Serum concentrations of oestradiol and progesterone and frequency of sexual behaviour during the normal oestrous cycle in the snow leopard (*Panthera uncia*)

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Serum oestradiol and progesterone concentrations were measured at weekly intervals for six months, and correlated with daily behavioural observations in two adult female snow leopards (*Panthera uncia*). Three oestradiol peaks ($> 21 \text{ pg ml}^{-1}$; interval 3.6 weeks) were identified in a snow leopardess housed alone (two more were probably missed because of the weekly sampling schedule), and three oestradiol peaks were identified in a snow leopardess housed with a male as a breeding pair (interval 6 weeks). Daily frequencies of feline reproductive behaviour averaged 1.77 observations per observation period during weeks of high oestradiol and 0.62 during weeks of low oestradiol. Progesterone concentrations did not rise above baseline values ($< 2 \text{ ng ml}^{-1}$) in the isolated animal, but 6 weeks of high progesterone concentrations ($4.9\text{--}38.8 \text{ ng ml}^{-1}$) was recorded in the paired snow leopardess following mating. No offspring were produced. Snow leopards were observed daily for an additional 4.5 years. Sexual behaviour peaks could be clearly identified from December through April, and average daily sexual behaviour scores were higher during these months than during the rest of the year. Intervals between sexual behaviour peaks for the isolated snow leopardess averaged 3.03 weeks. The sexual behaviour of the paired snow leopards decreased for 8–9 weeks following mating when no offspring were produced, and decreased for 13 weeks in one year when a single cub was born.

Introduction

Analyses of serum hormone concentrations from members of the Felidae have demonstrated a spectrum of patterns associated with their reproductive cycles. In domestic cats, seasonal, multiple oestrogen peaks occur which induce reproductive behaviour (Michael, 1961; Michael and Scott, 1964; Leyhausen, 1979) but these peaks are not followed by ovulation or progesterone secretion unless mating or direct multiple cervical stimulation occurs (Longley, 1910; Greulich, 1934; Concannon *et al.*, 1980; Wildt *et al.*, 1980). In lions, in contrast, progesterone secretion suggestive of ovulation was recorded after 6 of 8 oestrogen surges in females isolated from males, but housed together, and ovulation was recorded later in one isolated lioness (Schmidt *et al.*, 1979). Hormonal data collected from leopards in our laboratory suggest that female leopards will ovulate without cervical stimulation if housed together, but there was no evidence of ovulation in isolated females (Schmidt *et al.*, 1988). These data suggest that although some impulse may trigger ovulation, not all Felidae require cervical stimulation to induce ovulation. Reproductive behaviour in the absence of a male suggested that female leopards and female

lions in captivity cycled throughout the year (Schmidt *et al.*, 1979; Schmidt *et al.*, 1988).

Other female cats have not been studied in social situations. Hormonal data have been collected from a jaguar (Wildt *et al.*, 1979), isolated pumas (Bonney *et al.*, 1980) and isolated Siberian tigers (Seal *et al.*, 1985). In all these felids, oestrogen surges were accompanied by reproductive behaviour and none of the oestrogen surges was followed by progesterone concentrations suggestive of ovulation.

Information about the reproductive cycle of snow leopards is limited to observation of external events. Birth records and observed behavioural oestrous cycles strongly suggest that they are seasonal breeders (Freuh, 1968; Marma and Yunchis, 1968; Freeman, 1971, 1977, 1983; Kitchener *et al.*, 1975; Koivisto *et al.*, 1977; Rieger, 1982), which is logical considering their severe natural habitat. To our knowledge, serial oestradiol and progesterone concentrations have not been measured during phases of the normal reproductive cycle in this species.

This study was undertaken to provide information on serum oestradiol and progesterone concentrations in snow leopards housed as a breeding pair and in an isolated female during the normal cycle. We also attempted to correlate changes in reproductive behaviour with changes in hormone concentrations, and to monitor those reproductive behaviour patterns throughout

the year. We hoped to add information on the reproductive physiology and behaviour of this endangered species, and to use this information to manage our snow leopards more effectively for breeding.

Materials and Methods

Animals

Four adult snow leopards were observed over five years from 1985 to 1989. The three snow leopardesses were: Natasha, who was 8 years old at the start of the study and was nulliparous; Chuma, who was 8 years old and had had one litter of cubs; and Omaha 6 who was included in the final two years of the behavioural phase of this study. Omaha 6 was 7 years old and had previously had one litter of cubs. Piotr, the male snow leopard, was 11 years old at the start of the study, and had never sired cubs prior to the study, although he had been housed with three different females for breeding since 1978 and had been observed copulating with each of them. The intervals between breeding episodes were consistently 2 months, very similar to pseudopregnancy in the domestic cat (Paape *et al.*, 1975; Verhage *et al.*, 1976). All snow leopards were housed in the feline building of the Metro Washington Park Zoo in Portland, Oregon.

The breeding pair of snow leopards was housed together at all times, and was exhibited daily in an outdoor area measuring 12 m × 12 m. Natasha was exhibited daily in an outdoor area measuring 14 m × 7 m as an isolated female. The animals were fed in adjacent indoor areas measuring 3 m × 2 m. Commercial carnivore diet (Nebraska Brand Feline Diet, Animal Spectrum, Inc., Lincoln, Nebraska) was fed six times per week, bones were offered once per week. Water was available *ad libitum*.

Behaviour

Snow leopards were observed for 45 min in the outdoor area every day for the 5-year study except for the days on which serum samples were collected. Behaviour patterns were recorded in 1 min block periods. Each behaviour was scored by the number of blocks in which it occurred. These daily behavioural data were averaged for each week by summing the daily frequency of each behaviour for the 3 days before and after the day of serum collection, then dividing by the number of observation days for the week.

Daily frequencies of those behaviours generally agreed upon as characteristic feline reproductive behaviours (Cooper, 1942; Michael, 1961; Ulmer, 1966; Kleiman, 1974; Wildt *et al.*, 1978; Leyhausen, 1979; Schmidt *et al.*, 1979, 1988; Schille *et al.*, 1979; Seal *et al.*, 1985) were examined to determine which, if any, were consistently associated with hormonal oestrus in the snow leopards. These behaviours included: rolling, rubbing head, lordosis, being mounted, low growl, marking, urinating and calling (at night: from night keeper's reports). Average daily frequencies of behaviours were compared during weeks of increased oestradiol ($>21 \text{ pg ml}^{-1}$), and reduced oestradiol ($<21 \text{ pg ml}^{-1}$), concentrations. This value (21 pg ml^{-1}) was arbitrarily selected before behaviour analysis on the basis

of examination of the total data set and apparent grouping of values above and below this point. Blood samples were collected once per week for six months, and the serum was frozen at -20°C for later analyses. The animals were immobilized with ketamine HCl ($12\text{--}14 \text{ mg kg}^{-1}$) and xylazine ($0.5\text{--}1 \text{ mg kg}^{-1}$) by darting.

Hormone assays

Oestradiol and progesterone concentrations were measured as described previously (Schmidt *et al.*, 1979) using antisera purchased from Holly Hills Biologicals (Hillsboro, Oregon). The oestradiol antiserum was raised against 6-keto-oestradiol-17-6-oxime-BSA in rabbits and crossreacted 28% with oestrone, 3% with oestriol and $<1\%$ with common androgens, progestagens and corticoids. The progesterone antiserum was similarly raised against 11α -hydroxyprogesterone- 11α -hemisuccinate-BSA and crossreacted 2% with 17α - and 20α -hydroxyprogesterone, desoxy-corticosterone and $<1\%$ with other common androgens, oestrogens and corticoids. Chromatography (Resko *et al.*, 1975) on 1.0 g Sephadex LH-20 columns separated oestrone, oestradiol and progesterone from each other as well as from common androgens and corticoids before assay. Various aliquots of pooled snow leopard serum were assayed after chromatography and provided parallel displacement curves to the standards in both assays. Addition of $25 \text{ pg oestradiol ml}^{-1}$ and $12.5 \text{ ng progesterone ml}^{-1}$ to pools of snow leopard serum yielded recoveries of 23.6 pg ml^{-1} and 12.2 ng ml^{-1} after subtraction of pre-existing steroid concentration. Serum ($100\text{--}500 \mu\text{l}$) was extracted with freshly distilled diethyl ether and analysed for steroid content by radioimmunoassay after chromatographic purification. The values derived from the standard curve were corrected for reagent blanks and extraction/purification losses during chromatography. Average blank and recovery factors were determined in independent samples and were found to be $2.1 \pm 0.09 \text{ pg}$ and $72.3 \pm 1.4\%$ for the oestradiol assay and $26.5 \pm 4.2 \text{ pg}$ and $83 \pm 0.8\%$ for the progesterone assay, respectively. The limits of sample detection after correction were 2 pg per tube for oestradiol and $10\text{--}15 \text{ pg}$ per tube for progesterone. The intra- and interassay coefficients of variation for either assay did not exceed 12% as determined by repeated analysis of our standard quality control pools of rhesus monkey serum.

Results

Weekly oestradiol concentrations

During the 6 month study three oestradiol peaks $>21 \text{ pg ml}^{-1}$ suggestive of oestrus were recorded in the isolated snow leopard, Natasha (Fig. 1a) (range $33\text{--}60 \text{ pg ml}^{-1}$). Two more peaks (27 March 1985, 15 May 1985 Fig. 1a) were probably missed (note behavioural peaks) due to the weekly blood sampling schedule. Intervals between oestradiol peaks averaged 3.6 weeks. Three oestradiol peaks were recorded in Chuma (Fig. 1b). The interval between oestradiol peaks in the paired snow leopards where breeding was observed (Chuma) was 6 weeks. Because of the intermittent sampling schedule, true maximum concentrations were probably not recorded in many cases.

Fig. 1. Weekly oestradiol ($\square$ — $\square$), and progesterone ($\triangle$ — $\triangle$) concentrations and average daily observed frequency of feline sexual behaviour scores ($\bullet$ — $\bullet$) for snow leopards (a) Natasha (housed alone), and (b) Chuma (housed with Piotr as a breeding pair) during 1985.

Weekly progesterone concentrations

One period of notable progesterone secretion was recorded in Chuma, after mating, which lasted for 6 weeks. Progesterone values ranged from 4.93 to 38.81 ng ml⁻¹. Progesterone remained at baseline values (< 2 ng ml⁻¹) for the isolated snow leopardess, Natasha.

Behaviour

The mean daily frequencies of behaviours commonly associated with oestrus in cats were compared during weeks of increased and reduced serum oestradiol concentrations. Several feline reproductive behaviours tended to increase as oestradiol increased, and these behaviours together gave a good indication of oestrus. The most reliable single behavioural indicator of oestrus, particularly in isolated animals, was calling.

The mean daily observed frequency of sexual behaviour was averaged by month to determine the seasonality of sexual behaviour in our snow leopards (Table 1). Sexual behaviour appears clearly seasonal in these cats with peak activity in December–April.

Intervals between sexual behaviour peaks in the isolated snow leopardess averaged 3.03 (range 2–6) weeks for the 5

Table 1. Summary of average daily observed sexual behaviour scores for each month, showing seasonality of sexual behaviour in two snow leopardesses, Natasha (housed alone) and Chuma (housed with a male, and mated by him in March of each year)

Month	Snow leopard	
	Natasha	Chuma
January	1.39 (0–10)	1.08 (0–2.5)
February	1.55 (0–5.3)	0.91 (0–2.33)
March	1.50 (0–6.6)	1.66 (0–6.17)
April	1.23 (0–3.2)	0.17 (0–0.8)
May	0.66 (0–1.2)	0.34 (0–0.83)
June	0.44 (0–1.5)	0.29 (0–1.5)
July	0.52 (0–1.25)	0.68 (0–1.67)
August	0.49 (0–3.33)	0.53 (0–1.6)
September	0.46 (0–1.5)	0.43 (0–1)
October	0.32 (0–1.33)	0.62 (0–2.5)
November	0.76 (0–4.5)	0.54 (0–2)
December	1.40 (0–3.67)	0.32 (0–5)

Daily observations were made over five years. Figures in parentheses are ranges.

years (Fig. 1a). For paired snow leopards, a marked decrease in reproductive behaviour for 8 weeks followed mating in 1985 (Fig. 1b); similar patterns in behaviour occurred following matings in 1986, 1988 and 1989, and were similar to the pattern of breeding activity at 9 week intervals previously (1980–1984) recorded with Piotr and Natasha. In 1987, 13 weeks of decreased reproductive behaviour followed mating. At the end of that time the female, Chuma, produced a single kitten.

Discussion

The oestradiol pattern shown by the snow leopards in this study is similar to patterns determined for other Felidae. In the follicular phase of the oestrous cycle, oestradiol secretion increased for brief periods then fell rapidly to baseline values. Oestradiol concentrations during these increases ranged from 26 to 66 pg ml⁻¹ (from a baseline of < 20 pg ml⁻¹). These values compared with peak concentrations of 46.7 pg ml⁻¹ for Siberian tigers (Seal *et al.*, 1985), 19–108 pg ml⁻¹ for lions (Schmidt *et al.*, 1979), 65.8 pg ml⁻¹ for leopards (Schmidt *et al.*, 1988), 30–375 pg ml⁻¹ for pumas (Bonney *et al.*, 1980) and 58 pg ml⁻¹ for domestic cats (Verhage *et al.*, 1976). Intervals between peaks with no measurable progesterone increase (3.6 weeks) were similar to those of leopards (3 weeks: Eaton, 1977; 3.4 weeks: Schmidt *et al.*, 1988) and domestic cats (2–3 weeks: Paape *et al.*, 1975; Wildt *et al.*, 1978). The interval between oestradiol peaks for the cycle with a luteal phase but no production of young was 6 weeks, which was similar to that of the leopard (7.3 weeks: Schmidt *et al.*, 1988) and to pseudo-pregnancy in domestic cats (6 weeks: Paape *et al.*, 1975; Verhage *et al.*, 1976).

One period of progesterone secretion was observed in mated snow leopards during our study. That luteal phase lasted for 6

weeks, similar to the non-fertile progesterone surge described for leopards (5 weeks: Schmidt *et al.*, 1988) and pseudopregnancy as described in domestic cats (6 weeks: Paape *et al.*, 1975; Verhage *et al.*, 1976). We suspected that our male, Piotr, had low fertility and was causing a pseudopregnancy-like condition in our female snow leopards because, although he mated with the females, they came back into behavioural oestrus 8–9 weeks later, and never produced cubs. Progesterone concentrations during this luteal phase ($4.9\text{--}38.8\text{ ng ml}^{-1}$) were lower than those described for leopards ($13\text{--}98\text{ ng ml}^{-1}$: Schmidt *et al.*, 1988), but similar to those described for domestic cats (peak 25.8 ng ml^{-1} : Paape *et al.*, 1975; peak 34.9 ng ml^{-1} : Verhage *et al.*, 1976). We conclude that this period of progesterone secretion is induced by a non-fertile mating, similar to the pseudopregnant cycle of domestic cats.

Increases in sexual behaviour were observed 2–4 weeks apart when mating did not occur. After matings when no cubs were produced, intervals between peaks increased to 7–8 weeks. The mating in 1987 that did result in pregnancy and produced a cub was associated with a decrease in sexual behaviour for 13 weeks (data not shown). There was no significant difference in sexual behaviour intensity or intervals between peaks irrespective of whether animals were being immobilized for weekly collection of blood samples.

The behaviours that were associated with increased oestrogen concentrations in our snow leopards were similar to those described for domestic cats (Michael, 1961; Leyhausen, 1979) and other snow leopards (Marma and Yunchis, 1968; Koivisto *et al.*, 1977; Doherty and Wharton, 1986).

In our laboratory, as well as in the wild (Jackson and Ahlborn, 1986) and other captive situations (Blomqvist and Stern, 1982; Peters, 1982) isolated snow leopards call with increased frequency and intensity during oestrus than at other times. C. R. Lewis used this information to develop a method for timing the introduction of isolated animals for mating on the basis of calling patterns in the male (who begins calling first) and female which has led to the production of several litters of cubs.

During the five years of this study a seasonal pattern to reproductive behaviour with sexual behaviour peaks late and early in the year was clearly demonstrated and summer was a time of low activity. In our paired snow leopards, although increases in both sexual and social behaviours were noted during winter, mating was observed only once or twice per year during late winter to early spring. Perhaps the increases in reproductive behaviours late in the year represent a transitional period, where follicles form and regress, but do not mature until later in the season. This pattern of mating in snow leopards in the first months of the year has been well described in captivity (Freeman, 1971, 1977; Kitchener *et al.*, 1975; Blomqvist *et al.*, 1982; Rieger, 1984; Ruedi, 1984; Doherty and Wharton, 1986) and in the wild (Rieger, 1984; Jackson and Ahlborn, 1986).

On the basis of these data, the hormonal and behavioural reproductive biology of our snow leopards was similar to that of domestic cats in having seasonal multiple oestrogen and sexual behaviour peaks which, at least in the isolated snow leopard, were not followed by progesterone secretion suggestive of ovulation. After mating, progesterone concentrations suggestive of ovulation were observed for six weeks where no cubs were produced; this is similar to pseudopregnancy in domestic cats.

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