

# Puberty and oestral cycle length in captive female jaguars *Panthera onca*

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Habitat loss and fragmentation have been leading jaguars to constant conflicts with humans, and as a result, jaguar populations have been declining over the last decades. Captive breeding is often a tool for species conservation, and it is not different for jaguars. However, success is far from optimal due to the lack of basic knowledge about species' reproductive biology. In the present study, we assessed gonadal hormonal profiles of natural oestral cycles and puberty and compared our data to those of other wild felids. We collected faecal samples from two to seven times per week for 18 months from two adults and three pre-pubertal females. We defined baseline levels for progestins and oestrogens in order to estimate oestrous cycle length and age at puberty. We compared our data with 16 other species through generalized linear model, using weight and genus as two explanatory variables. Cycle length was  $38.28 \pm 2.52$  days, ranging from 25 to 44 days, while sexual maturity was attained within 22 months. Due to our analysis of both hormonal and behavioural data, there is a variation between this research from other studies that employed only behavioural observations. Such difference may be caused by the absence of behavioural oestrous at the peri-pubertal period. When compared to wild felids of similar size, puberty and oestral cycle durations of the jaguar females fell within the same range. Our modelling showed that age at maturity was influenced mostly by size and only *Leopardus* partially explained the observed variation. Conversely, oestral cycle length did not differ among genera or size categories. Our study adds to the body literature in the reproductive endocrinology of wild felids, and because female gametes are more challenging to collect and preserve, a strong understanding on the female reproductive physiology is essential to assisted reproduction and wild population viability assessment.

**Key words:** Felids, maturity, non-invasive, oestrogens, progestins, reproduction

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## Introduction

Felids are considered hypercarnivores, which are animals that present morphological specializations for a diet composed

almost exclusively of flesh. Big cats generally require substantial amounts of food and also large home ranges (Loveridge *et al.*, 2010). Species with these characteristics are often considered as top predators, regulating prey population and

providing essential ecosystem services (Treves & Karanth, 2003). However, deforestation often leads to increased contact with humans, converging to negative interactions and, together with illegal poaching, there is a worldwide trend in felid population decline (Wildt *et al.*, 1979; Lasley *et al.*, 1994; Loveridge *et al.*, 2010; Quigley *et al.*, 2018).

The most successful conservation strategies acknowledge the importance of reproductive physiology parameters (Cooke *et al.*, 2013). Understanding the reproductive processes under natural conditions (*in situ* populations) provides a guideline for *ex situ* conservation efforts. However, captive populations provide a more refined assessment of reproductive parameters and, consequently, the development of assisted reproductive techniques. Such knowledge is vital for the maintenance of viable and healthy wild populations (Cooke *et al.*, 2010).

The success in applying assisted reproductive technologies is <10% in most felids (Brown *et al.*, 1996). Much of it is due to the lack of knowledge about fundamental aspects of their reproductive biology so that we are unable to mimic or recognize natural requirements. In this sense, ovarian steroid profiles are still unknown for half of the felid species (Brown, 2011). The ovarian activity has two main aspects: when the activity begins (puberty), and the oestrus cycle length. For most of the felid species studied so far, age at maturity is of 1 to 2 years (Schmidt *et al.*, 1979, 1988; Bonney *et al.*, 1981; Santiapillai *et al.*, 1982; Bernard and Stuart, 1987; Graham *et al.*, 1995; Eisenberg and Redford, 1999; Brown, 2011; Barnes *et al.*, 2016), except for the tiger *Panthera tigris* (Nowell and Jackson, 1996; Graham *et al.*, 2006) that reaches maturity around 4 years of age.

As for oestrus cycle duration, it varies across genera, being as short as 7 days in the cheetah, *Acinonyx jubatus* (Graham *et al.*, 1995; Brown, 2011), and as long as 56 days in the lioness, *Panthera leo* (Schmidt *et al.*, 1979; Graham *et al.*, 1995). There are also differences regarding methods, and behavioural data usually provides a lengthier oestrus cycle than studies based on hormonal profiles (e.g. Tewes *et al.*, 1987).

The jaguar (*Panthera onca*) is the largest South American cat, and although charismatic, it is considered as the carnivore least compatible with anthropic reality (Cavalcanti *et al.*, 2010). Overall, there is a decreasing trend in this species' populations in several countries (de la Torre *et al.*, 2017), with a boost in captive breeding programs. Wildt *et al.* (1979) conducted one of the earliest studies on the reproductive physiology of jaguars 40 years ago. However, ovulation in the referred females was artificially induced, and few studies to date have tried to assess naturally occurring oestrus cycle, and none have addressed puberty (e.g. Barnes *et al.*, 2016).

Therefore, the present study provides, for the first time, the hormonal profiles of naturally occurring oestrus cycles, aiming to contribute to the knowledge on ovarian function

and sexual maturation in female jaguars. We hypothesize that sexual maturity will fall within the known range described for felids (1–2 years old). Considering oestrus cycle length, we believe that its duration will be shorter compared to studies reporting behavioural oestrus. Since it varied greatly, we predict that the observed range will either be closer to its sister-species (21–56 days, mean 33) or comparable with cats of similar size (7–56 days, mean 29).

## Material and methods

### Humane care guidelines

The present research agrees with Ethical Principles in Animal Research adopted by the Bioethics Commission of the School of Veterinary Medicine and Animal Science of the University of São Paulo under the protocol number 1425/2008.

### Subjects

We assessed five female jaguars, two adults (AA01 and AA02) and three pre-pubertal individuals (PP01, PP02 and PP03). Of the three pre-pubertal cats, two were sisters (PP01 and PP02). Adult females and PP03 were housed individually, while the sisters were housed together. However, the sisters slept in separate cages, allowing sample collection in the following morning without cross contamination. The estimated age, stimulatory cues (direct or indirect) received from conspecific, origin and holding institution are shown in Table 1.

All animals were exposed to natural climatic conditions (photoperiod, temperature, etc.). Water was provided *ad libitum*, and the diet was offered in the afternoon according to the institution's routine.

**Table 1:** List of the animals' identification, their estimated age, exteroceptive cues available to females (indirect—only visual, olfactory and auditory stimuli; direct—all plus tactile stimulus), their origin and current captive colony location

Animal	Age	Exteroceptive cues	Captive colony
AA01	>24 months	Indirect with unrelated males	FPZSP <sup>1</sup>
AA02	>24 months	Indirect with unrelated males	RioZoo <sup>2</sup>
PP01	11 months	Direct with PP brother	PZMQB <sup>3</sup>
PP02	11 months	Direct with PP brother	PZMQB <sup>3</sup>
PP03	15 months	Indirect with PP brother	CCFS <sup>4</sup>

Age was estimated according to Cavalcanti and Gese (2010) <sup>1</sup>Fundação Parque Zoológico de São Paulo (São Paulo, Brazil); <sup>2</sup>Fundação RioZoo (Rio de Janeiro, Brazil); <sup>3</sup>Parque Zoológico Municipal Quinzinho de Barros (Sorocaba, Brazil); <sup>4</sup>Centro de Conservação da Fauna Silvestre Ilha Solteira (São Paulo, Brazil)

## Sample collection and RIA analysis

We collected 953 faecal samples from all females two to seven times per week for 18 months (from January 1998 to August 1999). Because there were periods of intermittence, some of the cycles detected were incomplete and, therefore, precise cycle length was not always possible.

Only fresh samples and those that were not cross-contaminated between individuals were collected and stored at  $-20^{\circ}\text{C}$  until hormonal extraction. Extraction of faecal oestrogen and progesterone metabolites was performed using the protocol previously reported by Brown *et al.* (1994). Briefly, we added 90% ethanol to 0.2 g of dry faeces, which was incubated at  $90^{\circ}\text{C}$  for 25 min after boiling, refilling the evaporated ethanol to initial volume. Samples were homogenized and centrifuged for 15 min, with the supernatant transferred to a new test tube. The remaining pellet was resuspended in 5 mL 90% ethanol and centrifuged, with the two supernatants combined in the same test tube. The tubes were dried and reconstituted with 1 mL of 100% methanol, followed by an ultrasonic bath for 15 min, and then stored at  $-20^{\circ}\text{C}$  until analysis.

Analyses of gonadal hormone metabolites were performed using radioimmunoassay utilizing a commercial kit (Coat A Count, Diagnostic Products Corporation, Los Angeles, CA, USA) that had been previously validated for the jaguar (Viau *et al.*, 2005). Because it was published in Portuguese, we will provide the basic values necessary for validation. Serial dilutions of pooled faecal extracts paralleled to the standard curve ( $r=0.96$ ,  $P<0.001$  for progestins, and  $r=0.97$ ,  $P<0.001$  for oestrogens). Intra- and inter-assay coefficient variations were  $<13\%$  and  $<2.1\%$ , respectively. Assay sensitivity was 1.47 pg/mL for oestrogens and 0.005 ng/mL for progestins.

## Steroid stability over time

In order to assess whether progesterone metabolites would remain stable over time and to provide a safer, cheaper and eco-friendlier technique, we selected a subset of 20 samples and conducted an enzyme immunoassay (EIA) after 8 years of storage. According to Kumar and Umaphathy (2019), RIA employs radioactive material and waste and costly equipment.

For EIA, extraction followed and Illera *et al.* (2003). The assay used progesterone antibody CL425 and progesterone-HRP conjugate (Coralie Munro, UC Davis, California), and we also validated the assay through parallelism ( $r=0.98$ ,  $P<0.001$ ). The assay sensitivity was 0.14 pg/well, and all coefficients of variation were below 10%.

## Oestral cycle length

We considered peaks when oestrogen values exceed the mean  $\pm 1.5$  SD the baseline for three consecutive days. We defined the length of the oestral cycle based on the time between two consecutive oestrogen peaks. As stated previously, the interment sampling routine did not allow for

precise cycle duration and, thus, we only included cycles whenever they were complete.

To facilitate visualization, we aligned cycles assigning the first day that we detected baseline oestrogen levels as day zero (D0) and the preceding period as oestrus phase. Conversely, the following days were considered as inter-oestrus.

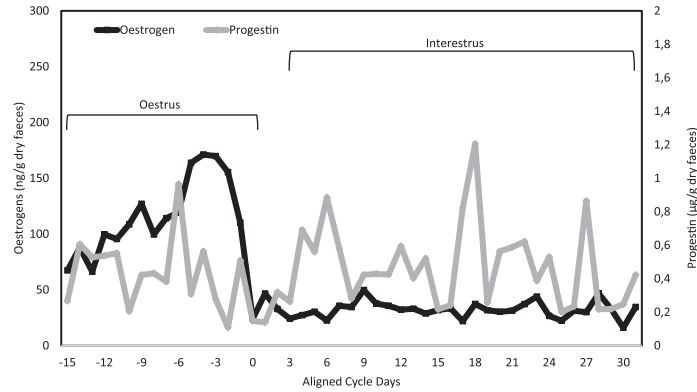
## Basal levels and oestral cycle definition

We defined baseline values of oestrogen by applying successive mean recalculations on only adult females. Whenever the values of the faecal metabolites exceeded the mean  $\pm 1.5$  SD, we excluded those values (Brown *et al.*, 1996) and calculated new means. After all the recalculations, the oestrogen baseline value was  $31.06 \pm 1.3$  ng/g dry faeces for AA01 and  $24.35 \pm 2.68$  ng/g dry faeces for AA02. For progesterone evaluation, we adopted the same procedure, although considering the mean  $\pm 2.0$  SD (Brown *et al.*, 1996). Progesterone baseline levels were  $0.10 \pm 0.003$   $\mu\text{g/g}$  dry faeces for AA01 and  $0.19 \pm 0.020$   $\mu\text{g/g}$  dry faeces for AA02. Because progesterone values oscillated around the basal values, we adopted oestrogen baseline levels as threshold to define oestrus and inter-oestrus. As most measurements from pre-pubertal females remained at the baseline level, the onset of ovarian activity was detected once oestrogen levels surpassed the mean  $\pm 1.5$  SD (Brown *et al.*, 1996).

## Statistical analysis

We analysed data using the SAS System for Windows software (SAS Institute Inc, Cary, NC, USA, 2000) and R (2019). Variables were tested for normality by Kolmogorov–Smirnov, and we employed Bartlett test for homogeneity of variances. Our data did not meet the premises for parametric tests, so we used Wilcoxon tests to compare groups (Shille *et al.*, 1983). All data referring to the hormonal concentrations were expressed as means  $\pm$  standard error of the mean.

In order to compare with other felids, we gathered available information on the same reproductive parameters (age at maturity—AgM—and oestral cycle length—ECL). Only 10 genera had available information on these variables, a reduced subset for proper statistical analysis. Regardless, this dataset and its residuals were normally distributed, allowing the use of Pearson's correlation to understand if weight and maturity, and weight and oestral cycle length, were correlated. We also employed one-way ANOVA and Tukey test *post hoc* to separate AgM and ECL mean values among groups. 'Groups' were genus and weight category (small  $<6.5$  kg, medium up to 20 kg and large above 20 kg, following Nowell and Jackson, 1996). Finally, we tested if AgM and ECL variation was explained by size category and genus with linear regression. Because some genera were underrepresented, we excluded those with less than three studies, namely *Caracal*, *Lynx*, *Otocolobus* and *Prionailurus*, from genus comparison, but not from size comparison. However, it is important to emphasize that this approach does not consider that some



**Figure 1:** Mean values of faecal oestrogen and progestins from seven oestral cycles of adult female jaguars (*Panthera onca*,  $n = 2$ ), discriminating oestrus and inter-oestrus phases (D0 = first basal value of faecal oestrogens).

**Table 2:** Basal and peak values of oestrogen (ng/g dry faeces) and progestins (µg/g dry faeces) for each adult female

Individual	Oestrogen (ng/g dry faeces)		Progestin (µg/g dry faeces)
	Basal	Peak	Basal
AA01 ( $n = 380$ )	31.06 ( $\pm 1.3$ )	120.34 ( $\pm 11.82$ )	0.44 ( $\pm 0.25$ )
AA02 ( $n = 129$ )	24.35 ( $\pm 2.68$ )	174.13 ( $\pm 18.70$ )	1.65 ( $\pm 0.31$ )

**Table 3:** Mean and range values of oestrogen (ng/g dry faeces) and progestins (µg/g dry faeces) for each group

Group	Oestrogen (ng/g dry faeces)		Progestin (µg/g dry faeces)	
	Mean	Range	Mean	Range
Adults ( $n = 509$ )	68.99 ( $\pm 6.55$ )	3.50–609.37	0.85 ( $\pm 0.09$ )	0.08–6.51
Pre-pubertals ( $n = 262$ )	10.97 ( $\pm 0.91$ )	0.28–59.16	0.26 ( $\pm 0.04$ )	0.02–4.44

genera may be more closely related than others. We could not assess the interaction of weight and genus due to our reduced sample size.

## Results

### Adult females

Based on seven oestral cycles from the two adult females, cycle length was  $38.28 \pm 2.52$  days, ranging from 25 to 44 days (Fig. 1). From a single animal, five complete cycles (AA01) were detected, and the cycle length was  $36.20 \pm 3.09$  days, ranging from 25 to 41 days. As for the AA02, two complete oestral cycles were detected, with a duration of  $43.50 \pm 0.5$  days.

The oestrus phase had an average duration of  $10.42 \pm 1.15$  days (ranging from 7 to 15), and the inter-oestrus phase lasted for  $\sim 28 \pm 1.43$  days (ranging from 28 to 31 days). The values for each adult female are provided in Table 2, while the mean

values for adult and pre-pubertal females are included in Table 3.

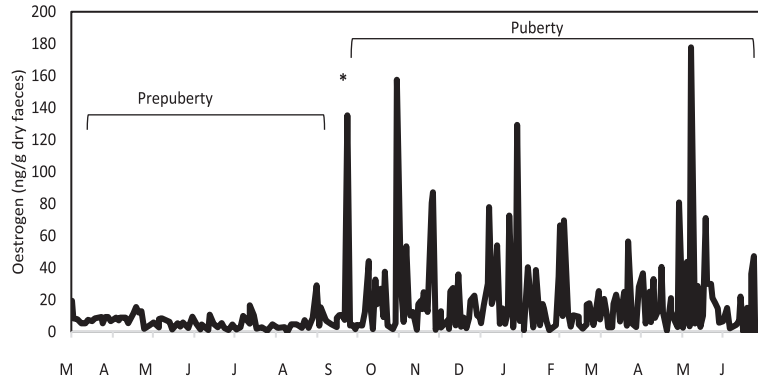
### Pre-pubertal females

The female PP03 was considered as a model for the other pre-pubescent females (PP01 and PP02) since it was possible to clearly detect the differences between pre-pubertal and pubertal periods (Figs 2 and 3; Table 4). All animals entered the peri-pubertal phase with an approximate age of 20 months (18–20 months of age).

Wilcoxon rank-test showed that there was a significant difference between pre-pubertal and adult females concerning both progestin and oestrogen levels. However, assessing pre-pubertal females only, oestrogen levels differed from pre-puberty and puberty phases, but not progestins.

### Steroid stability: RIA and EIA comparison

Comparing RIA results obtained from samples assayed 8 years apart, both results showed a significant positive



**Figure 2:** Longitudinal profile of faecal oestrogens for a pre-pubertal female (PP03) jaguar (*Panthera onca*). Asterisk refers to the onset of ovarian activity.

**Table 4:** Mean and range values of oestrogen (ng/g dry faeces) and progestins (µg/g dry faeces) for each maturation stage from female PP03

Maturation stage	Oestrogen (ng/g dry faeces)		Progestin (µg/g dry faeces)	
	Mean	Range	Mean	Range
Pre-pubescent (n = 109)	6.11 (±0.41)	0.28–29.99	0.10 (±0.003)	0.02–0.23
Pubescent (n = 165)	20.21 (±2.15)*	0.80–177.09	0.19 (±0.020)*	0.01–1.56

\*P < 0.05 (statistical difference between pre-pubertal and pubertal stages).

correlation ( $r = 0.98$ ;  $P < 0.001$ ). All the patterns (oestral cycle duration, puberty, as well as progestin and oestrogen profiles) demonstrated above through RIA were recovered using EIA.

### Comparison with other felids

Our survey showed that there is reproductive information for only 10 felid genera (Table 5). There was a strong correlation between AgM and size ( $P < 0.005$ ) and between ECL and size ( $P < 0.04$ ). Our GLM showed that only *Leopardus* genus had a positive influence on AgM ( $P < 0.05$ ). However, considering ECL, there was no effect either from genera or size. Finally, one-way ANOVA and Tukey *post hoc* test detected differences in AgM among size categories (large differentiates from both medium and small sized cats). Only two genera differed in AgM, namely *Leopardus* and *Panthera*. Conversely, ECL were statistically equal among genus and size categories. Hence, AgM is mostly influenced by weight and genus, while ECL is fairly constant among taxa and sizes.

## Discussion

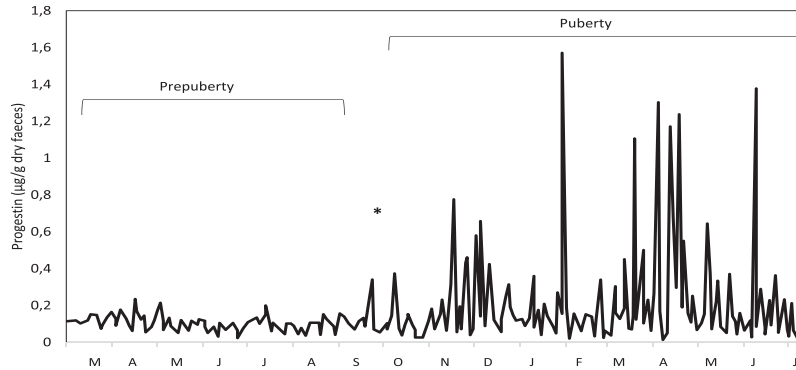
### Oestral cycle length and oestrus duration

The cycle duration of seven natural cycles from two adult jaguars was  $38.28 \pm 2.52$  days. This duration differs slightly from other studies. For instance, Wildt *et al.* (1979) estimated

an oestral cycle of  $47.2 \pm 5.4$  days. The referred study assessed only one female, associating behavioural observations with artificially induced ovulation protocols. As for studies based solely on behavioural observations, the cycle was longer, which lasted up to 65 days (Wildt *et al.*, 1979; Tewes *et al.*, 1987; Sadleir, 1996). By assessing oestrus duration, the work of Barnes *et al.* (2016), provided data for seven adult females in different reproductive situations. The females considered as a control in the referred study resembled the husbandry protocol applied in our study. The duration of oestrus found in our study was  $10.42 \pm 1.15$  days, while those females were of  $5.5 \pm 0.4$  days. Similarly, based on behavioural observations of four natural reproductive cycles, oestrous duration was 8 to 10 days (Jorge-Neto *et al.*, 2010). It is important to emphasize that unbalance in either behaviour or hormonal regulation may lead to reproductive failure, and therefore, both need to be synchronized and working adequately.

In our study, adult females were stimulated only indirectly by males (without physical contact). It seems that this situation did not suppress follicular growth since there was oestrogen fluctuation (Shille *et al.*, 1979). Nonetheless, due to the fluctuation pattern of progestins, it was not possible to detect ovulation, although it might have occurred. Traditionally, jaguars, like many other felids, are characterized as induced ovulators, which is when ovulation is induced by copulation (Wildt *et al.*, 2010). However, there is evidence that other exteroceptive stimuli (tactile, visual, olfactory or





**Figure 3:** Longitudinal profile of faecal progesterins for a pre-pubertal female (PP03) jaguar (*Panthera onca*). Asterisk refers to the onset of ovarian activity.

**Table 5:** List of species, its respective size and size category (based on Nowell and Jackson, 1996), oestral cycle length (ECL), age at maturity (AgM) and the references where the information is available

Species	Size (kg)	Size category	ECL (days)	AgM (years)	References
<i>Panthera tigris</i>	136	Large	29	3–4 years	a; b; c; d; e
<i>Panthera leo</i>	126	Large	38.5	2 years	a; b; f; g
<i>Panthera onca</i>	56	Large	37.5	2 years	a; h; this study
<i>Puma concolor</i>	41	Large	16	2 years	a; e; i; j
<i>Acinonyx jubatus</i>	43	Large	14	2–3 years	a; b; k; l
<i>Panthera pardus</i>	40	Large	24.5	2.5 years	a; e; f; m; n; o; p
<i>Panthera uncia</i>	37.5	Large	32.5	2–3 years	a; b; e; q
<i>Lynx</i> sp.	15	Medium	NA	1–2 years	a; e; l
<i>Neofelis nebulosa</i>	20	Medium	27.5	2 years	a; e; l; r
<i>Caracal caracal</i>	10	Medium	23.5	0.5–1.5 years	a; b; s
<i>Leopardus pardalis</i>	9.0	Medium	18.4	1 years	a; t
<i>Prionailurus viverrinus</i>	6.8	Medium	20	2 years	u; v; w
<i>Leopardus tigrinus</i>	2.0	Small	16.7	2 years	a; t
<i>Leopardus wiedii</i>	3.2	Small	17.6	0.5 years	a; t
<i>Otocolobus manul</i>	3	Small	14	1 years	a; x
<i>Felis margarita</i>	3	Small	11.1	1 years	y
<i>Felis nigripes</i>	3.5	Small	11.9	1 years	y

a Nowell and Jackson, 1996; b Graham *et al.*, 1995; c Graham *et al.*, 2006; d Seal *et al.*, 1985; e Brown and Comizzoli, 2018; f Schmidt *et al.*, 1979; g Putman *et al.*, 2010; h Barnes *et al.*, 2016; i Bonney *et al.*, 1981; j Eisenberg and Redford, 1999; k Brown *et al.*, 1996; l Brown, 2011; m Sadleir, 1996; n de Haas van Dorsser *et al.*, 2007; o Owen *et al.*, 2010; p Cunningham and Gross, 2000; q Schmidt *et al.*, 1993; r Yamada and Durrant, 1989; s Bernard and Stuart, 1987; t Moreira *et al.*, 2001; u Santymire *et al.*, 2011; v Kinzer & Groome, 2011; w Fazio *et al.*, 2016; x Brown *et al.*, 2002; y Herrick *et al.*, 2010.

auditory) may trigger ovulation (Wildt *et al.*, 1979; Sadleir, 1996; Rekwot *et al.*, 2001; Jorge-Neto *et al.*, 2020). Non-mating ovulations were recorded in females housed together in other species (Bristol-Gould and Woodruff, 2006; Brown, 2011). Hence, ovulatory stimulation in felids is variable not only across species but also at an individual level.

### Puberty

We have found that females entered the peri-pubertal phase with an approximate age of 20 months (18–20 months of age), a length similar to those reported in the literature (Wildt *et al.*, 1979). Based only on behavioural patterns, age at

puberty estimation fell between 24 and 30 months (Wildt *et al.*, 1979; Mondolfi and Hoogesteijn, 1986). The female PP03 was followed for 31 months, and her cycling was not like the adult females. Therefore, even after initiating ovarian cycling at 20 months, females may show irregular cycles for an extended period. In caracals, although the onset of the ovarian activity happened between 7–10 months, reproductive success was only achieved at the age of 14 months (Bernard and Stuart, 1987).

It is not uncommon that the hypothalamic–pituitary–gonadal axis is activated prior the expression of reproductive behaviour. Puberty is a process that, afterwards, an individual attains sexual maturity. In the review of Schultz and Sisk (2006), they have shown that individuals experimentally treated with steroid hormones did not express behaviour during pre-puberty. They concluded that the brain was not yet primed for reproductive behaviour activation.

### Comparison with other wild felids

Puberty (or maturity) is reasonably stable across Felidae (1–2 years old), with smaller species attaining sexual maturity at an earlier age (1 years old) than larger species (2 years old) (Schmidt *et al.*, 1979; Bonney *et al.*, 1981; Santiapillai *et al.*, 1982; Bernard and Stuart, 1987; Graham *et al.*, 1995; Brown, 2011). Some exceptions are, for instance, *Prionailurus*, an Asian wild cat of 6.8 kg (medium-sized) that attains maturity around 2 years of age (Kinzer & Groome, 2011; Fazio *et al.* 2016; Santymire *et al.* 2011). In our analysis, size explained a large portion of the differences observed in maturity, while the only genus that influenced variation was *Leopardus*.

In domestic cats, the onset of puberty varies according to breed and body weight (Root *et al.*, 1995; Johnston *et al.*, 2001). This trend is not restricted to felids but is ubiquitous to mammals. For instance, smaller mammalian species tend to attain maturity at relatively smaller sizes than larger mammals (McClintock, 1983; Plotka *et al.*, 1988). However, it is impressive that a smaller magnitude of just 20 kg is enough to explain differences in this reproductive parameter.

As for our findings on oestral cycle length, it diverged from other Neotropical felids (e.g., *Leopardus* spp. Moreira *et al.*, 2001; *Puma concolor*) or other *Panthera* species (*P. pardus*, Santiapillai *et al.*, 1982; Schmidt *et al.*, 1988; Schmidt *et al.*, 1993; Graham *et al.*, 1995). However, when compared to cats of similar size, the duration falls within the same range (*P. tigris*, Brown, 2011; *Puma concolor*, Eisenberg and Redford, 1999; *Pa. leo*, Schmidt *et al.*, 1979; Graham *et al.*, 1995; *Pa. uncia* Graham *et al.*, 1995). Although differences can be detected visually, our statistical analysis did not confirm such a trend.

Hence, while the phylogenetic signal (i.e. the effect of genus) exists, all size categories explained the variation observed in AgM. Interaction among genus, size, ECL and AgM could not be tested because of small sample size, though

it would be interesting to assess this interaction further. Allometric and phylogenetic influence on reproduction is widespread among animals (e.g. Harvey and Zammuto, 1985).

### Concluding remarks

It is necessary to emphasize that the *Panthera* genus is the most diverse Felidae genus and the most well-studied. However, many Felid species remain poorly known regarding its reproductive endocrinology, particularly those of smaller-sized and Asian species in order to understand the patterns in wild cats. Additionally, to collect and preserve female gametes is challenging, and a strong knowledge on physiology is necessary to be successful (Asa, 2012). Therefore, by adding to the body literature on hormonal assessment of wild felids, we can provide a starting point to sister-species. Many species rely on assisted reproduction to maintain viable captive and wild population and it is only possible by induction of oestrus and ovulation (Asa *et al.*, 2006). These parameters are of prime importance for wild population conservation and captive welfare.

### Conclusion

Our study is only the second study addressing naturally occurring oestral cycles in female jaguars and, to our knowledge, the first to assess puberty. Our findings agreed with literature that employed hormonal analysis but differed from those based solely on behavioural observations. Such difference could be related to the lack of oestrous behaviour at the beginning of the ovarian activity. Therefore, we emphasize that hormonal assessment is of prime importance for both the onset of ovarian activity but also as a follow-up on reproductive status of adult females. When compared to other felids, our data resembles other *Panthera* representatives if of similar size. An adequate functioning of ovarian hormones is of prime interest for assisted reproduction, occasionally a necessary step for endangered species.

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## References

- Asa CS. (2012) Reproductive biology and endocrine studies. In L Boitani, RA Powell, eds, *Carnivore Ecology and Conservation: A Handbook of Techniques*. Oxford University Press, New York pp 273–293.
- Asa C, Bauman K, Callahan P *et al.* (2006) GnRH-agonist induction of fertile estrus with either natural mating or artificial insemination, followed by birth of pups in gray wolves (*Canis lupus*). *Theriogenology* 66: 1778–1782 <https://doi.org/10.1016/j.theriogenology.2006.01.035>.
- Barnes SA, Teare JA, Staaden S, Metrione L, Penfold LM (2016) Characterization and manipulation of reproductive cycles in the jaguar (*Panthera onca*). *Gen Comp Endocrinol* 225: 95–103 <https://doi.org/10.1016/j.ygcen.2015.09.012>.
- Bernard RTF, Stuart CT. (1987) Reproduction of the caracal *Felis caracal* from the Cape Province of South Africa. *Afr Zool* 22(3): 177–182. <https://www.ajol.info/index.php/az/article/view/153778>.
- Bonney RC, Moore HDM, Jones DM. (1981) Plasma concentrations of oestradiol-17 $\beta$  and progesterone, and laparoscopic observations of the ovary in the puma (*Felis concolor*) during oestrus, pseudopregnancy and pregnancy. *J Reprod Fert* 63(2): 523–531. <https://doi.org/10.1530/jrf.0.0630523>.
- Bristol-Gould S, Woodruff TK. (2006) Folliculogenesis in the domestic cat (*Felis catus*). *Theriogenology* 66(1): 5–13. <https://doi.org/10.1016/j.theriogenology.2006.03.019>.
- Brown JL, Comizzoli P. (2018) Female Cat Reproduction. In M Skinner, ed, *Encyclopedia of Reproduction*. Academic Press, Cambridge, 692–701.
- Brown JL, Graham LH, Wu JL, Collins D, Swanson F (2002) Reproductive endocrine responses to photoperiod and exogenous gonadotropins in the pallas' cat (*Otocolobus manul*). *Zoo Biol* 21: 347–367 <https://doi.org/10.1002/zoo.10043>.
- Brown JL, Terio KA, Graham LA (1996) Fecal androgen metabolite analysis for non invasive monitoring of testicular steroidogenic activity in felids. *Zoo Biol* 15: 425–434 [https://doi.org/10.1002/\(SICI\)1098-2361\(1996\)15:4<425::AID-ZOO7>3.0.CO;2-D](https://doi.org/10.1002/(SICI)1098-2361(1996)15:4<425::AID-ZOO7>3.0.CO;2-D).
- Brown JL, Wasser SK, Wildt DE, Graham LH. (1994) Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. *Biol Reprod* 51(4): 776–786. <https://doi.org/10.1095/biolreprod51.4.776>.
- Brown JL. (2011) Female reproductive cycles of wild female felids. *Anim Reprod Sci* 124(3–4): 155–162. [10.1016/j.anireprosci.2010.08.024](https://doi.org/10.1016/j.anireprosci.2010.08.024).
- Cavalcanti SMC, Gese EM. (2010) Kill rates and predation patterns of jaguars (*Panthera onca*) in the southern Pantanal, Brazil. *J Mammal* 91(3): 722–736. <https://doi.org/10.1644/09-MAMM-A-171.1>.
- Cooke SJ, O'Connor CM. (2010) Making conservation physiology relevant to policy makers and conservation practitioners. *Conserv Lett* 3(3): 9–166. <https://doi.org/10.1111/j.1755-263X.2010.00109.x>.
- Cooke SJ, Sack L, Franklin CE, Farrell AP, Beardall J, Wikelski M, Chown SL. (2013) What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conserv Physiol* 1(1): 1–23, Cot001. <https://doi.org/10.1093/conphys/cot001>.
- Cunningham P, Gross C. (2000) Reproduction and development of the Arabian Leopard, *Panthera pardus nimr*, in captivity. *Zool Middle East* 20(1): 9–14. <https://doi.org/10.1080/09397140.2000.10637805>.
- de Haas van Dorsser FJ, Green DI, Holt WV, Picard AR (2007) Ovarian activity in Arabian leopards (*Panthera pardus nimr*): sexual behaviour and faecal steroid monitoring during the follicular cycle, mating and pregnancy. *Reprod Fert Develop* 19: 822–830 <https://doi.org/10.1071/RD07053>.
- de la Torre JA, González-Maya J, Zarza H, Ceballos G, Medellín R (2017) The jaguar's spots are darker than they appear: assessing the global conservation status of the jaguar *Panthera onca*. *Oryx* 52(2) 1–16 <https://doi.org/10.1017/S0030605316001046>.
- Eisenberg JF, Redford KH. (1999) Order Carnivora (Fissipedia). In: *Mammals of the neotropics*, 3rd vol. The Central Neotropics: Ecuador, Peru, Bolivia, Brazil. University of Chicago Press, Chicago. pp 279–311.
- Fazio JM (2016) *Assessment of Adrenal Activity and Reproductive Cycles during Captive Management in the Fishing Cat (Prionailurus viverrinus)* (Doctoral Dissertation). George Mason University, Fairfax.
- Graham LH, Byers AP, Armstrong DL, Loskutoff NM, Swanson WF *et al.* (2006) Natural and gonadotropin-induced ovarian activity on tigers (*Panthera tigris*) assessed by fecal steroid analyses. *Gen Comp Endocr* 147: 362–370 <https://doi.org/10.1016/j.ygcen.2006.02.008>.
- Graham LH, Goodrowe KL, Raeside RM, Liptrap RM. (1995) Non-invasive monitoring of ovarian function in several felid species by measurement of fecal estradiol-17 $\beta$  and progestins. *Zoo Biol* 14(3): 223–237. <https://doi.org/10.1002/zoo.1430140304>.
- Herrick JR, Bond JB, Campbell M, Levens G, Moore T *et al.* (2010) Fecal endocrine profiles and ejaculate traits in black-footed cats (*Felis nigripes*) and sand cats (*Felis margarita*). *Gen Comp Endocr* 165: 204–214 <https://doi.org/10.1016/j.ygcen.2009.06.021>.
- Harvey PH, Zammuto RM (1985) Patterns of mortality and age at first reproduction in natural population of mammals. *Nature* 315: 319–320 <https://doi.org/10.1038/315319a0>.
- Illera JCS, Munro CJ, Lorenzo PL, Illera MJ, Liu IKM, Illera M (2003) Amplified androstenedione enzymeimmunoassay for the diagnosis of cryptorchidism in the male horse: comparison with testosterone and estrone sulphate methods. *J Steroid Biochem* 84: 377–382 [https://doi.org/10.1016/s0960-0760\(03\)00057-8](https://doi.org/10.1016/s0960-0760(03)00057-8).
- Johnston SD, Kustritz MVR, Olson PNS. (2001) The feline estrous cycle. In SD Johnston, MVR Kustritz, PNS Olson, eds, *Canine and Feline Theriogenology*. Saunders Company, Philadelphia, pp 396–405.
- Jorge-Neto PN, Pizzutto CS, Araujo GR, Deco-Souza T, Silva LC, Salomão Jr JA, Baldassarre H. (2010) Copulatory behavior of the Jaguar *Panthera onca* (Mammalia: Carnivora: Felidae). *J Threat Taxa* 10(15): 12933–12939. <https://doi.org/10.11609/jott.4218.10.15.12933-12939>.



- Jorge-Neto PN, Luczinski TC, de Araújo GR, Júnior JAS, de Souza Traldi A *et al.* (2020). Can jaguar (*Panthera onca*) ovulate without copulation? *Theriogenology* 147. <https://doi.org/10.1016/j.theriogenology.2020.02.026>.
- Kinzer J, Groome C (2011) Population analysis and breeding and transfer plan: Fishing cat (*Prionailurus viverrinus*). In *AZA Red Program (Population Analysis and Breeding transfer plan)*, pp. 1–21.
- Kumar V, Umapathy G (2019) Non-invasive monitoring of steroid hormones in wildlife for conservation and management of endangered species—A review. *Indian J Exp Biol* 57: 307–314.
- Lasley BL, Loskutoff NM, Anderson GB. (1994) The limitation of conventional breeding programs and the need and promise of assisted reproduction in nondomestic species. *Theriogenology* 41(1): 119–132. [https://doi.org/10.1016/S0093-691X\(05\)80057-3](https://doi.org/10.1016/S0093-691X(05)80057-3).
- Loveridge AJ, Wang SW, Frank LG, Seidensticker J. (2010) People and wild felids: conservation of cats and management of conflicts. In DW MacDonald, AJ Loveridge, eds, *Biology and Conservation of Wild Felids*. Oxford University Press, Oxford, pp 161–195.
- McClintock M (1983) The behavioral endocrinology of rodents: a functional analysis. *BioScience* 33(9): 573–577. <https://doi.org/10.2307/1309208>.
- Mondolfi E, Hoogesteijn R. (1986) Notes on the biology and status of the jaguar in Venezuela. In SD Miller, DD Everett, eds, *Cats of the World: Biology, Conservation and Management*. National Wildlife Federation, Washington, pp 85–124.
- Moreira N, Monteiro-Filho ELDA, Moraes WD, Swanson WF, Graham, LH., Pasquali, OL, Gomes MLF, Morais RN, Wildt DE, Brown JL. (2001) Reproductive steroid hormones and ovarian activity in felids of the *Leopardus* genus. *Zoo Biol* 20(2): 103–116. <https://doi.org/10.1002/zoo.1010>.
- Nowell K, Jackson P (1996) *Wild Cats. Status Survey and Conservation Action Plan*. IUCN/SSC Cat Specialist Group, Gland.
- Owen C, Nuemann S, Slotow R. (2010) Copulatory parameters and reproductive success of wild leopards in South Africa. *J Mammal* 91(5): 1178–1187. <https://doi.org/10.1644/09-MAMM-A-256.1>.
- Plotka ED, Seal US, Zarembka FR, Simmons LG, Teare A, Phillips LG, Hinshaw KC, Wood DG. (1988). Ovarian function in the elephant: luteinizing hormone and progesterone cycles in African and Asian elephants. *Biol Reprod* 38(2): 309–314. <https://doi.org/10.1095/biolreprod38.2.309>.
- Putman SB, Brown JL, Franklin AD, Schneider EC, Boisseau NP, *et al.* (2010) Characterization of ovarian steroid patterns in female African lions (*Panthera leo*), and the effects of contraception on reproductive function. *Plos One* 10(10): 1–17. <https://doi.org/10.1371/journal.pone.0140373>
- Quigley H, Foster R., Petracca L, Payan E, Salom R, Harmsen B. (2018) *Panthera onca* The IUCN Red List of Threatened Species 2017: e. T15953A123791436. <http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T15953A50658693.en> (last accessed 6 June 2019)
- Rekwot PI, Ogwu D, Oyedipe EO, Sekoni VO. (2001) The role of pheromones and biostimulation in animal reproduction. *An Reprod Sci* 65(3–4):157–170. [https://doi.org/10.1016/S0378-4320\(00\)00223-2](https://doi.org/10.1016/S0378-4320(00)00223-2)
- Root MV, Johnston SD, Olson PN (1995) Estrous length, pregnancy rate, gestation and parturition lengths, litter size, and juvenile mortality in the domestic cat. *J Am Anim Hosp Assoc* 31: 429–433 <https://doi.org/10.5326/15473317-31-5-429>.
- Santymire RM, Brown JL, Stewart RA, Santymire RC, Wildt DE, Howard J. (2011) Reproductive gonadal steroidogenic activity in the fishing cat (*Prionailurus viverrinus*) assessed by fecal steroid analyses. *Anim Reprod Sci* 128(1–4): 60–72. <https://doi.org/10.1016/j.anireprosci.2011.09.001>
- Sadleir RMFS (1996) Notes on reproduction in the larger felidae. *Int Zoo Yearb* 6(1): 184–187. doi: 10.1111/j.1748-1090.1966.tb01746.x.z
- Santiapillai C, Chambers MR, Ishawaran N. (1982) The leopard *Panthera pardus fusca* (Meyer 1794) in the ruhuna national park, Sri Lanka, conservation. *Biol Cons* 23(1): 5–14. [https://doi.org/10.1016/0006-3207\(82\)90050-7](https://doi.org/10.1016/0006-3207(82)90050-7).
- Schmidt AM, Hess DL, Schmidt MJ, Lewis CR. (1993) Serum concentrations of oestradiol and progesterone and frequency of sexual behaviour during the normal oestrous cycle in the snow leopard (*Panthera uncia*). *Reproduction* 98(1): 91–95. <https://doi.org/10.1530/jrf.0.0980091>.
- Schmidt AM, Hess DL, Schmidt MJ, Smith RC, Lewis CR. (1988) Serum concentrations of oestradiol and progesterone, and sexual behaviour during the normal oestrous cycle in the leopard (*Panthera pardus*). *Reproduction* 82(1): 43–49. <https://doi.org/10.1530/jrf.0.0820043>.
- Schmidt AM, Nadal LA, Schmidt MJ, Beamer NB. (1979) Serum concentrations of oestradiol and progesterone during the normal oestrous cycle and early pregnancy in the lion (*Panthera leo*). *Reproduction* 57(2): 267–272. <https://doi.org/10.1530/jrf.0.0570267>.
- Schulz KM, Sisk CL (2006) Pubertal hormones, the adolescent brain, and the maturation of social behaviors: lessons from the Syrian hamster. *Mol Cell Endocrinol* 254: 120–126 <https://doi.org/10.1016/j.mce.2006.04.025>.
- Seal US, Plotka ED, Smith JD, Wright FH, Reindl NJ *et al.* (1985) Immunoreactive luteinizing hormone, estradiol, progesterone, testosterone, and androstenedione levels during the breeding season and anestrus in Siberian tigers. *Biol Repr* 32: 361–368 <https://doi.org/10.1095/biolreprod32.2.361>.
- Shille VM, Lundström KE, Stabenfeldt GH. (1979) Follicular function in the domestic cat as determined by estradiol-17 $\beta$  concentrations in plasma: relation to estrous behavior and cornification of exfoliated vaginal epithelium. *Biol Reprod* 21(4): 953–963.
- Shille VM, Munrot C, Farmer SW, Papkoff H, Stabenfeldt GH. (1983) Ovarian and endocrine responses in the cat after coitus. *Reproduction* 69(1): 29–39. <https://doi.org/10.1530/jrf.0.0690029>.

- Treves A, Karanth KU (2003) Human–carnivore conflict and perspectives on carnivore management worldwide. *Conserv. Biol* 17: 1491–1499 <https://doi.org/10.1111/j.1523-1739.2003.00059.x>.
- Tewes ME, Schmidly DJ. (1987) The Neotropical felids: jaguar, ocelot, margay and jaguarindi. In W Nowak, JA Backer, ME Obbard, B Malloch, eds, *Wild Furbearer Management and Conservation in North America*. Ontario Ministry of Natural Resources, Toronto, pp 697–711.
- Viau P, Felipe ÉCG, Oliveira CA. (2005) Quantificação de esteróides fecais de fêmeas de onça-pintada (*Panthera onca*) mantidas em cativeiro: validação da técnica. *Braz J Vet Res An Sci* 42(4): 262–270.
- Wildt DE, Platz CC, Chakraborty PK, Seager, SWJ. (1979) Oestrous and ovarian activity in a female jaguar (*Panthera onca*). *Reproduction* 56(2): 555–558. <https://doi.org/10.1530/jrf.0.0560555>.
- Wildt DE, Swanson W, Brown J, Sliwa A, Vargas A. (2010) Felids ex situ: managed programs, research, and species recovery. In DW MacDonald, AJ Loveridge, eds, *Biology and Conservation of Wild Felids*. Oxford University Press, Oxford, pp 217–235.
- Yamada JK, Durrant BS (1989) Reproductive parameters of clouded leopards (*Neofelis nebulosa*). *Zoo Biol* 8: 223–231 <https://doi.org/10.1002/zoo.1430080303>.