of meat-based diets. Most institutions feed a commercially prepared horsemeat based diet at a rate of 1-2 lbs. per day for 4 to 6 days per week.   Some facilities feed a variety of chunk meat and chicken products as well as whole animal supplements. Amounts offered vary dependent upon the condition of the individual cat.   As with other cat species one or two fast days per week are recommended. On fast days clouded leopard's can be offered knuckle bones and/or whole animal supplements such as chickens, mice or rats.

Any raw meat offered should not be allowed to warm to room temperature or above for extended periods of time. This may result in growth of harmful bacterial organisms. In general clouded leopards should be separated into individual enclosures for feeding. This reduces the risk of fighting over food and allows for accurate measurement of food consumption.

Environmental Enrichment

To combat stereotypic behaviors such as pacing, hair pulling, etc. it is recommended that clouded leopards be provided enrichment on an ongoing basis. Items suggested below should be offered at random so that a pattern is not developed as cats become easily desensitized. Also doing nothing is enrichment on some days. **All managers must review the list and set guidelines for appropriateness/safety/disease control. It is suggested that each institutions veterinarian review the items prior to use.** Food items should be used in quantities or frequencies that will not adversely affect the dietary needs of the animal.

1) Sand box (may become a defecation site)
2) Rib bones
3) Frozen feline diet balls
4) Knuckle bones
5) Rats/mice/rabbits-live or dead
6) Whole chickens
7) Feline diet blood trails
8) Straw/hay that has been used in ungulate exhibit/holding
9) Wood chips
10) Browse
11) Logs/stumps
12) Boomer ball
13) Spices and herbs--Russian sage, mint, cumin, nutmeg, oregano, cinnamon, vanilla, catnip, cloves, sage, basil, rose hips, rose petals
14) Peanut butter, jams and jellies, honey, finger Jell-O
15) Gelatin made with blood drippings from feline diet
16) Melons
17) Gourds
18) Pumpkins
19) Raccoon or elk urine-commercially purchased
20) Rope pulls-?

21) Telephone books
22) Cardboard tubes (smaller than head size)
23) Feathers from birds in collection
24) Christmas trees
25) Fish
26) Ice blocks containing food
27) Branches/wood chips from primate or small mammal exhibits
28) Cornstalks, cardboard boxes
29) Pine cones
30) Hard boiled eggs
31) Snow
32) Nothing
33) Animal skins and/or feet (deer/pig/domestic stock)-freeze for 90 days then cut into appropriate sizes

Housing

When designing housing areas for clouded leopards some important factors to consider are: their arboreal skills, their shy and reclusive nature, and the reputation of the males for aggression towards females. A successful housing situation will include areas where the animals feel secure, as well as a place where the female can feel unthreatened by the male and be able to defend herself. Because of the cat's extraordinary climbing and leaping abilities, both vertical and horizontal types of apparatus are necessary. Whether these are natural or artificial will depend upon individual design.

Exhibits

Height, complexity and proximity to the public are three key issues to consider when designing clouded leopard exhibits. Exhibits should be viewable by the public on no more than two sides. Animals need to be able to hide from each other and from the public. As much vertical height as possible needs to be available for climbing (8 ft. minimum). Minimum exhibit size for one pair of animals is 400 square feet with at least an 8 foot ceiling height (10-12 feet is recommended). Recommended containment barriers include 11-gauge behlen paneling (2 inch by 4 inch openings), 2-inch diameter chain link, glass or reinforced piano wire. All enclosures must have a top made of impenetrable material. If the exhibit perimeter makes contact with a natural substrate it is recommended that chain link or similar material be buried to a depth of 18 inches along that perimeter. Animal access to the exhibit should be through remotely operable shift doors with horizontal sliding doors preferable over the guillotine type.

Clean fresh water should be available at all times in the exhibit. Exhibit substrates should be able to be cleaned and sanitized. Overcleaning via use of strongly odored disinfectants is discouraged as this removes scent markings and can cause the cats undue stress. Natural substrates are encouraged as long as they can be cleaned periodically to prevent parasites. A varied topography in the exhibit is strongly encouraged. As clouded leopards are arboreal it is critical that large amounts of climbing structures and resting places above the ground be provided in the exhibit. Areas within the exhibit that allow the cats to hide from public view if and when they choose are also important. Those facilities not open to the public should use the exhibit parameters as a guideline when designing holding enclosures.

As clouded leopards are intolerant of temperatures below 50 degrees Fahrenheit institutions in northern climates are urged to consider indoor exhibits. All outdoor exhibits should provide shaded areas during hotter weather and humidity should be in the 30-70% range. Indoor exhibits should have a negative air pressure of 10 air exchanges per hour. If possible a separate ventilation system should be maintained from the public. Clouded leopards can be exhibited in either a diurnal or nocturnal setting. If not exposed to natural sunlight, artificial light cycles need to be provided. Supplemental heating should be provided for temperatures below 50 degrees.

Holding enclosures

If animals are part of an exhibit program each cat needs to have its own off-exhibit holding area measuring a minimum of 6 ft by 6 ft by 8 ft high. Substrates should be such that the area can be readily cleaned. Overcleaning via use of strongly odored disinfectants is discouraged as this removes scent markings and can cause the cats undue stress. Quaternary ammonia compounds should not be used. In addition each cat should have its own resting/nesting box within the off exhibit enclosure. These boxes can vary in size and materials but should be mounted off the ground (4 ft minimum) and include a remotely operable closing mechanism if at all possible. Nesting box materials are not always necessary but hay is recommended over straw. All boxes should have drainage and vent holes. Avoid large nestboxes as most clouded leopards (particularly females) feel comfortable in a box just large enough to allow them to stand and turn around. One suggested nestbox design used successfully by the Minnesota Zoo can be found at the end of this section. It is strongly recommended that pregnant females be offered multiple nestboxes. Some elaborate nestboxes have included mechanisms for weighing, observing and examining cats.

Each enclosure needs to have clean, fresh water available at all times. Lix-its are not recommended as cats cannot always utilize them effectively.

Facilities breeding clouded leopards need to provide the female a secluded off exhibit area. This area can be the cats' holding den, however, it needs to be of sufficient size to allow for at least one nestbox and should have an adjacent area allowing the female the opportunity to move away from the kittens when she desires. Females should not be moved from their normal holding area nor their routine changed close to parturition so careful planning is essential to assure the female’s comfort prior to parturition.

Video equipment and other remote monitoring devices are useful, especially in the maternity den and during introductions.

Pest Control

An active and aggressive pest control program should be followed. Rodent control can be accomplished using snap traps, live traps, glueboards, etc. Rodent baits should only be used if there is no possibility of the clouded leopard gaining access to the bait. Insect control can include fly or pest strips, natural or synthetic pyrethrins and growth inhibitors. All chemicals used should be approved by the institution's veterinarian prior to use. Applications should be performed by a licensed pest control technician accompanied by staff members to assure the safety of the animals and staff.

Social organization

The Clouded leopard is a secretive cat that inhabits dense forests and is difficult to locate in the wild. As a result wild populations have been virtually unstudied. Most of what is known about the behavior of clouded leopards has been learned from captive observations. Those who manage these cats in captivity have described them as shy, secretive, and nervous, often being slow to adapt to changes in their environment.

Group Composition

For reproductive purposes the optimal grouping is one male and one female. Occasionally trios have worked with both females producing cubs. Same sex pairs, both male and female, have been maintained but these animals were either siblings or animals that were hand raised together. Most successful pairings have been the result of introducing young animals ranging from preweaning to one year old. These cats form strong pair bonds and are usually managed as a pair for life. Mixed species exhibits are not recommended for this species.

Male / Female Interaction

Male/female pairs have been successfully housed together year round 24 hours a day. However, many clouded leopard managers feel a separation at night is necessary to prevent aggressive incidences from occurring during times of little supervision.

It has been noted that when cats are housed continuously as pairs many species lose interest in mating. A separation period, followed by reintroduction period can be beneficial to breeding success in some felids [Law et al, 1997]. However, in clouded leopards the risks associated with this separation oftentimes outweigh the benefits.

Compatible males and females are able to coexist in the same enclosure with little incident. However the compatibility of the pair may change if they are separated for any length of time. If a period of separation has occurred a gradual introduction should take place.
It is thought that males who are successful breeders are able to "read" females for signs of reproductive receptiveness.
As a female begins to come into estrus, a change should be noticeable in the male. The male may begin watching the female intently from a distance, but not stare at her aggressively. It is a good idea to alternate

cats through areas so males may investigate the area a female has inhabited. An increase in urine marking, cheek marking or claw marking should be demonstrated by the male. Increased marking behaviors are often seen in females as well. Males sniffing objects a female has marked will often flehmen and make a calling vocalization. If in close contact, cats will often prusten to each other. The female may roll or assume the lordosis posture. An increase in these positive behaviors should be noted prior to physically introducing the cats. Once physically together if a female is not receptive and indicates this by hissing, growling, or swatting at the male, the successful male will back down and resume watching the female for signs of receptiveness

Female / Offspring Interaction

Both mother-reared and handreared females have reared their young successfully. Mothers are watchful and protective of their young. As cubs learn to climb the females often climb up below them as if to guard them from a fall [Fellner, 1965]. Some females have been observed carrying their young down a tree if they have ventured up too high, or could not make their way back down.

As with most animals, care should be taken if any change is to occur in the clouded leopard's environment while rearing young. A female, with 1.1 five month old cubs, became ill and was removed from the area while she was treated and recovering. After a 20-day separation an attempt was made to reintroduce her to her cubs. Several attempts failed and it was noted that the male cub was very aggressive towards his mother and defensive of his female sibling, not allowing the mother near them. This was felt to be due to the fact that the cubs were at an age when they were reaching independence [Breitbeil, pers. ob].

Another incident occurred when a female with a four-month-old male cub was separated from the cub for her annual physical. She was returned to the enclosure later that same day, but was aggressive towards her offspring. A second attempt that same day, and a third the subsequent day both failed, as the female showed marked aggression. This same female and cub had been separated for a day when the cub was three months old and required medical treatment. At that time there was no difficulty in reuniting them [Breitbeil, pers. ob].

Male / Offspring Interaction

Typically female clouded leopards are housed separately from males prior to parturition, and during the rearing process. However there is documentation of a dam and sire rearing offspring together. The male tolerated the presence of the cubs, most notably the male cub, for approximately three months [Geidel et al, 1976]. This three-month length of tolerance time was also observed in a male who lived in close proximity to a female with cubs and passed the maternity area daily. Although no aggression was demonstrated toward the female cub, aggression was shown toward the male cub when the cub reached three months of age [Breitbeil, pers. ob].

Dispersal of young

Dam and litters can be kept together up to one year; however, extended periods of separation from the male may hamper reintroduction. Some institutions have had success with leaving males in with females and kittens; however, this is only in unique situations. Dam and kittens generally are kept separate from the male until kittens are removed. The male should be kept in visual and olfactory contact with the female in most situations.

Communication

Vocalizations: Clouded leopards have several vocalizations. Close contact calls consist of a short, high-pitched meow, and a prusten which is a soft expulsion of air through the lips making an 'iff, iff' sound. These vocalizations are used in greeting and as reassurance. The cry or call is a loud extended meow that can be heard over a distance of 100 meters [Law et al., 1997]. This call is often used when one cat is attempting to locate another. Growling and hissing are used as aggressive or warning vocalizations.

Marking: Clouded leopards like many species utilize olfactory markings as a form of communication. Cheek marking, when a cat rubs its head or cheek along an object, is seen in this species. Law, MacDonald and Reid [1997] found upon histological examination that these cats have enlarged sebaceous glands in the cheek region that are more prominent in males than females.
Urine marking, the spraying of urine on the ground or an object, is used to mark territory. This marking behavior is often accompanied by the scraping of substrate with the hind feet. This scraping behavior creates mounds that can serve as visual signals as well. Males will check for indicators of a female's reproductive receptiveness by sniffing her urine. Claw marking is another territorial mark and consists of scratching an object, usually wood with the front claws. Not only does this scratching leave visual marks, but also cats have glands on their front paws that leave an olfactory mark on the object.

Behavioral indicators of stress

Hair plucking has been noted in many clouded leopards and is believed to be associated with stress. This behavior was noted in a female prior to parturition, in a situation where it was felt the maternity quarters did not meet the needs of the cat [Murphy, 1976]. It was also been observed in a female after her cubs were pulled for handrearing. The behavior subsided after approximately a month’s time [Breitbeil, personal ob]. Another plucking episode occurred when the cat was exposed to new personnel in the area. She began plucking fur prior to parturition. This cat continued plucking her tail until it was denuded, and after the birth of the cub she began over-grooming the cub. This behavior continued even after the staff member was removed from the area. The situation was resolved after offering the cat a fully feathered dead chicken, which the cat was allowed to pluck and consume [Breitbeil, pers. ob].

Care must be taken when making changes to the environment of clouded leopards, as these cats appear to require long adjustment periods.

Cats and their keepers

It has been noted in both mother reared and handreared cats the tendency to bond with their primary caretakers. Fellner [1965] wrote of a cat that formed a strong bond to a keeper. The cat was able to distinguish the keeper from other people. He greeted the keeper by mewing, and drove all other people away by spitting at them. This behavior is not thought to be unusual, 25 out of 29 of those surveyed found clouded leopards to have formed bonds with their caretakers [Breitbeil, unpubl. data].

A difficulty in adjusting to new caretakers has also been noted. A male cat reacted strongly when a new male keeper was brought into the area. The male's aggression toward the keeper still had not subsided after six months [pers. obs].

Clouded leopards seem to react positively to meowing and prusten greetings when mimicked to them by their caretakers. This vocalizing may in some instances calm a nervous cat.

Introduction of Pairs

One component of the Clouded leopard SSP program is the development of introduction guidelines for opposite sex pairs. As this species is one of the most challenging felids to manage there is no one “right way” to manage all introductions. Management challenges center around mate incompatibility that has resulted in the SSP recommending that, whenever possible, clouded leopards be paired with future breeding partners before both animals reach one year of age. This pairing of juveniles has historically resulted in less likelihood of mate incompatibility as the animals reach sexual maturity. However, there are cases where clouded leopards that were paired when very young went on to experience incompatibility problems. Establishing lifelong pairs that will never be separated limits the SSP’s ability to maximize genetic diversity in the population. Due to aggression clouded leopard managers are hesitant to pair older unpaired cats, or to re-pair a cat whose long term mate has died. In a 1997 international survey 15 out of 19 facilities reported exclusive mating among their cats (Breitbeil, unpub.)Data). This high percentage could reflect the tendency for managers to create these long-term pairs and the hesitancy to tamper with this bond once it has been established. Therefore a successful procedure for introducing adult animals is needed. Establishing this procedure within strict guidelines is prohibitive due to the large variance of behavior within the species.

It is the recommendation of the SSP that the guidelines below be used for introducing younger animals. These guidelines may also apply to older adult animals; however, it is essential that all parties involved in the process be aware of the potential risks. The occurrence of male aggression towards females is high. In an international survey conducted in 1997 18 out of 28 survey respondents reported having females injured or killed by males. Only in two of these cases were there indications that an attack was imminent. Sudden and unexplained instances of males fatally attacking females have occurred during the process. Managers who have little experience introducing clouded leopards are strongly encouraged to contact those facilities that have had success in this regard for advice. Once physical introductions begin veterinary care should be available immediately in case of injury.

Basic keys to success for the introduction of pairs are:

- STABLE ROUTINE, FEEDING, CLEANING. STAFF WORKING WITH THE PAIR SHOULD REMAIN ON A FAMILIAR SCHEDULE TO MAKE THE ANIMALS COMFORTABLE IN THEIR SURROUNDINGS.
- Adjacent caging of adequate size for each animal (12 ft by 12 ft by 8 ft high). Solid walls between cages with two shift doors-one solid, the other allowing visual and olfactory access minimally. Some facilities prefer small diameter mesh walls separating enclosures thereby allowing each cat total visibility to each other to enable them to observe and become comfortable with each other’s movements and behaviors.

- Nestboxes for each cat with remote closing capabilities.
- Large amount of cage props such as sleeping boards, tree limbs, cargo nets, etc. to allow multi-level use of the enclosure and areas to retreat to or defend.
- Seclusion from other animals who are not part of the introduction.
- Minimal outside disturbance.
- Remote viewing capabilities (via video camera) for keeper staff.

Suggested introduction protocol:

Any small changes in routine can result in the cats becoming agitated and increasing the likelihood of aggression. If the introduction process has to be stopped or curtailed for any reason it is suggested that the process be slowed or started anew:

1. Acclimatization: Allow sufficient time for each new animal to adjust to its new surroundings before implementing changes or beginning the introduction process. This period can take a month or more depending upon the individuals involved.

2. Visual Introductions: Once animals are at ease with their surroundings, limited visual introductions can begin. The first visual introductions should be for short periods of time and always with familiar staff present. Behavioral cues such as postures, vocalizations, etc. should be monitored. Number and length of exposures can be increased over the next several days. At this stage some facilities rotate the pair between the two enclosures. This allows close exploration of smells and may help reduce territoriality. Aggressive behaviors, particularly by the male should abate before proceeding to tactile introductions.

3. Tactile Introductions: Tactile introductions through a common wall is the next step. This wall should be constructed of wire or similar materials with very small openings (1/2 inch or less) to prevent injuries to either cat. Many institutions modify the shift doors between the adjacent enclosures to facilitate this stage. Clouded leopards usually show one of three different behaviors when housed in close proximity. Antagonistic or aggressive behavior such as growling, hissing or spitting may be shown initially and usually passes within a short period. Do not move forward with introductions until this period passes. The second behavior is one of disregard for one another characterized by cats spending little time in close proximity. The third behavior may be shown when the female is in estrus: prusten, cheek rubbing, rolling, crying and lordosis. In some cases females who previously showed no signs of heat when isolated may act differently when put in close proximity to a male.

It is thought that males who are successful breeders are able to "read" females for signs of reproductive receptiveness.

As a female begins to come into estrus, a change should be noticeable in the male. The male may begin watching the female intently from a distance, but not stare at her aggressively. It is a good idea to alternate cats through areas so males may investigate the area a female has inhabited. An increase in urine marking, cheek marking or claw marking should be demonstrated by the male. Increased marking behaviors are often seen in females as well. Males sniffing objects a female has marked will often flehmen, and make a calling vocalization. If in close contact, cats will often prusten to each other. The female may roll or assume the lordosis posture. An increase in these positive behaviors should be noted prior to physically introducing the cats. Once physically together if a female is not receptive and indicates this by hissing, growling, or swatting at the male, the successful male will back down and resume watching the female for signs of receptiveness.

4. Physical Introductions: When the pair appears to be at ease with each other at the tactile access point as demonstrated by lying side by side or one animal presenting itself in a vulnerable position while the other animal reacts non-aggressively they are ready for partial physical introductions. Make sure both cats are fully aware of each other's presence before they are physically put together. Catching one or both of the cats off guard can trigger and immediate and sometimes fatal defensive reflex.

   Prior to the first partial physical introduction remove the female from her enclosure by either shifting her to an adjacent enclosure or locking her in her nestbox. Allow the male access to her empty enclosure. The male's reaction to female's urine/feces markings should be monitored closely. Males who show no reaction or ignore these markings <u>may</u> be more aggressive towards females when introduced. Males who investigate these markings and seem interested in the females <u>may</u> be less aggressive. A positive reaction may include flehmen, prusten and vocalizations.

   If the male is reacting in a positive fashion open the dividing door a few inches to test how the pair react. <u>Clouded leopards that give every indication of non-aggression and/or compatibility while separated may exhibit the exact opposite behavior when the physical barrier is removed. NEVER THROW THE DOOR OPEN AND LET THE MALE IN WITH THE FEMALE WITHOUT TESTING THEM FIRST</u>. If aggressive behavior is displayed, limit this type of access to short periods and repeat for several days until the cats become non-aggressive. Once that has been achieved the door can be opened a few inches at a time until the animals have full access to one another. All parts of the enclosure should be clearly visible to both animals. Make sure ample escape routes exist for both cats so that neither can be trapped or cornered by the other. This full access should be done with staff members present to separate the animals if necessary. Staff must be extremely vigilant in observing the cats as fatal attacks have occurred when staff has momentarily turned their backs. Hoses, CO2 fire extinguishers or any object that makes a loud noise should be kept close by in case of a fight. If a fight ensues separate the cats immediately.

Periods of supervised access can be increased in duration as long as the cats continue to feel comfortable with each other. This increase should be slow and careful attention should be paid to the pair's behavior during this time. Changes in environment (new cats in the vicinity, new keepers, veterinary procedures, etc.) could result in an increased risk of aggression. It is recommended that these changes be kept to an absolute minimum during this time. Over time the pair can be allowed short periods of unsupervised access. The use of remote monitoring equipment at this stage will give valuable insight to the pair's behavior when alone. Some clouded leopards react very differently when keepers are not present.

5. Post Introduction management: Some managers leave their clouded leopards together 24 hours a day once the pair feels comfortable with each other. Others continue to separate pairs at night or only introduce their pairs when the female is in estrus. Each pairing and situation is unique. The procedure outlined above is a guide only. As these cats tend to be crepuscular or nocturnal the use of video recording equipment is encouraged. Some managers feel it takes a year of more to successfully introduce pairs. Cues from the pair are the most important factors in successful introductions. It is critical that keepers caring for the animals know the behaviors of the cats and be able to distinguish changes in behaviors. It is recommended that introductions take place in off exhibit areas. Once the pair has been introduced off exhibit the process of introducing the pair to the exhibit can begin. The female should be allowed access to the exhibit first. After she has had time to explore her surroundings the male can be re-introduced to her on exhibit.

Care must also be taken when reintroducing pairs that have been split for births, medical or management reasons. The introduction process may need to begin anew in cases where cats have been separated for as little as one day.

Current Knowledge about the Reproductive Biology of the Clouded Leopard

(Authors: Budhan Pukazhenthi, JoGayle Howard and David Wildt, Conservation & Research Center, National Zoological Park, Smithsonian Institution, Front Royal, Virginia.)

Introduction

Nothing is known about the reproductive biology or mating strategies of free-living clouded leopards. However, there is substantial data on the reproductive physiology and endocrinology of clouded leopards maintained under zoo conditions.

Most research has fallen under the following categories: 1) semen and sperm characteristics within and among clouded leopards over time; 2) hormonal patterns in both males and females, especially longitudinal reproductive cyclicity in females; 3) developing methods to stimulate ovarian activity and ovulation; 4) artificial insemination (AI); 5) *in vitro* fertilization (IVF); and 6) semen cryopreservation technology and genome resource banking (GRB). Although generating a substantial database, these studies also have identified some unique characteristics of the species that may present challenges to captive management and the use of both natural and assisted breeding. Thus, like all good research, these systematic studies have spawned a host of potential high priority research issues.

Reproductive biology studies are a high priority because reproduction, of course, is the essence of species survival. An ability (for example) to detect: 1) reproductively active from inactive animals; or 2) if an animal is a seasonal breeder allows managers to attempt propagation using only appropriate individuals at appropriate times. This appears particularly relevant for the clouded leopard because of the species' high propensity for sexual incompatibility. Using current breeding regimens, it is quite common for males to make lethal physical attacks on females, even those that are demonstrating overt estrus. Therefore, a common problem with captive breeding of clouded leopards is an unwillingness of managers to pair genetically-appropriate individuals because of fear that the female will be injured, traumatized or killed. One result has been a worldwide distribution of 'isolated' females that are not paired with males. The problem is confounded further by a general lack of genetically valuable males in zoo populations. For example, it is known that most clouded leopards from North America are descended from only a few founders, leaving an extraordinarily few genetically valuable animals (n < 10) in the region.

As a result, there is general consensus that well-conducted reproductive studies eventually could lead to developing consistently effective assisted reproductive techniques like AI, IVF and embryo transfer. For example, the advantages of AI for the clouded leopard would be profound, not the least would be the ability to utilize all reproductively and genetically healthy singleton females. Because the ultimate goal is to maintain a high level of genetic diversity in all extant clouded leopard populations, AI also could be used to move genes among populations (via germ plasm) rather than take the more dangerous and stressful route of moving living animals. This strategy also might eventually allow valuable genes to be 'captured' from wild populations, introduced into zoo populations, the result being that no additional animals would need to be removed from the wild. This approach, in fact, would have the benefit of boosting the genetic viability of captive populations while ensuring that wild clouded leopards remain in native habitat to protect nature.

Male Reproductive Biology

The technique of electroejaculation conducted under a surgical plane of anesthesia is safe for collecting semen/sperm from clouded leopards [Wildt et al., 1986a; Howard, 1993]. Many males have been evaluated using this technique, and there is considerable data in the literature on semen volume, sperm count, sperm motility, number of motile sperm per ejaculate and the incidence of structurally abnormal sperm in an ejaculate [Table 1]. Repeated electroejaculations over time can identify males that produce different quality semen samples. However, although there is some natural variation within individuals, most clouded leopards produce approximately 1-3 ml of ejaculate containing ~30 million total sperm of which about 70% are motile. Semen samples are not milky in appearance, but rather slightly opaque, and it is essential that the seminal fluid be removed from the ejaculate as soon as possible by low speed centrifugation (300 x *g*; 8 min). Maintaining the sperm and seminal fluid together is lethal to sperm with most cells exhibiting a loss in motility within 30 min. Following centrifugation, the sperm pellet can be resuspended and motility sustained in common tissue culture medium, especially Ham's F10 supplemented with 5% heat-inactivated clouded leopard serum.

The functional capacity of clouded leopard sperm is determined by assessing the: 1) ability of sperm to acrosome react (acrosome is a membrane bound, cap-like structure located on the anterior region of the sperm head required for fertilization); and 2) ability of sperm to bind to the egg, penetrate the zona pellucida (outer covering of the egg) and enter the perivitelline space. For assessing the ability to acrosome react, sperm are incubated in tissue culture medium for various time intervals and then exposed to a calcium ionophore (A23187; 4 μM) to induce the acrosome reaction [Long et al., 1996a].

Subsequently, the acrosomal status is evaluated with an acrosome-specific fluorescent stain [Long et al., 1996a]. To assess sperm-oocyte interaction, in vitro-matured, salt-stored domestic cats oocytes are co-incubated with clouded leopard sperm for 6 h (38°C, 5% CO_2 in air) and the extent of zona penetration assessed using differential interference contrast microscopy [Howard et al., 1993]. The ability to acrosome react and penetrate an oocyte is considered a reliable measurement of fertilizing ability.

Protein source in the culture medium is known to influence sperm capacitation (activation) and ability of sperm to undergo the acrosome reaction. Therefore, the effect of heterologous proteins (fetal calf serum, human serum albumin) and homologous protein (clouded leopard serum) on sperm motility and zona penetration has been assessed in clouded leopards [Long et al., 1996b]. Clouded leopard serum improves sperm motility and longevity compared to fetal calf serum, however, zona penetration is not improved. Clouded leopard serum also enhances acrosome reaction and zona penetration compared to human serum albumin. Although ~30% of clouded leopard sperm acrosome react in the presence of homologous serum, this is considerably lower than results reported in a variety of other species.

Interestingly, almost 70% of all clouded leopard sperm from an average ejaculate are malformed, the two predominate abnormalities being abnormal acrosomes and tightly coiled tails. It now is well-known that these 'abnormally-shaped' sperm cannot penetrate and fertilize eggs, so this condition of 'teratospermia' in the clouded leopard is not normal [Howard et al., 1993]. However, it is similar to the condition observed in the cheetah (*Acinonyx jubatus*), which is thought to be related to the remarkable and historic loss in genetic diversity in that species [Newman et al., 1985]. These findings would be consistent with the impact of inbreeding which also has been measured in other felids like the Florida panther (*Felis concolor coryi*) and Asiatic lion (*Panthera leo persica*) where poor sperm quality occurs simultaneously with a measurable lack of genetic diversity [Wildt, 1994]. Based on molecular analysis, percent polymorphism (the frequency of polymorphic loci) is lower in the clouded leopard (6%) than in the domestic cat (22%), ocelot (21%), free-ranging Serengeti African lion (11%), captive tiger (10%) and captive leopard (10%) [Newman et al., 1985; Miththapala et al., 1991]. Percent average heterozygosity per individual also is reduced in the clouded leopard (2.3%) compared to other felid species (domestic cat, 8.2%; ocelot, 7.2%; Serengeti African lion, 3.8%; captive tiger, 3.5%; captive leopard, 3.1%) [Newman et al., 1985; Miththapala et al., 1991]. Interestingly, ejaculates collected from wild-caught animals maintained in Thailand zoos also contained a high proportion (>85%) of structurally abnormal sperm [Table 1]. This lends support to the possibility that the sperm abnormalities routinely observed in this species also is due to diminished genetic diversity. Finally, substantial studies have been conducted on circulating concentrations of adrenal (or stress) hormones in this species [Wildt et al., 1986b]. Compared to some other felid species, the clouded leopard has high peripheral concentrations of glucocorticoids which may be consistent with this species' shy temperament. Stress can adversely affect reproductive capacity, and it is worth noting that there could be a relationship between what appears to be physiological evidence of stress sensitivity and semen characteristics.

Female Reproductive Biology

A survey in 1989 of the international studbook revealed that 75% of all clouded leopard litters were born to females between 1 and 5 years of age. Sexual maturity ranged from 17 to 28 months with gestation ranging from 85 to 121 days (mean, 93 days) [Yamada and Durrant, 1989]. An analysis of international breeding records for captive female clouded leopards reveals that 46% of parturitions occur in March and April, indicating that most estrual periods occur from late December through February. These birth records largely have been corroborated by recent monitoring of fecal hormone metabolites in a substantial sized population of zoo-maintained clouded leopards [Brown et al., 1995]. On the basis of fecal estradiol profiles, duration of the estrous cycle was 24 ± 2 days (mean ± S.E.M.), with estrus lasting 6 ± 1 days and the duration of the nonpregnant luteal phase being 47 ± 2 days. If females are maintained under a 12 hour light:dark cycle, they will cycle regularly throughout the year. Females under natural light fluctuations experience a seasonal anestrus during the late summer and early fall. One female has been shown to demonstrate a lactational anestrus after birth of 3 cubs [Brown et al., 1995]. Of the 14 clouded leopard females monitored to date, ~40% were observed to spontaneously ovulate based on elevated excreted progestagen in the absence of mating [Brown et al., 1995; Figure 1]. The use of fecal steroid metabolite monitoring is extremely important because it offers a method of longitudinally assessing reproductive activity using a completely atraumatic approach, thereby eliminating the potential confounding impact of stress.

Ovulation Induction

Exogenous hormone treatments are effective in animals and humans for artificially stimulating the ovary to grow ovarian follicles and ovulate. These females then can mate naturally or embryos can be produced by assisted reproduction techniques. Like most mammals, the clouded leopard responds to intramuscular injections of the hormones (gonadotropins) routinely used for ovulation induction in many felid species. These include equine chorionic gonadotropin (eCG) to provoke follicle growth and human chorionic gonadotropin (hCG) to stimulate ovulation [Table 2]. These hormones have been used in conjunction with AI to produce offspring in 7 felid species, including the clouded leopard [Howard, 1999; Howard et al., 1996]. However, it now is well established that this species is exquisitely sensitive to these gonadotropins. For example, although weighing 4 times the body mass of the domestic cat, the clouded leopard actually requires less eCG and hCG to stimulate a comparable ovarian response. Ovarian hyperstimulation can sometimes occur, and there is concern that this could result in abnormal ovarian steroid secretion, influencing estrogen/progesterone ratios, thereby reducing the chance of eggs to fertilize and/or embryos to implant and develop normally. The problem is accentuated by the relatively high incidence of spontaneous ovulation within the species. These ovulating females secrete high concentrations of progesterone which can negate the effectiveness of the administered eCG/hCG [Howard et al., 1997]. The result can be normal follicular stimulation, but an absence of ovulation, so that successful AI cannot occur. These findings have highlighted the importance of determining the stage of estrus cycle in individual females prior to starting the hormonal therapy for assisted reproductive technologies.

Artificial Insemination

As described above, it is necessary for electroejaculated semen (designated for AI) to be processed to remove seminal plasma. This can be accomplished by low speed centrifugation (300 x *g*, 8 min) with re-suspension of the sperm pellet in Ham's F10 culture medium containing 5% heat-inactivated clouded leopard serum. The female can be induced to ovulate with intramuscular injections of 75-100 i.u. eCG followed by 75 i.u. hCG given 80 hours after hCG. Thirty-eight to 40 hours after hCG, the female will ovulate [Howard et al., 1996, 1997]. It now is well known that AI conducted after the time of ovulation is preferred to pre-ovulatory AI. This is because various anesthesia used at the time of AI (to restrain and sedate the female) may block ovulation in preovulatory females (i.e., females whose ovaries only contain follicles). Thus, AI in the clouded leopard should not be attempted before 38 hours after hCG. Furthermore, studies conducted during the non-breeding season demonstrate that clouded leopard females exhibit a normal follicular response to exogenous hormones, but fail to ovulate suggesting an ovarian refractoriness to hCG induced ovulation during the non-breeding season.

Site of Insemination

In most species, there are several options available for the site of sperm deposition for artificial insemination. These include the vagina, cervix or the uterus. Although pregnancies have resulted in domestic cats after vaginal insemination of anesthetized females, the incidence of pregnancy is low (~10%) [Platz et al., 1978]. Numerous insemination attempts in cheetahs and clouded leopards using nonsurgical vaginal or transcervical insemination were not successful [Howard, 1999; Howard et al., 1996]. It has been determined that anesthesia inhibits sperm transport to the oviduct after vaginal or transcervical insemination resulting in failure of fertilization. Therefore, a laparoscopic intrauterine insemination technique that permits direct deposition of sperm in the uterine horn has been developed for insemination of felids [Howard et al., 1992].

For laparoscopic insemination, females are anesthetized and subjected to laparoscopy. Ovaries are assessed for ovarian response to gonadotropins and the presence of unovulated follicles and post-ovulatory corpora lutea (ovulation sites). After confirmation of fresh ovulations, the uterine horn is stabilized with an accessory grasping forceps and cannulated with a sterile indwelling catheter inserted percutaneously. Then a sterile polyethylene tubing attached to a syringe containing the sperm suspension is inserted into the catheter and the semen expelled into the lumen of the uterine horn. The entire procedure is then repeated on the contralateral horn. With the advent of this technique, the overall pregnancy rate has increased significantly (~50%) in the domestic cat and cheetah [Howard et al., 1992, 1997]. Furthermore, use of this intrauterine insemination strategy has resulted in the production of offspring in the leopard cat, ocelot, snow leopard, puma, cheetah, tigrina and tiger [Howard, 1999]. However, only one pregnancy has been achieved in the clouded leopard [Table 3], suggesting the need to further examine factors (such as spontaneous ovulation) that may influence the efficiency of AI in the clouded leopard [Howard et al., 1996]. Studies are underway examining various methods to control the female reproductive cycle (inhibit ovarian function) to allow a predictable ovulatory response to exogenous gonadotropins which in turn, could improve the AI success in clouded leopards.

Genome Resource Banking and Sperm Cryopreservation

A Genome Resource Bank (GRB) is the organized collection, storage and use of biomaterials (sperm, embryos, oocytes, tissue, blood products and DNA) used for the purposes of conservation. The advantages of GRBs are profound, including: 1) allowing the easy and safe movement of genes among populations to help maintain genetic diversity, 2) helping reduce the total number of animals needed in captivity to achieved targeted levels of genetic diversity, 3) providing 'insurance' against catastrophes, including disease epidemics, and 4) as a resource for other biomaterials like tissue, blood by-products and DNA that are useful for addressing issues related to taxonomy, subspeciation, paternity, disease surveillance and forensics.

Clouded leopard sperm can survive freezing, showing reasonable post-thaw motility. In early studies to determine an optimal cryodiluent in clouded leopards, PDV-egg yolk diluent was chosen for its cryoprotective abilities in felids [Platz et al., 1978]. However, recent studies have 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. Modified TYB diluent is prepared from 2 commercially-available (Irvine Scientific, Santa Ana, CA) cryodiluents marketed for human sperm cryopreservation: 1) "Refrigeration Medium-TEST Yolk Buffer" containing 0% glycerol (catalog#9972); and 2) "Freezing Medium-TEST Yolk Buffer" containing 12% glycerol (catalog#9971).

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: one part of Freezing Medium-TEST Yolk Buffer with 12% glycerol and two parts of Refrigeration Medium-TEST Yolk Buffer with 0% glycerol.

The minimum acceptable criteria for freezing a clouded leopard sperm sample are ejaculates containing: 1) at least 2 million motile sperm; and 2) 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 AI or IVF. 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 AI or IVF, it may have important alternative value in the future especially in conjunction with sperm injection technology.

Ejaculates to be cryopreserved are washed by centrifugation, and the sperm pellet is slowly resuspended in 100-200 µl of 'Test Yolk Buffer diluent' containing 4% glycerol. The diluted aliquot is cooled slowly for 30 min at 5°C and then pelleted (~30 µl/pellet) on dry ice for 3 min 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 100-150 µl Ham's F10 culture medium. Individual pellets are held in air for 10 sec and then dropped into test tubes containing the media with vigorous mixing at 37°C for 30 sec. Thawed semen suspension is transferred into a 1.5 ml plastic, microcentrifuge tube and centrifuged at 300 x *g* for 8 min. Supernatant is removed, and sperm pellet is resuspended in fresh Ham's F10 medium. Aliquots are evaluated for sperm motility and forward progressive motility over time. Although clouded leopard offspring have not yet been produced by AI with thawed sperm, success has been achieved in the leopard cat, ocelot and cheetah [Howard, 1999].

Conclusions

As a result of more than 15 years of systematic research, there now is a valuable database on the reproductive biology of the captive clouded leopard male and female. Among other accomplishments: 1) viable sperm can be collected routinely and safely by electroejaculation; 2) sperm processing techniques have been developed that allow sperm motility to be sustained; 3) the reproductive cycle (duration and interval of estrus) has been characterized in detail by fecal hormone technology, a tool that allows the accurate, noninvasive determination of reproductive status; 4) it now is known that this species can ovulate spontaneously at a relative high frequency compared to other felid species; 5) artificial insemination has been successful on one occasion, and it is known that sperm must be deposited in the anterior aspect of the uterine horn after ovulation has commenced; 6) one hormonal regimen has been identified to induce ovulation; and 7) sperm can withstand the stress of cryopreservation by demonstrating post-thaw motility.

On the contrary, there remains concern about the: 1) homozygosity in the captive population; 2) inability of managers to routinely pair animals for natural breeding because of sexual incompatibility; 3) high percentage of malformed sperm in electroejaculates; 4) inability to routinely produce an ovarian response mimicking 'normal' by the use of exogenous gonadotropins, in part, because the species is exquisitely sensitive to these hormones; and 5) difficulty in achieving successful *in vitro* fertilization as a result of poor sperm and/or egg function or interaction. The greatest unknown is the absolute total lack of information on the reproductive biology of wild, free-ranging clouded leopards.

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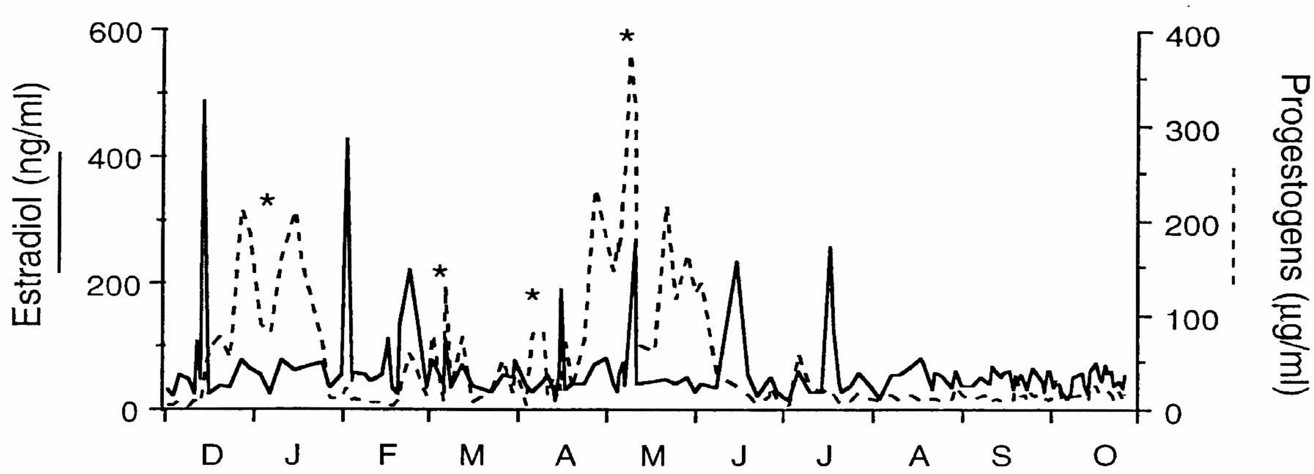

Figure 1. Fecal steroid profiles in a clouded leopard exhibiting spontaneous ovulation. Asterisk denotes incidence of ovulation. (From Brown et al., 1995).

Table 1. Ejaculate traits in captive-born and wild-born clouded leopards.

	North American Zoos Captive-Born (60 males; 134 ejaculates)	Thailand Zoos Wild-born (17 males; 22 ejaculates)
Ejaculate volume (ml)	2.72 ± 1.9	0.81 ± 0.1
Sperm concentration/ml (x 10^6)	41.6 ± 4.7	28.4 ± 4.2
Total sperm/ejaculate (x 10^6)	29.9 ± 2.9	27.7 ± 6.2
Sperm motility (%)	70.2 ± 1.1	52.6 ± 4.4
Sperm progressive motility [a]	3.6 ± 0.1	3.1 ± 0.2
Structurally normal sperm (%)	23.9 ± 2.2	11.4 ± 1.4

[a]Sperm progressive motility is based on a scale of 0-5 (5= rapid forward progression).

Table 2. Influence of dosages of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) on ovarian activity in clouded leopards.

	Dosages of eCG/hCG (IU)				
	200/140 or 400/280 (n=6)	100/75 (n=5)	75/75 (n=5)	50/75 (n=7)	25/75 (n=4)
No. post-ovulatory females/total number of females (%)	6/6 (100%)^{a}	4/5 (80%)^{a}	4/5 (80%)^{a}	7/7 (100%)^{a}	1/4 (25%)^{b}
No. fresh corpora lutea/female	5.3 ± 1.3^{a}	3.6 ± 1.2^{a,b}	3.2 ± 0.9^{a,b}	2.6 ± 0.6^{b}	0.8 ± 0.7
Mean diameter of fresh corpora lutea (mm)	3.7 ± 0.1^{a}	5.1 ± 0.3^{b}	5.4 ± 0.2^{b}	5.6 ± 0.3^{b}	3.7 ± 0.3^{a}
No. unovulated follicles/female	6.2 ± 2.2	7.2 ± 2.5	5.2 ± 2.3	5.1 ± 1.7	8.5 ± 2.2
Mean diameter of follicles (mm)	4.6 ± 0.2	4.9 ± 0.3	4.8 ± 0.2	4.1 ± 0.1	4.0 ± 0.1

[a,b]Within rows, values with different superscripts differ ($p < 0.05$). (From Howard et al., 1997)

Table 3. Ovarian activity and inseminate traits of a successful laparoscopic intrauterine artificial insemination in a clouded leopard.

Ovarian stimulation	
Dosage of eCG/hCG (IU)	100/75
Time of insemination (h post-hCG)	45.0
No. fresh corpora lutea	5.0
Mean diameter of fresh corpora lutea (mm)	7.0 ± 1.0
No. unovulated follicles	0.0
Inseminate traits	
Sperm motility (%)	70.0
Sperm progressive motility[a]	4.5
No. of motile sperm (x 10^6)	88.5
No. of cubs born	2

[a]Sperm progressive motility is based on a scale of 0-5 (5=most rapid forward progression). (From Howard et al., 1996)

Birth, Growth and Development

Contraception

Currently methods of reversible contraception center around the use of Melengesterol acetate (MGA) implants that prevent females from becoming pregnant. Recent studies have found that felids continually exposed to MGA may develop severe endometrial hyperplasia and have higher risk for mammary gland and uterine cancers. Managers considering the use of these implants in clouded leopards need to consider the long term effects of these implants.

Currently the most effective contraceptive measure in clouded leopards is separation of the pair while the female is in estrus. However, due to the highly sensitive nature of this species to changes in routine and the problems associated with separating pairs this method is not always practical.

Management of the pregnant female

In general females should be separated from the male two to four weeks prior to parturition. Strongly bonded pairs may be left together longer but should be separated at night during the last week of gestation. Some facilities chose to leave the male with visual and olfactory access to the female as long as the female does not become stressed. There should be multiple nestboxes available to the female well before parturition. Some facilities mount the nestbox five feet off the ground. These boxes can be bedded with grass hay. Some females will remove all bedding from the nestbox or may bury the kittens in the bedding in the box. Diet for the female should be increased slightly (5-10%) in the last trimester with an equivalent increase post-parturition.

No significant changes to the female's routine should be made in the four weeks prior to anticipated parturition. It is strongly suggested that only experienced keepers whom the female is used to care for the female. It cannot be emphasized enough that changes in the female's environment can be detrimental. Every effort should be made to give the female clouded leopard as much privacy as she needs. Managers may want to consider reducing the frequency of cleaning in the female's enclosure. Nestbox materials should not be changed unless soiled and no more than 40-50% of the bedding should be changed at any given time.

Remote monitoring of the nestbox via video camera is recommended. This can be accomplished by modifying the nestbox to include a camera and low level lighting. Again these nestboxes should be in place weeks before births are anticipated.

Usually within 24-48 hours before birth the female will exhibit changes in behavior such as anorexia, increasing amounts of time in the nestbox and restlessness. Once birth has occurred access to the female's den and the holding area should be strictly limited and if at all possible the female should be left totally alone for 24 hours. If video monitoring is used nursing should be observable. In general, if the female is spending large amounts of time in the nestbox and food is disappearing many managers feel everything is okay. Disturbances at this early stage and as late as eight weeks post-partum may cause the female to neglect or become aggressive to the kittens. If the decision is made to hand rear the kittens, females should be allowed to nurse the kittens for the first 12-24 hours to provide colostrum to the kittens.

Females may not eat for the first few days after birth but freshwater should be available at all times. After the initial post-partum period (7 days minimum) as the female becomes more comfortable a gradual return to normal cleaning and activities in the area can begin. Cubs should be left undisturbed for the first week minimally depending upon the behavior of the female. When the opportunity arises cubs can be weighed and sexed but this should only occur when the female is voluntarily out of the box and when kittens are at least 2-3 weeks old. It is important to keep these encounters to a minimum of time and with the people that the female is most familiar. Cubs should initially be handled with rubber gloves that have been soiled with feces from the den. As the cubs get older it is wise to have adequate room for the female to get away from them if she chooses.

The protocol below for care of the pregnant female and neonates has been successfully used at the Minnesota Zoo.

Clouded leopard-Pregnant/Lactating Female Management

1. Two to four weeks prior to parturition the male should be separated from the female allowing visual access only. Nestboxes can be bedded with straw or grass hay.
2. General diet for a 12-13 kg* gestating female:
   a) 250-300 grams meat based diet twice daily, knucklebones twice weekly.
   b) Approximately two weeks prior to parturition, add 50 grams meat based diet to each feeding.
   c) One week post parturition an additional increase of 50gms per feeding should be added.

      ***Diet amounts and increases should be customized to each female and situation. Changes are made by monitoring how quickly the female responds and consumes each feeding. Knuckle bones are withheld for one week from all animals in the immediate area following a birth. All but the dam can resume bones after one week.**
3. First 24 hours following a birth:
   a) the service area for the dam is closed off as much as possible.
   b) Staff traffic and noise in the area is restricted.
   c) Cleaning of adjacent cages is minimal with no hosing.

   After the first 24 hours, cleaning of cages can be resumed if it does not cause stress or annoy the dam and cubs. The nestbox with the cubs and nursing female are off limits unless otherwise approved.
4. A log is started on the day of birth for the dam's activity and cub vocalizations. Audio and visual monitoring equipment is used at the discretion of the curator.
5. Vaccination schedule:
   a) at two weeks of age, the cubs are vaccinated with modified live (intranasal) vaccine.
   b) The are vaccinated at three week intervals thereafter until six months of age.
6. Physical examinations:
   a) at two weeks of age the cubs are separated briefly from the dam for vaccinations and a quick physical exam which includes: sexing, heart and respiration rate, temperature, weight, blood sampling and a stool sample if available.
   b) Cubs need to be identified by fur clipping or tattoos for purposes of record keeping.
   c) Nails can be trimmed
   d) Nestbox bedding material can be changed.
   e) Cubs and dam should be reunited as quickly as possible
   f) Physical exams can be given opportunistically in conjunction with the vaccinations

7. Knuckle bones can be offered to the dams once the cubs are coming out of the nestbox on their own. Offering the bones in the PM allows the cubs to participate (outside of the nestbox) in the chewing/investigating of the bone with the dam.
8. Cub diet*:
    a) between six and eight weeks of age the dam's diet should again be increased as the cubs will be eating some of the meat offered.
    b) Increase 50 grams per feeding per week for two weeks once cubs are observed sharing dams diet regularly.
    c) As soon as the dam shifts for food during this period, allow the cubs to be separated for 15-30 minutes to eat on their own.
    d) The group can be reunited if the cubs or the dam become alarmed or after the cubs return to the nestbox.

    ***Again diet increases and food amounts offered should be determined on a individual basis. Litter size, cub and/or dam condition are the key indicators.**

9. Tree branches and other natural props should be added to shift cages to stimulate climbing and aid in development and coordination of the cubs.

Infant Development

As with other carnivores clouded leopards are born blind and helpless. Umbilical cords usually fall off within 3-7 days after birth. Eyes open at 10-14 days. Teeth begin erupting at three weeks and shortly thereafter the cubs will begin chewing on all surfaces so care must be exercised to prevent ingestion of splinters, etc. By one month cubs should be moving steadily on all four legs and by five weeks most are jumping, running and attempting to climb. They also will begin to venture out of the nestbox at this stage.

Littermates become increasingly independent at six weeks. Intense play with siblings begins during this time and the first "chuff" vocalizations may occur. Most kittens begin eating solids at about nine weeks and are weaned at 90 to 100 days. Increase the female's rations when kittens are six to eight weeks old. Increase diet again when kittens are observed eating regularly. Kittens should be allowed to eat on their own (shift female away) for 15-30 minutes once they begin eating. It should be mentioned that females may become intolerant of kittens as they age so careful observation is needed to avoid aggressive encounters. Aggression between littermates at 60 to 90 days is not unusual and kittens may need to be separated for feeding.

Hand rearing

Many facilities chose to hand raise their clouded leopard kittens for a number of reasons. Due to this species secretive nature, some female's unwillingness to raise their kittens and aggression to the kittens by the female there is a large base of information concerning hand raising clouded leopards. Some managers feel hand raised clouded leopards are better exhibit animals as they tend to exhibit fewer signs of stress in an exhibit situation. Hand raised female clouded leopards have gone on to raise their own offspring (Fletchall, unpublished data).

If the decision is made to hand rear cubs it is essential that all necessary formulas and equipment be on hand. Records of animal weight, weight gain, rectal temperature, formula composition, amount eaten, behavior, ambient temperatures, etc. should be maintained throughout the rearing process. As clouded leopards tend to be a challenge to get to suck and are more susceptible to aspiration it is recommended that only experienced keepers work with cubs during the first weeks.

In the event of a singleton birth every effort should be made NOT to rear the kitten alone. A domestic kitten or another cat species can be introduced at three to four weeks of age. This companionship provides valuable play experience necessary for proper socialization and normal developmental skills. It is a critical ingredient to the animal's later success in dealing with conspecifics. Ultimately a "nursery group" of clouded leopard kittens is preferable to even a small litter. The more opportunity kittens have to interact with conspecifics the more adaptable they are to pairing and bonding.

Clouded leopard kittens are very active and moving well by four weeks of age and climbing and leaping skills develop shortly thereafter. Hand reared clouded leopards should be provided ample space to improve climbing and leaping skills. Padding an enclosure floor with straw and providing several levels of shelving or other climbing apparatus, and well as "toys" for pouncing, chasing and carrying are recommended. These activities are an important beginning to ongoing environmental enrichment. Clouded leopards can also be offered shallow water pools for play.

The following protocols have been submitted by three facilities who have had success in hand raising clouded leopards. Other successful methods have been used and managers are encouraged to discuss hand rearing techniques with other clouded leopard managers. Historically Esbilac and KMR have been the primary milk replacer products used for clouded leopards. In 1995 butterfat replaced coconut (vegetable) oil in both formulas. Some felids have experienced severe constipation when on this new formula. In the past few years many institutions have had success with Pet-Ag's Zoologic Milk Matrix as the primary milk replacer. Managers are encouraged to contact the SSP Coordinator or other facilities experienced in hand raising felids before purchasing replacer for the most up to date information.

Hand rearing protocol for Clouded leopards

Feeding procedures

1. Weigh cub before first feeding daily and before stimulating urination/defecation. This weight will be the one used to determine amount of formula to be offered the next day.
2. Check body temperature at least once daily. Body temp. should range between 96-99 degrees. Do not feed if temp. is below this range and notify vet staff. Incubator temp. should be 90 degrees initially then gradually reduced as kittens grow.
3. Check gums for pink color (should return to pink quickly after touching gums with finger).
4. Check hydration (pull skin gently at scruff of neck, should bounce back if hydration is adequate).
5. Based on weight taken above, mix days formula (add 1 drop lactaid/100cc formula). Quantity offered determined by; (cub's weight) x (% of body weight to be offered)=quantity for 24 hr. period. Divide this number by the number of feedings per day=the amount of food offered per feeding.
6. Feed appropriate amount of Esbilac formula. Place twice the amount to be fed in bottle to avoid kitten sucking air. Warm bottle by placing in hot water before feeding. Weigh bottle periodically throughout feeding until proper amount has been fed. Keep kitten upright with head raised during feeding.
7. After feeding stimulate to urinate and defecate by rubbing cotton balls on anogenital area. Note and record amts., color and quantity of stool. Specific gravity of urine can be monitored to assess hydration.

Formula concentrations/amounts to feed

1. Week 1: Feed every 3 to 4 hours. Feed 25% of body weight over a 24 hour period. Concentration of formula should be 8% (8 grams powdered Esbilac and 92cc distilled water). Add 1 drop Lactaid to each 100cc formula.* Gradually increase concentration of Esbilac to 15% by Day 7.

   *1st day only: Heat formula for 90 minutes at 90° F to predigest lactose. Day 2 on add Lactaid and make 24 hours in advance, refrigerate.
2. Day 8-21: 7 feedings per day at 23-25% of body weight. Increase concentration of formula to 18% by Day 21.
3. Day 22-35: 6 feedings per day at 20-23% of body weight. Formula concentration to 21% by Day 35.
4. Day 36-60: Decrease feedings to 5 per day. Feed at 20% of body weight. Formula concentration 22%. Begin eliminating bottle feedings and substitute solid food/formula mixture offered in saucer or flat pan.
5. Day 60 on: Gradually eliminate formula feedings. Kitten weaned and eating all solids by Day 90.

Clouded leopard Hand rearing protocol

Initial incubator temperature should be between 88°-99° F. Remove kittens from incubator at 1 ½ months and keep at room temperature.

- Day 1: Offer 15-25% of body weight divided into five feedings. Formula is Esbilac mixed at ½ normal strength with water. Add 1/2cc serum from mother's blood per feeding to formula to provide antibodies.
- Day 2: Full strength Esbilac. Feed 15-25% of body weight dispersed into 5 feedings. Add ½ cc serum at each feeding. Add Poly-vi-sol to formula.
- Day 9: Feeding full strength Esbilac. 5 feedings per day at 15-25% body weight. Begin adding Lactaid to formula.
- Day 41: Start offering 1 tsp. of meat once daily.
- Day 56: Decrease number of formula feedings to 4x daily.
- Day 63: Decrease formula feedings to 3x's daily. Offer solid meat twice daily.
- Day 82: Decrease formula feedings to 2x's daily.
- Kittens weaned completely at 15 weeks.

Clouded leopard Hand-rearing protocol

Formula: 1 cup distilled water
½ cup powdered Zoologic milk matrix
¼ tsp. dicalcium phosphate (add beginning the end of the first week)
granules of one lactobacillus capsule

- Day 1: 1st feeding-Offer Pedialyte or 5% dextrose only
- Day 1-3: Offer mixture of distilled water:Zoologic matrix at a 3:1 ratio.
- Day 4-7: Gradually increase ratio to 2 parts distilled water to 1 part powdered Zoologic matrix. In general kittens eat 10-20% of body weight per day.

- Days 1-14: Kittens eat every 3 hours/ 7 times per day. ¼ to ½ ounce per feeding. Let go six hours at night between feedings.
- Day 14-21: Kittens eat every 4 hours/ 6 times per day. ¾ to 1 ounce per feeding. Let go eight hours at night.
- Week 3: Kittens eat every 4 hours/ 5 times per day. 1 to 1.5 ounces per feeding.
- Week 4: Kittens eat every 5 hours/ 4 times per day. 1.75 ounces per feeding. Add turkey baby food to formula at a rate of 1 tablespoon per cup of formula offered.*
- Week 5: Kittens eat every 5 hours. 1.75 ounces per feeding.
- Week 6: Kittens eat every 5 hours. 1.75 ounces per feeding. Parboiled chicken "drumettes" with skin removed are offered at this stage to stimulate chewing activity.

*Increase amount of baby food gradually until feeding ½ jar baby food per cup of formula at 4-5 weeks of age; 1 jar to 1 cup formula at 5-6 weeks. 2 jars to 1.5 cups formula from 6 weeks until weaning. Introduce solid food with some formula poured over it in a saucer or bowl at 8-9 weeks. Kittens should be off bottle entirely by 11 weeks.

Do not use incubator as the temperature and humidity seem to be the major cause of neonatal hair loss. For the first two weeks kittens are kept in a small carrier with a heating pad on low. Heating pad is covered with a towel or heavy flannel and one half is covered with an extra layer to allow kittens to move away from the heat. After the first week the heating pad can be turned off during the day. Try not to keep the kittens too warm. A kitten should feel comfortably warm to the touch and should not make the caregiver conscious of hot or cold. Kittens' feet should be warm to the touch, never sweaty.

Kittens should be stimulated to urinate and defecate. All kitten stools are somewhere between mustard and pudding consistency ("milk stools") during the period that they are on formula. Worrisome diarrhea is the consistency of water and will dehydrate a neonate very rapidly. If this occurs take them off formula and feed only Pedialyte for 12 hours, gradually reintroducing formula beginning with a 3:1 ratio and working back to 2:1 as stools firm up. Stool color can be an indicator of problems: yellow or brown is normal, greenish indicates too much food, white indicates kitten not digesting milk.

Use a Four Paws pet nursery with a small rounded nipple. Small size of nipple seems to work better than preemie or regular baby nipples.

If the kitten has nursed even once from the mother (you will know this because the stool will be a sticky consistency and yellow in color) it will be difficult to get them interested in a rubber nipple. The longer the amount of time it has been with the mother, the longer it will take to get them on a nipple. Do not assume that when kittens get hungry enough they will eat-they won't. Take time to let them feel the nipple in their mouth and slowly drip milk onto the tongue. If a kitten is not closing its' mouth around the nipple and creating good suction put your thumb and index finger on either side of its mouth by the nipple and this will create suction. Give the kitten a rest during the feeding, don't let it drink everything all at once.

Always hold baby flat on it's stomach with head tilting only slightly upward. NEVER HOLD A BABY ON IT'S BACK WHILE NURSING OR IT WILL ASPIRATE FLUID INTO ITS LUNGS.

Growth rates

Weights of hand-reared clouded leopards*		
Days of age	Average weights (grams)	Range of weights (grams)
0-5	214	166-260
6-10	276	200-345
11-15	370	280-457
16-20	518	367-669
21-25	633	475-790
26-30	800	613-988
60	1795	1500-2075
90	3100	2700-3500
*Includes weights of both sexes. Males tend to be slightly above the average, females slightly below. (N=20+)		

Weights of mother-reared clouded leopards*		
Days of age	Average weights (grams)	Range of weights (grams)
0-5	190	160-230
6-10	418	220-550
11-15	546	292-776
16-20	757	658-812
21-25	885	728-1008
26-30	1000	583-1360
60	1556	1358-1820
90	2470	2100-2600
*Includes weights of both sexes. Males tend to be slightly above the average, females slightly below. (N=4)		

Veterinary Care

Quarantine

Ideally newly arriving clouded leopards should be quarantined separate from other species of carnivores, particularly other felids. If animals were housed together at the previous institution they may be housed together at the receiving institution, however, they should be watched closely for signs of aggression and separated if needed. Clouded leopards are easily stressed so care should be taken to keep the quarantine area quiet and keep contact with keepers to only those who will routinely care for the cats. It may take several days or weeks for clouded leopards to settle into their new environment. If the diet at the new institution differs from the old a gradual transition should be made to the new diet.

During quarantine the individual cat should be evaluated for internal and external parasites, and the following serological tests performed: feline leukemia virus, Feline Immunodeficiency Virus (FIV), Feline Infectious Peritonitis (FIP), toxoplasmosis and Feline heartworm antigen and antibody. A CBC/sera profile should be done and evaluated. It is also recommended that sera be stored from females if possible.

A minimum of 30 days quarantine is recommended.

Chemical Anesthesia

In general Clouded leopards have been anesthetized using different chemical agents as outlined below:

1. 10mg/kg body weight ketamine in combination with 0.5mg- 1 mg/kg body weight xylazine (yohimbine used as a reversal).
2. Medetomidine at a rate of 0.05-0.08 mg/kg in combination with 2-3 mg/kg ketamine. Atipemazole is used as reversal agent.

NOTE: One veterinarian reported bradycardia and apnea in a clouded leopard anesthetized with a combination of 3mg/kg telazol, 2.5mg/kg ketamine and 0.6 mg/kg xylazine.

Inhalant Anesthesia

Clouded leopards are routinely placed under inhalent anesthesia with normal recoveries. Isoflourane is recommended as the gas anesthesia of choice. One should be aware that clouded leopards frequently become apnic after intubation and therefore must be ventilated manually.

CLOUDED LEOPARD VETERINARY PROTOCOLS

The clouded leopard veterinary protocols that follow are suggested guidelines that will vary based on institution, geographic location and disease prevalence.

VACCINATION: ADULTS – Should be vaccinated annually or every two years against the following diseases.
Feline panleukopenia (parvo virus)
Feline viral rhinotracheitis (herpes virus)
Calicivirus
Rabies

NEONATES - Should be vaccinated at least three times if possible between the ages of 8-10, 12-14, and 16-20 weeks. Rabies vaccine should be given at 16 weeks and then again at one year.

Members of Felidae are susceptible to canine distemper virus, but vaccination is not recommended at this time.

Vaccines used: FVR, Calici, Panleukopenia (Killed Vaccine), Fel-o-Vax PCT, Fort Dodge, Fort Dodge, Iowa
FVR, Calici, Panleukopenia, Rabies (Killed Vaccine), Fel-o-Vax PCT-R, Fort Dodge, Fort Dodge, Iowa
Rabies vaccine (Killed Vaccine), Imrab, Rhone Merieux, Inc. Athens, Ga.

References: 1998 Report of the American Association of Feline Practitioners, 1998; Bush et al., 1981; Crawshaw et al., 1996; Eberle et al., 1991; Junge et al., 1991; Spencer, 1991; Spenser and Burroughs, 1991; Wack, 1991; and Wack et al., 1993.

PARASITE SURVEILLANCE : Fecal flotation and fecal smear performed annually or biannually or as clinical signs warrant.

Deworming: **Pyrantel Pamoate** (Given orally at 5mg/kg. This dose should be given starting at two weeks of age and repeated every two weeks until six weeks of age).
Ivermectin (Has been used at a rate of 200µg/kg without adverse effects both subcutaneously and intramuscularly. Ivermectin has also been tolerated at the same dose injected into a rodent carcass as a food source).

INFECTIOUS DISEASE SURVEILLANCE : During quarantine procedures as well as annual physical examinations, blood should be submitted for the following seriologic testing.

Feline Leukemia Virus
Feline Immunodeficiency Virus
Feline Infectious Peritonitis
Toxoplasmosis
Feline Heartworm Antigen and Antibody

Questions regarding Neofelis SSP veterinary protocols should be addressed to:

Norah B. Fletchall

SSP Coordinator
John Ball Zoo
1300 W. Fulton
Grand Rapids, MI 49504

Analysis of International Studbook Data

An analysis of historical data contained in the International Studbook for the Clouded leopard is summarized in Charts 1 through 4. Median age at first reproduction is 38 months in females and 35 months in males. The earliest recorded age of reproduction is 16 months in females and 18 months in males. Average litter size is 2. 83 males and 91 females have produced offspring.

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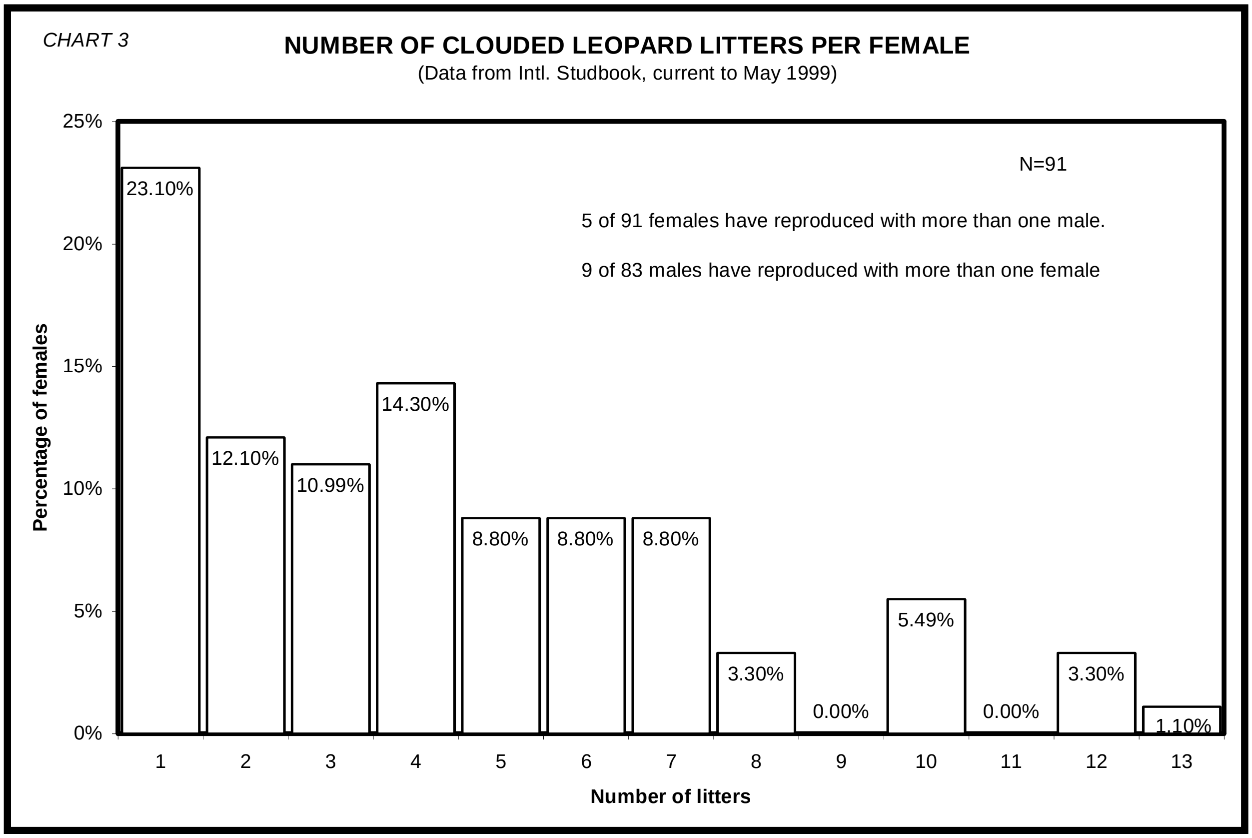

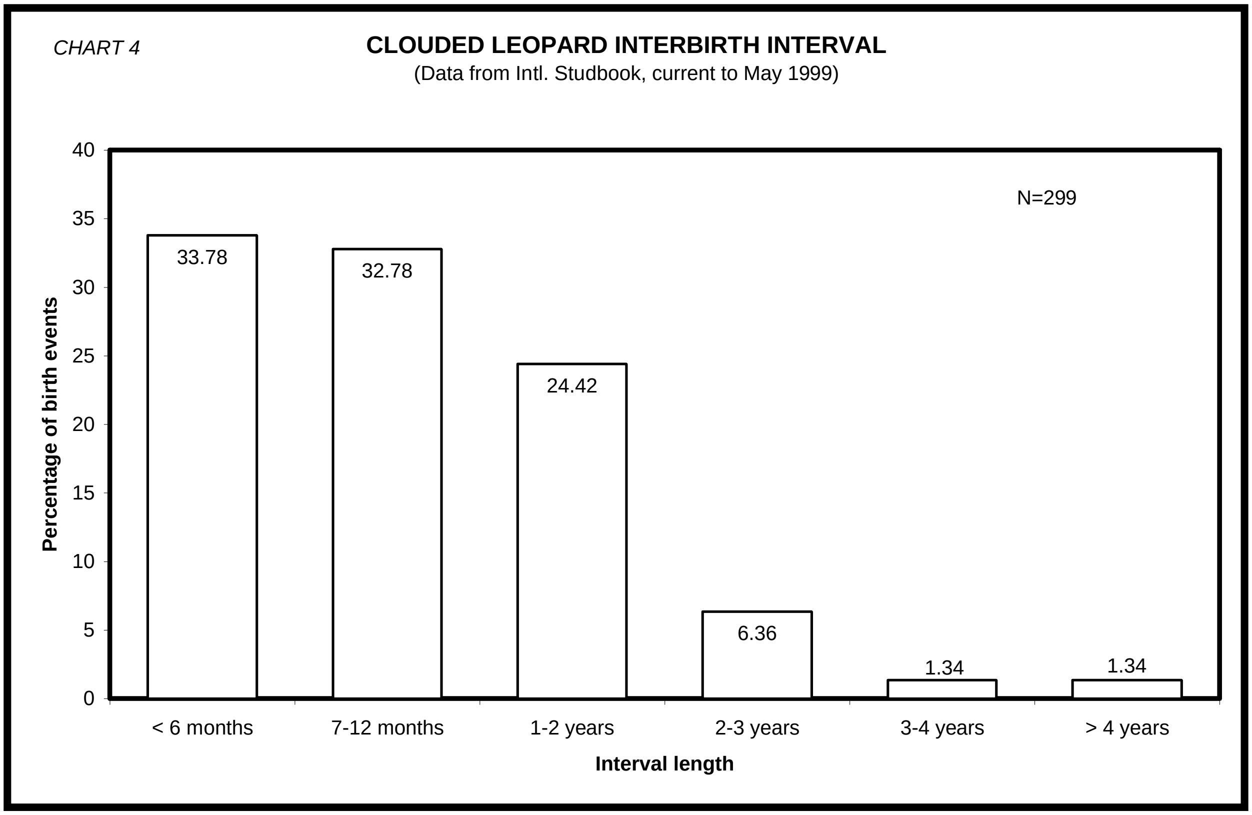
CHART 4
CLOUDED LEOPARD INTERBIRTH INTERVAL
(Data from Intl. Studbook, current to May 1999)
N=299
Percentage of birth events
40
35
30
25
20
15
10
5
0
33.78
32.78
24.42
6.36
1.34
1.34
< 6 months
7-12 months
1-2 years
2-3 years
3-4 years
> 4 years
Interval length

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