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Abstract: Detailed evaluation of the history of cheetah reproduction in captivity is prerequisite to defining the questions to be asked and direction the orientation of scientific protocols. Comparison of successful and unsuccessful reproductive attempts will provide valuable insight if the following factors are analyzed: age, housing, mate selection, introduction protocol, estrus, diet, seasonality, necropsy. Cheetahs are the least pugnacious cats in captivity and as such are excellent candidates for non-invasive sample collection techniques which can provide significant information concerning normal and abnormal reproductive function.

An Integrative Approach to Reproduction in the Captive Cheetah

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It is a well-accepted fact that the broader the scientific base applied to a problem, the faster it will be solved. We are fortunate to be able to tap into a long recorded history and a large scientific talent pool with which to create and implement a coordinated, multidisciplinary approach to address reproductive failure in the captive cheetah.

The cheetah research council, on the other hand, is severely handicapped in its endeavors by an appalling dearth of basic information concerning its subject. We simply have not compiled the data necessary to begin to design a "whole-animal" reproductive research protocol. For example, preliminary data from the San Diego Wild Animal Park indicates that failure to breed rather than male or female factor infertility is responsible for low reproductive rates. In fact, only one male has been shown to be infertile and the vast majority of breedings result in pregnancy. It is imperative to be certain that the cause of infertility is correctly diagnosed for each individual to avoid treating symptoms without resolving underlying problems.

Species" Reproductive History

Detailed evaluation of the history of cheetah reproduction in captivity is prerequisite to defining the questions to be asked and directing the orientation of scientific protocols. Comparison of successful and unsuccessful reproductive attempts will provide valuable insight if the following factors are analyzed:

1. AGE: There is some anecdotal evidence that captive cheetahs cease to reproduce at a relatively young age. This factor is confounded with duration of captivity for many individuals but these easily obtained data will help to define the reproductive profile of the species.

2. HOUSING: The effects of proximity to other cheetahs on reproduction are unknown. In some mammalian species, cyclicity in certain females is dampened or suppressed by the presence of a cycling female. This mechanism would not be operational under normal conditions for a solitary species such as the cheetah, but the possibility of its action on captive females should be investigated. Comparison of the breeding records of all institutions will indicate if solitary females are more likely to cycle than those in multiple female facilities. In other latter, suppression may be suspected if all or most litters are produced by just one or two females. These data may also uncover a stimulatory effect on reproduction in multi-female housing situations. Male-female proximity is likely to influence breeding in this species. A comparison of reproduction in always-paired individuals with those housed within visual, auditory and/or olfactory distance of males and those completely separate between introductions will be most informative.

The theory that the illusion of prey availability stimulates breeding activity

should be explored.

3. MATE SELECTION: Many cheetah breeders describe female mate preference. Compilation of statistics may indicate if females require 2, 3, or more males from which to choose for breeding. In our multi-male facility, male mate preference is also observed: an estrus female may be completely ignored by one male, then immediately bred by a second male.

4. INTRODUCTION PROTOCOL: In addition to allowances for mate selection, the method of introduction may stimulate or discourage breeding.

5. ESTRUS: Duration of estrus in the cheetah has been variously reported as 1-4 days and as long as 14 days. Information from individual facilities will clarify this issue and should include criteria for determining estrus (breeding, vocalization, posturing, vulvar swelling or discharge). Copulation frequency associated with successful and unsuccessful breedings may further define the differences. Introduction protocols for natural breeding as well as timing and frequency of artificial insemination (AI) will benefit from more precise data concerning these important reproductive parameters.

6. DIET: As Dr. Caro has observed, virtually all captive cheetahs are obese, or at least overweight. It is unknown how excess weight may affect reproduction. The correlation of body weight, diet and feeding schedules with successful breedings may indicate areas of concern that can be corrected.

7. SEASONALITY: Conflicting reports describe the cheetah as a seasonal and a year-round breeder. Re-examination of data from the wild combined with captive breeding records will indicate if temperature, rainfall, photoperiod and/or latitude affect reproduction. Artificial reproduction protocols will be designed differently for seasonal versus non-seasonal breeders: seasonal anestrus is not the same as failure to cycle in a non-seasonal species.

8. NECROPSY: Greater use can be made of necropsy records and specimens. Ovarian activity at the time of death can be correlated with age, season, diet, housing and reproductive history.

Non-invasive Data Collection

Cheetahs are the least pugnacious cats in captivity and as such are excellent candidates for non-invasive sample collection techniques which can provide significant information concerning normal and abnormal reproductive function.

It is well established that male cheetahs exhibit poor semen quality. Spermiograms of fertile, infertile, captive and wild cheetahs are indistinguishable. Poor semen quality is a fact to be considered when designing protocols for its handling and cryopreservation.

Obviously, the ultimate proof of sperm viability is the production of offspring. The captive situation does not lend itself to extensive testing of various handling and freezing protocols through AI, thus, development of optimum methods must be carried out in vitro.

Collection of large numbers of samples for testing can be accomplished by training males to provide semen voluntarily thus obviating the stress of capture and anesthesia necessary for electro-ejaculation. Both hand-raised

and mother raised males have been trained to service an artificial vagina. The technique allows weekly semen collection for extensive AI and freezing studies and provides data concerning seasonal effects on spermatogenesis.

Vaginal cytology evaluation of female cheetahs immediately following natural breeding revealed a typical feline estrus smear. Cytological monitoring of a hand-raised female showed cyclicity undetectable by the behavior of the female herself or males in adjoining enclosures. No evidence of vulvar swelling or discharge accompanied cytological estrus. These data further reinforced the theory that many females may be cycling without behavioral or pheromonal expression of estrus. Vaginal smears may be used to time introductions for natural breeding or to coordinate AI with physiological estrus. Vaginal cytology is currently being correlated with fecal hormone levels. The latter can be utilized to non-invasively monitor the estrous cycle of females not habituated to vaginal swabbing. The perfection of fecal analysis techniques will be hastened by the contribution of samples from known cyclic females from as many institutions as possible.

If vaginal cytology and/or fecal analysis fails to delineate an estrus cycle in an individual animal, more invasive analysis must be pushed including frequent serum collection, hormonal challenge and manipulation and laparoscopic examination of ovaries and reproductive tract.

The non-invasive techniques of semen collection and vaginal cytology, can be utilized by personnel at many institutions following some specialized training. Samples obtained may be analyzed at each institution or sent to a laboratory with a more experienced staff. Institutions interested in participating in semen evaluation or vaginal cytology studies can learn collection techniques at a workshop to be hosted by the San Diego Wild Animal Park's cheetah propagation group.

Prioritzed Reproductive Studies

Male
1. Non-invasive semen collection
2. Longevity in various extenders at several temps
3. AI with fresh semen
4. Cryopreservation
5. In vitro fertility assessment
6. In vitro fertilization

Female
1. Non-invasive cycle information
   vaginal cytology and/or fecal analysis
2. Timed breedings/AI
3. Serum hormone evaluation of acyclic individuals
4. Opportunistic GnRH challange of acyclic individuals
5. Hormonal induction of estrus and ovulation. Superovulation, embryo collection, culture, transfer and cryopreservation
7. In vitro fertilization