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 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 ng ml^{-1}) in the isolated animal, but 6 weeks of high progesterone concentrations (4.9–38.8 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 pg ml^{-1}), and reduced oestradiol (< 21 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 (12–14 mg kg^{-1}) and xylaxine (0.5–1 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 as 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 pg oestradiol ml^{-1} and 12.5 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 (100–500 µl) 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 ± 0.09 pg and 72.3 ± 1.4% for the oestradiol assay and 26.5 ± 4.2 pg and 83 ± 0.8% for the progesterone assay, respectively. The limits of sample detection after correction were 2 pg per tube for oestradiol and 10–15 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 pg ml^{-1} suggestive of oestrus were recorded in the isolated snow leopard, Natasha (Fig. 1a) (range 33–60 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.
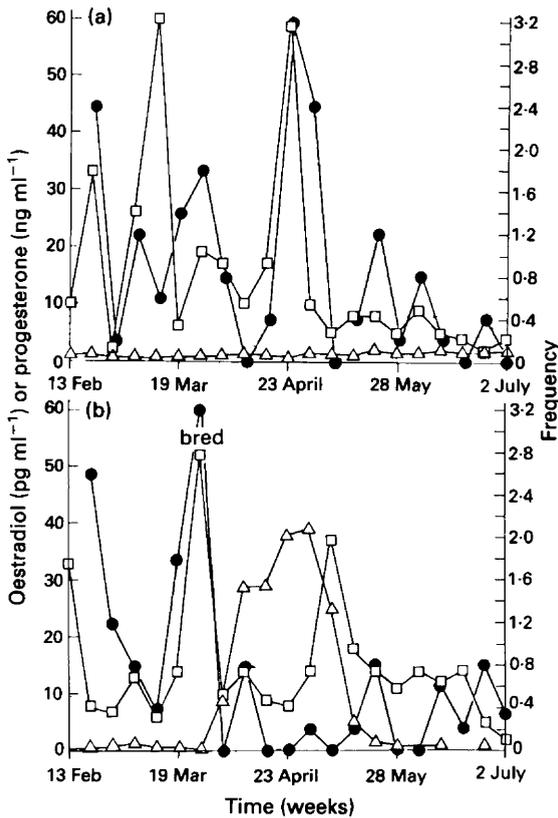
Fig. 1. Weekly oestradiol (□—□), and progesterone (△—△) concentrations and average daily observed frequency of feline sexual behaviour scores (●—●) for snow leopards (a) Natasha (housed alone), and (b) Chuma (housed with Piotr as a breeding pair) during 1985.

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)

| | Snow leopard | |
Month	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.

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

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 pseudopregnancy 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 pseudo-pregnancy 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 (4.9–38.8 ng ml^{-1}) were lower than those described for leopards (13–98 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.

References

Blomqvist L and Stern I (1982) Reproductive biology of the snow leopard *International Pedigree Book of Snow Leopards* **3** 71–79

Bonney RC, Moore HDM and Jones DM (1980) Plasma concentrations of oestradiol-17β and progesterone, and laparoscopic observations of the ovary in the puma (*Felis concolor*) during oestrus, pseudopregnancy and pregnancy *Journal of Reproduction and Fertility* **63** 523–531

Concannon P, Hodgson B and Lein D (1980) Reflex LH release in estrous cats following single and multiple copulations *Biology of Reproduction* **23** 111–117

Cooper JB (1942) An exploratory study of African lions *Comparative Psychology Monographs* **16** 1–49

Doherty J and Wharton D (1986) Breeding management of the snow leopard at the New York Zoological Park *Proceedings of the Fifth International Snow Leopard Symposium*, pp 173–179 Ed. H Freeman. Conway Printers Private Ltd, Bombay

Eaton RL (1977) Reproductive biology of the leopard *Zoologische Garten* **47** 329–351

Freeman HE (1971) A preliminary study of the behaviour of captive snow leopards (*Panthera uncia*) *International Zoo Yearbook* **11** 217

Freeman H (1977) Breeding and behavior of the snow leopard *The World's Cats* **3** 36–44

Freeman H (1983) Behavior in adult pairs of captive snow leopards (*Panthera uncia*) *Zoo Biology* **2** 1–22

Freuh RJ (1968) A note on breeding snow leopards (*Panthera uncia*) at St Louis Zoo *International Zoo Yearbook* **8** 74–76

Greulich WW (1934) Artificially induced ovulation in the cat (*Felis domestica*) *Anatomical Record* **58** 217–222

Jackson RM and Ahlborn GG (1986) Observations on the ecology of snow leopard in West Nepal *Proceedings of the Fifth International Snow Leopard Symposium*, pp 65–87 Ed. H Freeman. Conway Printers Private Ltd, Bombay

Kitchener SL, Meritt DA and Rosenthal MA (1975) Observations on the breeding and husbandry of snow leopards (*Panthera uncia*) at Lincoln Park Zoo, Chicago *International Zoo Yearbook* **15** 212–217

Kleiman DG (1974) The estrous cycle in the tiger (*Panthera tigris*) *The World's Cats* **2** 60–75

Koivisto I, Wahlberg C and Muuronen P (1977) Breeding the snow leopard (*Panthera uncia*) at Helsinki Zoo 1967–1976 *International Zoo Yearbook* **17** 39–44

Leyhausen P (1979) *Cat Behavior* Garland STPM Press, New York

Longley WH (1910) Factors which influence the maturation of the egg and ovulation in the domestic cat *Science* **31** 465–466

Marma BB and Yunchis VV (1968) Observations on the breeding, management and physiology of snow leopards (*Panthera uncia*) at Kaunas Zoo from 1962–1967 *International Zoo Yearbook* **8** 66–74

Michael RP (1961) Observation upon the sexual behaviour of the domestic cat (*Felis cattus* L.) under laboratory conditions *Behaviour* **18** 1–24

Michael RP and Scott PP (1964) The activation of sexual behaviour in cats by the subcutaneous administration of oestrogen *Journal of Physiology* **171** 254–274

Paape SR, Shille VM, Seto H and Stabenfeldt GH (1975) Luteal activity in the pseudopregnant cat *Biology of Reproduction* **13** 470–474

Peters G (1982) The vocal repertoire of the snow leopard (*Uncia uncia* [Schreber 1775]) *International Pedigree Book of Snow Leopards* **2** 137–158

Rieger I (1982) Breeding Ounces, *Panthera uncia* (Schreber 1775) in Zoological Gardens *International Pedigree Book of Snow Leopards* **3** 49–50

Rieger I (1984) Oestrous timing in ounces, *Panthera uncia*, Schreber (1775) *International Pedigree Book of Snow Leopards* **4** 99–103

Resko JA, Ploeom JG and Stadelman JL (1975) Estrogens in fetal and maternal plasma of the rhesus monkey *Endocrinology* **97** 425–430

Ruedi D (1984) Zoo Basel – the first breeding success with snow leopards *International Pedigree Book of Snow Leopards* **4** 73–74

Schmidt AM, Nadal LA, Schmidt MJ and Beamer NB (1979) Serum concentrations of oestradiol and progesterone during the normal oestrous cycle and early pregnancy in the lion (*Panthera leo*) *Journal of Reproduction and Fertility* **57** 267–272

Schmidt AM, Hess DL, Schmidt MJ, Smith RC and Lewis CR (1988) Serum concentrations of oestradiol and progesterone and sexual behaviour during the normal oestrous cycle in the leopard (*Panthera pardus*) *Journal of Reproduction and Fertility* **82** 43–49

Seal US, Plotka ED, Smith JD, Wright FJ, Reindl MJ, Taylor RS and Seal MF (1985) Immunoreactive luteinizing hormone, estradiol, progesterone, testosterone and androstenedione levels during the breeding season and anestrus in Siberian tigers *Biology of Reproduction* **32** 361–368

Shille VM, Lundstrom KE and Stabenfeldt GH (1979) Follicular function in the domestic cat as determined by estradiol-17β concentrations in plasma: relation to estrous behaviour and cornification of exfoliated vaginal epithelium *Biology of Reproduction* **21** 953–963

Ulmer FA (1966) Voices of the Felidae *International Zoo Yearbook* **6** 259–262

Verhage JF, Beamer NB and Brenner RM (1976) Plasma levels of estradiol and progesterone in the cat during polyestrus, pregnancy and pseudopregnancy *Biology of Reproduction* **14**, 579–585

Wildt DE, Guthrie SC and Seager SWJ (1978) Ovarian and behavioral cyclicity of the laboratory maintained cat *Hormones and Behavior* **10** 251–257

Wildt DE, Platz CC, Chakraborty PK and Seager SWJ (1979) Oestrus and ovarian activity in a female jaguar (*Panthera onca*) *Journal of Reproduction and Fertility* **56** 555–558

Wildt DE, Seager SWJ and Chakraborty PK (1980) Effect of copulatory stimuli on incidence of ovulation on serum luteinizing hormone in the cat *Endocrinology* **107** 1212–1217