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Reproductive gonadal steroidogenic activity in the fishing cat (*Prionailurus viverrinus*) assessed by fecal steroid analyses

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ABSTRACT

Non-invasive fecal steroid analyses were used to characterize gonadal activity in the fishing cat (*Prionailurus viverrinus*). Estrogen, progestagen and androgen metabolites were quantified in fecal samples collected for 12 months from four males and 10 females housed at seven North American zoological institutions. Male reproductive hormone concentrations did not vary ($P > 0.05$) among season, and estrogen cycles were observed year-round in females and averaged (±SEM) 19.9 ± 1.0 days. Mean peak estrogen concentration during estrus (460.0 ± 72.6 ng/g feces) was five-fold higher than baseline (87.3 ± 14.0 ng/g feces). Five of seven females (71.4%) housed alone or with another female demonstrated spontaneous luteal activity (apparent ovulation without copulation), with mean progestagen concentration (20.3 ± 4.7 μg/g feces), increasing nearly five-fold above baseline (4.1 ± 0.8 μg/g feces). The non-pregnant luteal phase averaged 32.9 ± 2.5 days ($n = 13$). One female delivered kittens 70 days after natural mating with fecal progestagen concentrations averaging 51.2 ± 5.2 μg/g feces. Two additional females were administered exogenous gonadotropins (150 IU eCG; 100 IU hCG), which caused hyper-elevated concentrations of fecal estrogen and progestagen (plus ovulation). Results indicate that: (1) male and female fishing cats managed in North American zoos are reproductively active year round; (2) 71.4% of females experienced spontaneous ovulation; and (3) females are responsive to exogenous gonadotropins for ovulation induction, but a regimen that produces a normative ovarian steroidogenic response needs to be identified.

1. Introduction

The fishing cat (*Prionailurus viverrinus*), one of 37 distinct felid species, is a skillful swimmer that often dives underwater to catch fish. This cat has grey/brown fur with dark brown or black spots and black lines running up the forehead and over the head crown. The species is stout with females (6–7 kg) weighing notably less than males (10–12 kg). In nature, the fishing cat prefers tropical, broad-leaf forests associated with marshes, mangroves and swamps (Nowell and Jackson, 1996) where it consumes a variety of aquatic prey, including fish, crustaceans, mollusks, rodents, frogs and snakes. Historically believed to range throughout Southeast Asia, extending from Pakistan to peninsular Malaysia (Seidensticker and Lumpkin, 1991), there is limited information on the current status of wild fishing cats, although the species is believed to be in decline due to wetland destruction, water pollution, pesticide poisoning and hunting (Macdonald, 1950; Acharjyo and Misra, 1975; Nowell and Jackson, 1996). The species recently was upgraded from 'vulnerable' to 'endangered' on the

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International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (IUCN, 2008).

The North American *ex situ* fishing cat population consists of 15 unrelated founders and a total of 60 animals at 25 institutions with a population genetic diversity, which is the metric of genetic variation within a population, of 89.7%. All founders have surviving offspring in the population; however, genetic representation among founders is unequal and <20% of the recommended breeding pairs produce offspring each year (Swanson, 2007). With improved genetic management through natural breeding and/or assisted reproduction (which would ensure that each individual's genes are represented), genetic diversity could be increased to 95% (Swanson, 2007). This is important for reducing inbreeding over time, particularly in populations with limited opportunities for the addition of new genes (via new individuals). Establishing genetically healthy, self-sustaining *ex situ* populations can be difficult in felids due to behavioral incompatibility and lack of information on appropriate husbandry standards and basic species biology (Wildt et al., 2010). Besides breeding challenges, male fishing cats also have been known to attack females (Bill Swanson, personal communication), as occurs in *ex situ* collections of other rare felids (e.g., clouded leopard, *Neofelis nebulosa*; Howard et al., 1996; Pelican et al., 2006).

Captive management of any species benefits from understanding its basic reproductive biology, including endocrinology (Wildt et al., 2001, 2010). Such information is useful for enhancing husbandry and natural breeding or using assisted reproduction techniques to help maintain genetic diversity (Howard, 1999; Pope et al., 2006b). Using non-invasive fecal steroid monitoring (see review, Brown, 2006), estrous cycle length has been characterized in multiple felid species (range, 10–20 days). Some felid species are known to spontaneously ovulate, although most are believed to be induced ovulators (Brown, 2006). Additionally, season can influence reproduction as observed in the Pallas' cat (*Otocolobus manul*) and clouded leopard (Brown et al., 1995, 2002). However, there is a surprising lack of information on fishing cat reproduction. Behavioral observations of zoo-managed animals have suggested that the species is polyestrous (Roberts, 1997) with a gestation ranging from 63 (Nowak, 1999) to 70 days (Mellen, 1991). Births have occurred throughout the year in North America under various artificial lighting protocols (Swanson, 2007). However, in the coastal wetlands of northeastern India, peak mating activity occurs in January and February with births observed in March through May (Nowell and Jackson, 1996). Recently, fishing cat seminal characteristics have been characterized, resulting in the development of sperm cryopreservation and heterologous *in vitro* fertilization techniques (Thiangtum et al., 2006). Additionally, *in vitro* embryo development and transfer has been reported, but with poor success (Pope et al., 2006a).

The aim of the present study was to characterize gonadal steroidogenic activity in male and female fishing cats throughout the year using non-invasive fecal hormone monitoring. Specific objectives were to: (1) assess the influence of season on gonadal hormone metabolite patterns in both the male and female; (2) characterize fundamental reproductive cycles and pregnancy in the female; (3) determine the species 'ovulatory' type (induced versus spontaneous); and (4) evaluate an ovulation induction protocol that may be useful for developing assisted reproduction approaches that could contribute to genetic management of the *ex situ* population.

2. Materials and methods

2.1. Animals

Fourteen adult (1–12 years old) fishing cats (4 males, 10 females) were studied at seven zoological institutions in North America, including the Minnesota Zoological Garden (Apple Valley, MN; latitude 44.7°N; fishing cats studbook numbers 193, 303, 325, and 305), Cincinnati Zoo & Botanical Garden (Cincinnati, OH; latitude 39.2°N; studbook numbers 29 and 397), Sacramento Zoo (Sacramento, CA; latitude 38.6°N; studbook numbers 265 and 323), San Francisco Zoological Gardens (San Francisco, CA; latitude 37.8°N; studbook number 38), Happy Hollow Zoo (San Jose, CA; latitude 37.3°N; studbook numbers 268 and 400), Oklahoma City Zoological Park (Oklahoma City, OK; latitude 35.5°N; fishing cats studbook numbers 356 and 392), and San Antonio Zoological Gardens and Aquarium (San Antonio, TX; latitude 29.4°N; studbook number 53). All cats had been born in North American zoos and, with the exception of one male and one female, none had reproduced (Table 1). Animals were housed as singletons or in multiconspecific cat enclosures on or off public display (Table 1). All individuals were exposed to natural (outdoor) lighting. Enclosures contained one to three hiding places and some a water feature. Animals were provided water *ad libitum* and Toronto Carnivore and/or Nebraska Brand Feline commercial diet supplemented with bones and fish. Fresh fecal samples were collected from the floor of the enclosure or the ground throughout the study and stored at −20 °C in individual, labeled plastic bags for subsequent hormonal analyses of androgens in males and estrogens and progestagens in females.

2.2. Reproductive hormone patterns and seasonal influences

To characterize reproductive traits and seasonality, four male and seven female fishing cats were monitored by collecting feces five to seven times per week for 12–14 months. All animals were exposed to a natural outdoor photoperiod with two of four (50%) males and three of seven (42.9%) females also having artificial (indoor) lighting. Three of four males were housed as singletons with the remaining individual maintained during the mornings with a female and otherwise alone. Females were kept as singletons, except for two that were maintained with a female conspecific. Additionally, fecal hormones were monitored opportunistically during translocation of individuals into new exhibits and also during a veterinary procedure, providing the chance to investigate the effects of alteration in management practices on reproductive hormone patterns.

An additional 6-year-old female housed with a male became pregnant after copulations and, thus, provided data