REVIEW

Reproductive biology of the 38 extant felid species: a review

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ABSTRACT

1. Knowledge of reproductive biology is crucial to improving *in situ* and *ex situ* breeding programmes for felids. We reviewed the available literature (223 publications) on the reproductive biology of all 38 felid species.

2. We found that 78% of the publications (173) were focused on either or both the oestrous cycles (84) or ejaculate traits (92) of felids. Literature was biased towards the domestic cat *Felis catus* (31), the cheetah *Acinonyx jubatus* (27), and the panthera lineage (66). There was a paucity of literature on the caracal lineage (7), the bay cat lineage (3), members of the domestic cat lineage other than the domestic cat (11), and several species of the ocelot lineage.

3. The mean duration of oestrus varies little between the different lineages and species (mean 5.2 days, range 1–10 days, $n_E = 2265$). However, the duration of interoestrus varies greatly in most species (e.g. 1–118 days in the domestic cat). Gestation length also varies significantly between species, but is similar within each lineage and related to adult body size. Non-pregnant luteal phases appear to persist for half the duration of pregnant luteal phases (48%, 21–71 days, $n_E = 256$; c.f. previous reports of one-third the duration of pregnant luteal phases).

4. Sperm motility (sperm motility index), sperm viability, and acrosome intactness are high in the fresh ejaculates of most felid species [69% (26–90%, $n_E = 2104$), 69% (49–87%, $n_E = 443$), and 84% (21–100%, $n_E = 1763$), respectively]. Teratospermia is highly prevalent within Felidae, but is particularly problematic for the puma and lynx lineages [ejaculates with 76% (63–94%) and 79% (63–98%) abnormal sperm, respectively]. Teratospermia appears to be linked to low genetic diversity.

5. The maintenance and enhancement of genetic diversity through the use of assisted reproductive technologies should be a long-term goal for felid conservation management. A short-term management goal should be to improve the success of assisted reproductive technologies in felids by minimising captivity-related stress, which can adversely affect fertility and ovarian activity.

INTRODUCTION

Felidae is a diverse family consisting of 38 extant species that inhabit a wide variety of environments and exhibit a diverse range of lifestyles (Nowell & Jackson 1996, Johnson et al. 2006, IUCN 2017). Twenty-five species are considered endangered or threatened in at least part of their natural geographic range, and all 37 non-domestic species are listed in the Convention on International Trade in Endangered Species (CITES) treaty (Appendix S1; Nowell & Jackson 1996, Nowell 2002, Swanson 2003, Johnson et al. 2006). Poaching (either directly or of prey species),

culling, and habitat destruction or fragmentation are the main reasons cited for the high proportion of threatened species within Felidae (Appendix S1). The impacts of these factors are so severe that *in situ* conservation efforts are not sufficient to ensure the persistence of many species.

Captive breeding programmes have become an important component of the conservation strategies for many species, although the conservation value of such programmes is often questioned. Captive animals are rarely released into the wild, and, even if they are released, post-release survival rates are low due to poor hunting success or predator avoidance (Jule et al. 2008). However, the maintenance of felids in captivity does provide opportunities for researchers to investigate the behaviour and physiology of felids, and findings can be applied to improve both *ex situ* and *in situ* conservation efforts. In fact, the vast majority of the published literature on the reproductive biology and physiology of felids has been conducted on captive animals. A major complication with captive breeding programmes has been the poor reproductive performance of most felids in captivity (Mellen 1991, Terio et al. 2004, Brown 2006, Moreira et al. 2007, Fanson et al. 2010, Brown 2011). Factors leading to poor reproductive success of felids in captivity, and whether wild populations are also affected by such factors, can only be determined by having a thorough understanding of the reproductive biology of felids, and of similarities and differences between species (Brown 2006). Until now, however, there has been no comprehensive review of their reproductive biology.

This review summarises literature on the reproductive biology of all 38 extant felid species and identifies knowledge gaps. Major factors that appear to affect the reproductive biology and fertility of felids are identified, and the implications of these for captive management are discussed.

METHODS

Literature review

Peer-reviewed literature and secondary papers (e.g. book chapters) were reviewed for the period from 1941 to December 2017. Searches were conducted in both Web of Science and Google Scholar using the following terms:

Female felids – 'common name' OR 'scientific name' OR 'alternative name' AND breed* OR cycl* OR estradiol OR estrogens OR estrous OR estrus OR felid* OR ovar* OR pregnan* OR progest* OR pseudopreg* OR reproduct*.

Male felids – 'common name' OR 'scientific name' OR 'alternative name' AND andro* OR ejaculat* OR breed* OR electroejaculat* OR motil* OR morpholog* OR pleiomorphic OR reproduct* OR sperm* OR teratosperm* OR testosterone OR 'uret* catheteri$ation'.

The citation lists of all publications we found were checked for additional publications until no new relevant literature was discovered.

Summary statistics

The values reported are weighted means of the values presented in Appendices S2 and S3, with 'n_E' representing the number of reproductive events [entire oestrous cycles, oestrus, interoestrus, non-pregnant luteal phases (NPLP; often referred to as pseudopregnancies), or ejaculates] and 'n' being the number of individuals. In Appendix S2, all values were calculated as weighted means of the values reported in each of the publications cited, and are presented with a mean, range, and sample size (n, n_E). The ejaculate assessments from individual publications are presented in Appendix S3, with values presented as means ± standard errors. The data from some publications in Appendix S3 were categorised according to the collection method (see Appendix S4), sperm quality (teratospermic vs. normospermic males), season, habitat status (wild, captive on-exhibit, or captive off-exhibit), or genetic diversity (GD; i.e. males from populations with high or low GD), in order to determine whether and how these factors affect sperm quality in felids.

Statistical analyses were conducted using RStudio version 1.0.143 (R Foundation for Statistical Computing, Vienna, Austria) and a significance level of $P < 0.05$. Normality was tested using a Shapiro-Wilk test. Parametric data were analysed using either a two-sample *t*-test or an analysis of variance and Tukey's *post-hoc* test. A Kruskal-Wallis test or pairwise Wilcoxon rank sum test was used to analyse non-parametric data.

RESULTS

Literature analysis

A total of 223 papers on felids were used for this review and for Appendices S1–S5; 173 of these focused on the general reproductive biology of felids. The literature is heavily biased towards the domestic cat *Felis catus*, cheetah *Acinonyx jubatus*, and the panthera lineage (Fig. 1). Research on the reproductive biology of non-domestic felids in the bay cat, caracal, and domestic cat lineages is restricted to a few studies (Fig. 1), many of which have very small sample sizes. The small amount of literature on caracals *Caracal caracal* and servals *Caracal serval* is surprising (only six and three publications, respectively) given the numbers of both these species in captivity.
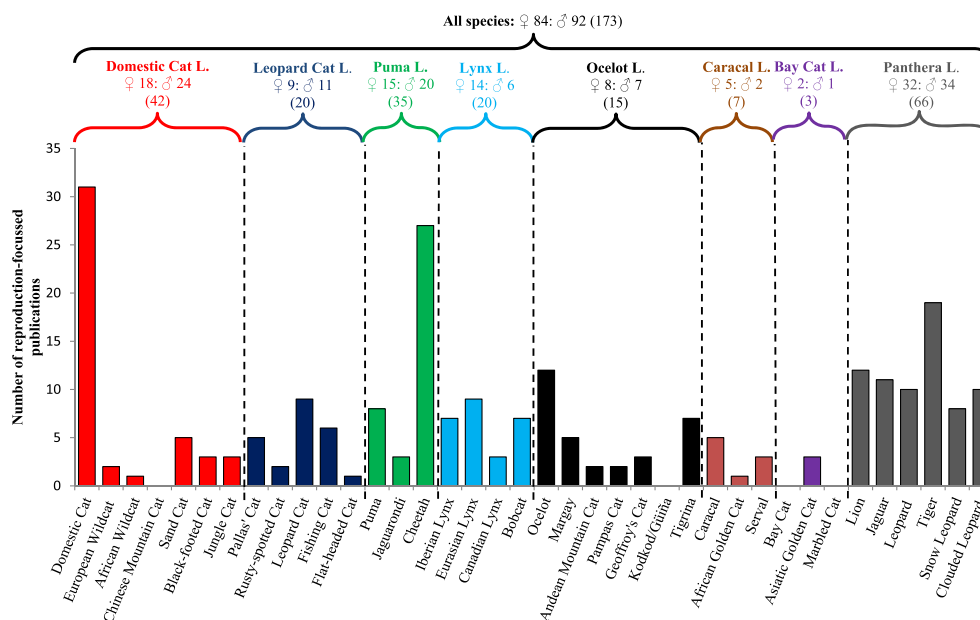
Fig. 1. Numbers of publications on the reproductive biology of each of the 38 felid species, based on the publications listed in Appendices S2 and S3. The total numbers of publications for each lineage (abbreviated as L.) do not necessarily equal the sum of the numbers of publications, because many publications included species from multiple lineages and/or information on the reproductive biology of both male and female felids. For the same reason, the total number of publications, number of female-focused (♀) publications, and number of male-focused (♂) publications for all species does not equal the sum of these values across lineages. [Colour figure can be viewed at wileyonlinelibrary.com]

The generalised feline oestrous cycle

A detailed summary of the literature (84 publications) on the basic female reproductive biology and oestrous cycles of all 38 felid species is provided in Appendix S2. The oestrous cycle of felids consists of four phases: anoestrus, follicular phase (pro-oestrus and oestrus), interoestrus (while technically part of follicular phase, interoestrus has been considered separately herein), and luteal phase/di-oestrus (Fig. 2; Brown 2011).

THE FOLLICULAR PHASE: PRO-OESTRUS AND OESTRUS

The follicular phase of the oestrous cycle, which includes both pro-oestrus and oestrus, is defined by the presence of developing ovarian follicles (Chatdarong 2003). Pro-oestrus is relatively short in most felids (<24 hours), and is marked by the presence of small, developing, primary or secondary follicles (Bristol-Gould & Woodruff 2006, Brown 2011). Developing follicles secrete oestradiol at an increasing rate, so pro-oestrus is also accompanied by a gradual rise in plasma oestradiol concentrations (Griffin 2001, Chatdarong 2003, Malandain et al. 2011). The indirect positive feedback of oestradiol on hypothalamic gonadotrophin-releasing hormone neurons via hypothalamic kisspeptin neurons up-regulates the activity of the hypothalamic-pituitary-gonadal axis to stimulate ovarian

folliculogenesis and steroidogenesis further (Smith et al. 2007, Popa et al. 2008). This positive feedback loop is responsible for the continued growth and development of follicles during the follicular phase.

Endocrine oestrus is defined by the presence of one or more mature tertiary or dominant follicles and peak oestradiol concentrations (Bristol-Gould & Woodruff 2006, Brown 2011, Malandain et al. 2011). Oestradiol is the primary stimulant of reproductive behaviours in domestic cats, with exogenous oestradiol treatments triggering the expression of oestrous behaviours in ovariectomised domestic cats (Michael & Scott 1964, Whalen & Hardy 1970). As with other induced ovulators, oestradiol appears to be the only reproductive hormone required to stimulate the expression of oestrous behaviours in felids (c.f. the need for progesterone priming in spontaneous ovulators; Michael & Scott 1964, Whalen & Hardy 1970, Bakker & Baum 2000). Oestrous behaviours are relatively consistent among felid taxa, with most species exhibiting an increase in the frequency of grooming, allogrooming, rubbing, rolling, scent-marking, locomotor activity, vocalisation or calling, and lordosis (Michael & Scott 1964, Blomqvist & Sten 1982, Mellen 1993, Umapathy et al. 2007, Kinoshita et al. 2009, Brown 2011). The intensity of behavioural oestrus, however, differs considerably within and between felid species, and oestrus signs range from overt and easily detectable to hidden and undetectable (Blomqvist & Sten 1982, Mellen 1993, Foreman
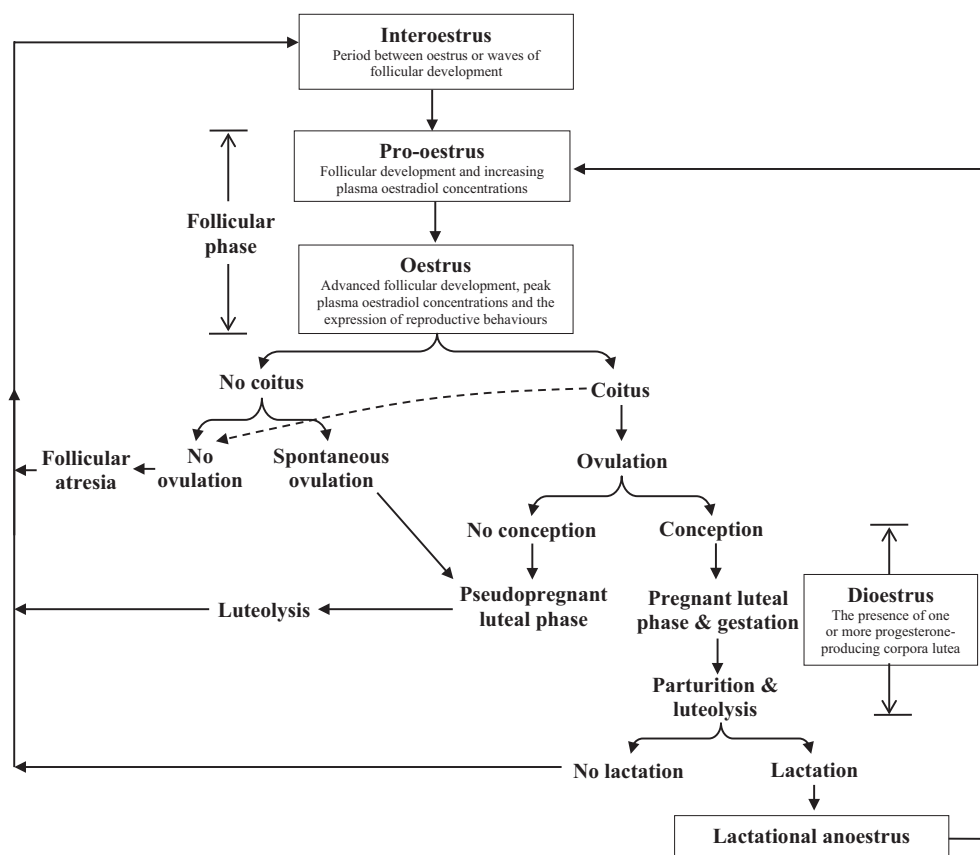
Fig. 2. The generalised polyoestrous cycle of non-seasonal felids.

1997, Wielebnowski & Brown 1998, Morato et al. 2001, Brown et al. 2002, Henriksen et al. 2005, Umapathy et al. 2007, van Dorsser et al. 2007, Bell 2009, Kinoshita et al. 2009, Siemieniuch et al. 2012, Putman et al. 2015).

Felids are primarily induced or reflex ovulators, with coital stimuli required for the induction of ovulation. Coitus activates tactile neurons in the vagina and cervix, initiating an afferent signalling pathway that leads to the stimulation of hypothalamic gonadotrophin-releasing hormone neurons, and thus the pre-ovulatory gonadotrophin-releasing hormone/luteinising hormone surge (Bakker & Baum 2000, Richards et al. 2002). In felids, the magnitude and duration of the pre-ovulatory gonadotrophin-releasing hormone/luteinising hormone surge is dependent on the number and frequency of copulations, and multiple matings are typically required to induce ovulation (Concannon et al. 1980, Shille et al. 1983, Glover et al. 1985, Schramm et al. 1994, Foreman 1997, Bakker & Baum 2000).

INTEROESTRUS

Most felids are polyoestrus, exhibiting multiple oestrous events throughout the year or breeding season (Graham

et al. 1995, Brown 2011, Jewgenow et al. 2014). If ovulation does not occur during oestrus then there is a period of ovarian quiescence before another oestrous event. The period between consecutive oestrous events is referred to as interoestrus and is associated with basal oestradiol concentrations.

THE LUTEAL PHASE: DIOESTRUS

If ovulation occurs, the remaining theca and granulosa cells of the ruptured follicles are transformed into corpora lutea, a process called luteinisation (Richards et al. 2002, Feldman & Nelson 2004). This phase of the oestrous cycle is referred to as dioestrus, and is associated with a rise in plasma progesterone concentrations. Elevated progesterone concentrations have an important role in the maintenance of pregnancy in most species (Senger 1997, Griffin 2001).

If conception occurs following ovulation, then dioestrus will consist of a pregnant luteal phase (PLP). Progesterone concentrations generally remain elevated throughout gestation; however, temporary mid-gestational decreases in plasma progesterone concentrations have been observed in felids (Czekala et al. 1994, Brown et al. 1995, Graham

et al. 1995, van Dorsser et al. 2007, Brown 2011, Malandain et al. 2011). The physiological significance of this drop in progesterone is not entirely clear, but it may reflect a switch from luteal to placental progesterone production (Briggs et al. 1990, Brown et al. 1995, Feldman & Nelson 2004, van Dorsser et al. 2007). Indeed, placental progesterone appears sufficient for the maintenance of pregnancy during late gestation, since a mid-gestational ovariectomy does not terminate pregnancy in domestic cats (Malassiné & Ferré 1979, Brown et al. 1995). Regardless of the source, progesterone concentrations recover quickly from any mid-gestational decreases and remain elevated through to parturition, returning to baseline levels just after birth (Czekala et al. 1994, Graham et al. 1995).

Felids also exhibit prolonged NPLPs, which occur following spontaneous ovulations or sterile coitus-induced ovulations (Feldman & Nelson 2004, Brown 2011, Malandain et al. 2011). Plasma progesterone concentrations observed during PLP and NPLP are similar, although NPLPs are shorter (Appendix S2; Brown et al. 1995, Graham et al. 1995, Dehnhard et al. 2012).

ANOESTRUS

Anoestrus is a prolonged phase of reproductive acyclicity that is normally associated with the non-breeding season. Felids also exhibit a long period of reproductive inactivity following parturition and while young are suckling, referred to as lactational anoestrus (Feldman & Nelson 2004, Brown 2011).

A species-specific account of felid oestrous cycles

The oestrous cycle has been described for only 24 of the 38 felid species, although for many species information is available on the duration of oestrus only (Appendix S2). Furthermore, many studies on the oestrous cyclicity of felids have been conducted on small sample sizes (either in terms of numbers of animals or numbers of events).

The duration of oestrus does not appear to differ significantly between lineages (d.f. = 7, P = 0.08) or species (d.f. = 19, P = 0.11). While there is some intra- and inter-species variation, the duration of oestrus generally ranges from 2 to 10 days (Table 1). Longer periods of oestrus have been observed in some species, such as domestic cats (weighted mean, range, sample size: 7.3 days, 1–118, n_E = 438), sand cats *Felis margarita* (2.9 days, 1–11, n_E = 109), rusty-spotted cats *Prionailurus rubiginosus* (5.6 days, 1–11, n_E = 50), jaguars *Panthera onca* (6.5 days, <15, n_E = 201), snow leopards *Panthera uncia* (4.3 days, 1–19, n_E = 145) and clouded leopards *Neofelis nebulosa* (5.2 days, 1–17, n_E = 237), but the mean durations of oestrus for these species still fall within a 2–10-days range (Appendix S2).

Given that the duration of oestrus appears to be rather consistent, it seems that most of the inter- and intra-specific variation in oestrous cycle length of felids is associated with the highly variable interoestrus intervals (Table 1). While the mean duration of interoestrus did not differ significantly between lineages (d.f. = 7, P = 0.31) or species (d.f. = 18, P = 0.45), a high degree of variability has been reported for each lineage (Table 1) and species (Appendix S2) – possibly because the duration of interoestrus has rarely been reported. In most instances, the length of interoestrus has been estimated based on the mean durations of oestrus and the entire anovulatory oestrous cycle.

Spontaneous ovulations have been observed in all felid lineages (except the caracal and bay cat lineages, for which there is a general paucity of published research on reproductive biology). The frequency of spontaneous

Table 1. Weighted means (in days) of reproductive parameters of the eight felid lineages.

Lineage	Mean length of anovulatory oestrous cycle, in days (range)	Mean duration of oestrus, in days (range)	Range of inter-oestrus interval, in days	Mean duration of NPLP, in days (range)	Mean duration of gestation, in days (range)	NPLP as % duration of gestation
Domestic cat	14.5 (2–69)	5.8 (1–118)	2–65	32.2 (12–55)	65.7 (60–71)	48.7
Leopard cat	20.4 (7–43)	6.0 (1–11)	0–39	34.8 (18–60)	68.9 (56–76)	50.2
Puma	14.2 (3–55)	4.1 (1–9)	3–50	51.7 (38–62)	92.8 (72–98)	55.9
Lynx	Monoestric	ND (1–10)	Monoestric	>2 years	65.7 (60–72)	>1111.0
Ocelot	18.7 (1–52)	3.2 (1–6)	6–60	35.6 (28–60)	72.2 (66–83)	44.6
Caracal	18.3 (4–54)	4.5 (3–6)	13–51	47.5 (47–48)	78.2 (75–81)	60.3
Bay cat	39.0	6.0	~33	ND	79.0 (74–84)	ND
Panthera	21.4 (5–148)	5.0 (1–19)	1–145	43.8 (11–72)	101.8 (50–127)	44.1
Overall mean	17.7 (1–148)	5.2 (1–118)	1–145	40.9*	78.0	47.6*

The values presented have been calculated from Appendix S2.
ND, no data; NPLP, non-pregnant luteal phase.
*Excludes the lynx lineage.

ovulations appears to differ considerably between felid species. Non-pregnant luteal phases have been described for 19 of the 38 felid species. In most felid species, NPLP typically persists for about half the duration of PLP (48%, 22–71%, $n_E = 256$). The exception is the *Lynx* spp. which exhibit prolonged NPLP, with corpora lutea and elevated progesterone concentrations persisting for two years or more (Göritz et al. 2009, Jewgenow et al. 2014, Painer et al. 2014). Female *Lynx* spp. can re-enter oestrus during subsequent breeding seasons since old corpora lutea are structurally present, but functionally suppressed, until after the breeding season (Jewgenow et al. 2014, Painer et al. 2014). Pregnant luteal phases are also prolonged (two years or more) in this lineage, despite a gestation length of 60–70 days (Jewgenow et al. 2014, Painer et al. 2014).

The duration of gestation differs considerably within Felidae (d.f. = 30, $P < 0.001$), but is generally similar within each lineage (d.f. = 7, $P = 0.2$; Table 1). Nowell and Jackson (1996) categorised felids according to body type into small (<6.5 kg), medium (7–20 kg), and large (35–135 kg) species. Weighted means of the gestation periods of these groups support the hypothesis that larger cats have longer gestation periods (d.f. = 2, $P < 0.001$), with small, medium, and large cats exhibiting different ($P < 0.05$) mean gestation lengths of 67.8 days (60–84, $n_E = 79$), 79.4 days (60–121, $n_E = 138$), and 98.3 days (50–127, $n_E = 504$), respectively.

Reproductive biology and the ejaculate quality of male felids

A full description of the approaches used to collect and assess male ejaculates and fertility is presented in Appendix S4. Literature on the ejaculate quality is available for 28 of the 38 felid species (Appendix S3); we found no published research on the ejaculate traits of European wild cats *Felis silvestris*, African wild cats *Felis lybica*, Chinese mountain cats *Felis bieti*, rusty-spotted cats, Andean mountain cats *Leopardus jacobita*, kodkod *Leopardus guigna*, African golden cats *Caracal aurata*, bay cats *Pardofelis badia*, and marbled cats *Pardofelis marmorata*. The methods used to collect ejaculates or spermatozoa from domestic cats have a considerable effect on sample volume and sperm concentration (d.f. = 2, $P < 0.05$ and d.f. = 3, $P < 0.05$, respectively), and include electroejaculation (EE), artificial vaginas (AV), urethral catheterisation (UC), and the post-mortem or post-castration collection of epididymal sperm (EP; Axnér & Linde-Forsberg 2002). Methods other than EE have been used in only a few felid species (see Appendix S3); comparable data are available only for domestic cats and lions *Panthera leo*. In the domestic cat, EE and EP result in similar concentrations of sperm, while AV and UC provide lower sample volumes but substantially higher sperm concentrations (Fig. 3). This is also apparent in the lion, where UC results in 'ejaculate' samples that are about 80 times more concentrated than those obtained using EE [1940.0×10^6 sperm per ml ($n_E = 7$) vs. 24.4×10^6 sperm per ml ($n_E = 48$), respectively]. The much higher spermatozoa concentrations observed in 'ejaculates' collected by UC could be partly associated with the considerably smaller sample volumes obtained using this technique (Fig. 3; Appendix S3). The method of collection does not appear to have a significant effect on the number of abnormal sperm or on sperm motility, at least in the two species studied.
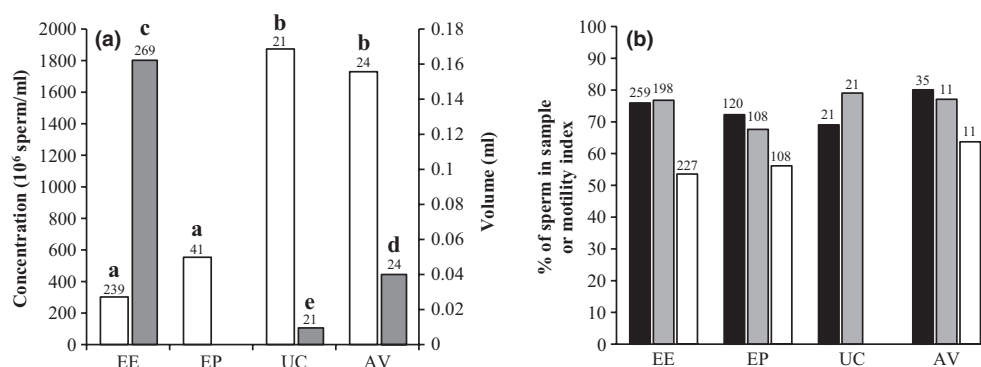

Fig. 3. (a) Volume (grey) and sperm concentration (white) of ejaculates collected from domestic cats using four techniques: electroejaculation (EE), post-mortem epididymal sperm collection (EP), urethral catheterisation (UC), and artificial vagina (AV). Different letters indicate statistical significance ($P < 0.05$), within each variable. Sample sizes (i.e. number of ejaculates) are indicated as a number above each bar. Data on sample volume were not reported for epididymal collections. (b) Percentage of motile sperm (black), sperm motility index (SMI; grey) and percentage of morphologically abnormal sperm (white) in the ejaculates of domestic cats collected using EE, EP, UC, and AV. Sample sizes (i.e. number of ejaculates) are indicated as a number above each bar. Data on the percentage of abnormal sperm in samples collected using UC were not reported. The percentage of motile sperm, SMI, and percentage of morphologically abnormal sperm were similar between collection methods ($P > 0.05$).

Table 2. Weighted means of morphologically abnormal sperm in the ejaculates of species from each felid lineage.

Lineage	n_E (number of ejaculates)	Total morphological abnormal sperm (%)			Primary abnormalities (%)	Secondary abnormalities (%)
		Weighted mean	Minimum	Maximum		
Domestic cat	473	53.0	23.8	90.7	7.7 ($n_E = 150$)	30.1 ($n_E = 150$)
Leopard cat	130	43.9	3.2	80.4	20.3 ($n_E = 40$)	28.4 ($n_E = 40$)
Puma	1055	75.6	63.2	93.5	34.5 ($n_E = 543$)	44.6 ($n_E = 543$)
Lynx	84	78.8	63.2	98.2	ND	ND
Ocelot	312	41.9	17.6	71.0	9.2 ($n_E = 157$)	24.5 ($n_E = 157$)
Caracal	7	29.4	12.0	36.4	ND	ND
Bay cat	1	61.0	ND	ND	ND	ND
Panthera	830	54.0	8.8	84.7	26.7 ($n_E = 506$)	22.3 ($n_E = 506$)
Overall mean	2882 (total)	60.6	3.2	98.2	25.5 ($n_E = 1396$)	32.3 ($n_E = 1396$)

Values calculated from data in Appendix S3.
ND, no data.

Two methods of assessment of sperm motility, the percentage of motile sperm and progressive motility (PM), are often combined into a standardised sperm motility index (SMI) value. The SMI is calculated as follows: SMI = (% motile sperm + (20 × PM))/2. While SMI values differ between species (d.f. = 22, $P < 0.01$), a Tukey's *post-hoc* test revealed that pumas *Puma concolor* and tigers *Panthera tigris* were the only species to have significantly different SMI values (lowest and highest mean SMI values of all species, respectively). In general, sperm motility is relatively high among felids (Appendix S3), with a mean SMI across species of 69% (26–90%, $n_E = 2104$).

Sperm vitality (69%, 49–87%, $n_E = 443$) and acrosome intactness (84%, 20–100%, $n_E = 1763$) also appear to be high in fresh ejaculates of most felids, although only 13 of the 92 studies investigating the felid ejaculate quality reported sperm vitality. In contrast, the percentage of morphologically abnormal sperm (assessment described in Appendix S4) in felid ejaculates seems high (61%, 3–98%, $n_E = 2882$; Table 2), although there is a considerable variation between the different felid lineages (d.f. = 7, $P < 0.001$) and species (d.f. = 27, $P < 0.001$). In fact, all publications from the puma and lynx lineages report teratospermia (Table 2; Appendix S3).

It is difficult to elucidate the aetiology of teratospermia in felids, since both primary (26%, 3–63%, $n_E = 1396$) and secondary (32%, 3–54%, $n_E = 1396$) sperm abnormalities (defined in Appendix S4) are highly prevalent (Table 2). While secondary abnormalities generally appear to be more prevalent (d.f. = 117, $P = 0.028$), there is considerable variability between species. It is possible that the high incidence of teratospermia is due, in part, to poor GD, as species with poor GD (e.g. cheetah and puma) exhibit much higher percentages of morphologically abnormal sperm (Table 2; Appendix S3). Furthermore, lions from populations with low GD produce ejaculates with much higher percentages of abnormal sperm than individuals from populations with greater genetic variance (60%, $n_E = 23$ and 27%, $n_E = 18$, respectively; Wildt et al. 1987, Brown et al. 1991).

An alternative cause for the high proportions of abnormal sperm in felid ejaculates is the captive environment itself (e.g. captivity-related stress or diet). The ejaculate traits of captive and wild individuals have only been compared in puma, cheetah, Iberian lynx *Lynx pardinus*, lions, and jaguars (Appendix S5). Captive jaguars and lions appear to produce ejaculates with higher percentages of morphologically abnormal sperm than their wild counterparts, while the ejaculate quality of captive pumas, cheetah, and Iberian lynx do not appear to differ greatly from that of their wild conspecifics (Appendix S5). This inconsistency is possibly related to the small number of ejaculates that have been collected and assessed from wild felids.

DISCUSSION

In this first comprehensive review of the reproduction-focused literature spanning all felid species, a strong research bias is apparent towards the domestic cat, puma, and panthera lineages (83% of the literature; Fig. 1). Seventy four per cent of the publications on the domestic cat lineage are on the domestic cat, while within the puma lineage, 86% are on cheetah. Panthera is the only lineage for which a reasonable number of publications exist for all representatives. There is a paucity of research for many species in the domestic cat, leopard cat, ocelot, caracal, and bay cat lineages (Fig. 1). Literature on the female oestrous cycle is absent for the European wildcat, African wildcat, Chinese mountain cat, Andean mountain cat, Pampas cat *Leopardus colocolo*, African golden cat, bay cat, and marbled cat (Appendix S2). Literature on the ejaculate quality of these species is also limited (Appendix S3).

In general, reproduction-focused literature is limited for the smaller felids, with a number of studies reporting a strong positive correlation between body size and research effort (Brodie 2009, Brooke et al. 2014). This may be because larger cats are more commonly held in captivity, and thus are more accessible for research (Brodie 2009, Inskip & Zimmermann 2009, Brooke et al. 2014). Indeed, the species for which reproductive biology literature is limited are rarely held in captivity, are restricted to low-density populations in the wild, and are generally cryptic and difficult to study (Nowell & Jackson 1996, Sunquist & Sunquist 2002, Brodie 2009, Brooke et al. 2014, IUCN 2017). However, peer-reviewed literature on caracals and servals is also lacking, despite an abundance of these species in captivity, so it may be more accurate to state that research is biased towards the more popular and iconic species (e.g. *Panthera* spp. and cheetah; Fig. 1).

Considerable inter- and intra-specific variation in the oestrous cycles and ejaculate traits of felids is evident (Appendix S2), so extrapolation of knowledge across even closely related species is not recommended. For example, the frequency of spontaneous ovulation differs greatly within the domestic cat, ocelot, and panthera lineages, so the frequency of spontaneous ovulations is unlikely to be predictable from data from other species within a lineage.

The variability reported for the oestrous cycle length of many felids is likely to be underestimated, as variability appears to increase as more females and cycles are observed; greater oestrous cycle length variation is reported for well-studied species such as the domestic cat and *Panthera* spp. In fact, the oestrous cycles of only 15 of the 38 species have been described from sample sizes of five or more animals, even when sample sizes are combined across all relevant publications (Appendix S2). This suggests that large sample sizes are critical when aiming to describe the reproductive biology of female felids, to ensure that the extreme variability of most feline oestrous cycles is captured. For example, the duration of NPLP has typically been reported to be about one-third that of gestation (Brown 2011); however, our more comprehensive literature analysis indicates that the duration of NPLP is about half that of pregnancy in all felid lineages (Table 1). Factors that appear to drive variation in felid oestrous cycle length include the captive environment (enclosure size or enrichment), time spent with keepers, number of veterinary treatments, body weight, age, diet, and season (Setchell et al. 1987, Mellen 1991, Swanson et al. 1996b, Brown et al. 2002, Rodrigues da Paz et al. 2006, Fanson et al. 2010, Balme et al. 2013). Many felid species are highly seasonal with respect to the frequency of oestrus (i.e. the length of interoestrus; Foreman 1997, Moreira et al. 2001, Brown et al. 2002, Kinoshita et al. 2009).

Most studies have been conducted on captive animals, but the captive environment has been shown to affect reproductive seasonality (Swanson et al. 1996a, Foreman 1997, Brown et al. 2002). For example, Geoffroy's cats *Leopardus geoffroyi* are thought to be seasonal, monoestrous breeders in the wild, but have been found to cycle all year round in captivity (although the frequency of oestrus still peaks between February and August in the northern hemisphere; Foreman 1997). The increased ovarian cyclicity of Geoffroy's cats in captivity is thought to be associated with a more consistent food source (Foreman 1997). Many wild felids live at high altitudes or latitudes, where prey densities fluctuate seasonally (Johnston et al. 1994, Swanson et al. 1996b, Brown et al. 2002, Newell-Fugate et al. 2007, Göritz et al. 2009), but this is seldom mirrored in feeding regimes for captive-held felids. However, if food availability or body weight were the only factors regulating seasonal reproduction in felids, then all felid species would be expected to breed all year round in captivity (since their diet is consistent), but this is not the case (Schmidt et al. 1993, Swanson et al. 1996b, Brown et al. 2002, Göritz et al. 2009).

There is considerable evidence for photoperiod-regulated reproduction in felids (Shille et al. 1979, Foreman 1997, Graham et al. 2004). Artificial lighting has been shown to stimulate follicular development in Pallas' cats *Otocolobus manul* temporarily, during the non-breeding season (Swanson et al. 1996a, Brown et al. 2002). Both artificial lighting and exogenous melatonin treatment can influence the frequency of oestrus in domestic cats (Michel 1993, Graham et al. 2004, Tsutsui et al. 2004). While this seemingly provides putative support for the photoperiodic control of seasonal reproduction in felids, factors such as body weight, temperature, prey availability, and social stimuli have all been associated with seasonal reproduction and cannot be ignored (Michel 1993, Swanson et al. 1996a, Foreman 1997, Brown et al. 2002, Jansen & Jenks 2012). Regardless, it is clear that seasonality may contribute towards the highly variable oestrous cycles described in many felids (Appendices S2 and S3) by altering the frequency of oestrus.

More quantitative evidence exists for male felids on the effects of reproductive seasonality on variability in ejaculate traits, such as volume and concentration (e.g. Pallas' cats, *Lynx* spp. and snow leopards; Appendix S3; Donoghue et al. 1992, Johnston et al. 1994, Swanson et al. 1996b, Jewgenow et al. 2006, Newell-Fugate et al. 2007, Erofeeva et al. 2014). The percentage of pleiomorphic sperm (mostly secondary sperm abnormalities) in the ejaculates of seasonally breeding felids also appears to be lower during the breeding season (Johnston et al. 1994, Swanson et al. 1996b, Newell-Fugate et al. 2007). This may be due to the higher testosterone concentrations during the breeding

season in some felids, since testosterone levels influence normal spermatogenesis (Newell-Fugate et al. 2007).

Species throughout Felidae have high proportions of morphologically abnormal sperm, regardless of the method of collection. In fact, teratospermia is probably the most significant factor affecting the fertility of male felids and has been described multiple times in all felid lineages except for the caracal lineage, for which only two publications are available (Table 2, Appendix S3). This is problematic given that morphologically abnormal sperm are rarely involved in the fertilisation process (Appendix S4; Howard et al. 1990, Long et al. 1996, Pukazhenthi et al. 2006). However, despite the prevalence and severity of teratospermia in felids, its aetiology remains unknown.

The progressive loss of GD has been identified as one of the major challenges for felid conservation (Obrien et al. 1985, Facemire et al. 1995, Nowell & Jackson 1996). The effects of low GD on fertility have been studied more in male felids than in females (Wildt et al. 1987, Barone et al. 1994). Cheetahs, for example, experienced a major genetic bottleneck approximately 10000 years ago and are now almost genetically monomorphic (Obrien et al. 1985, Menotti-Raymond & O'Brien 1993, Pukazhenthi et al. 2006). The high prevalence of teratospermia in this species may be linked to low GD, but a causal link is difficult to prove due to a lack of more genetically diverse populations for comparison. Data from other species support a causal relationship (Brown et al. 1991, Pukazhenthi et al. 2006). For instance, lions from populations with poor GD produce ejaculates with a higher proportion of pleiomorphic sperm than lions from populations with better GD (Wildt et al. 1987, Brown et al. 1991). Inbred domestic cats have also been found to exhibit severe teratospermia (>85% abnormal); a single generation of inbreeding is sufficient to decrease semen quality (Howard et al. 1990, Pukazhenthi et al. 2000, Neubauer et al. 2004). This is particularly concerning, as it suggests that the negative effects of inbreeding can occur over a short period, and is especially problematic given the severity of felid habitat loss and fragmentation (Nowell & Jackson 1996, Wolf & Ripple 2017). It is unlikely, however, that poor GD is the only cause of the high prevalence of teratospermia within Felidae, since some species (e.g. Canadian lynx *Lynx canadensis*, Eurasian lynx *Lynx lynx*, and some *Felis* spp.) produce teratospermic ejaculates despite reasonably high GD (Schwartz et al. 2003, Pukazhenthi et al. 2006, Swanson 2006, Schmidt et al. 2011).

Captivity-related stress (the activation of the hypothalamic-pituitary-adrenal axis in response to the captive environment) has been shown to suppress the hypothalamic-pituitary-gonadal axis and adversely affect male fertility (Morgan & Tromborg 2007, Koester et al. 2015). Glucocorticoids are physiological markers of stress

(Mostl & Palme 2002), and captive cheetahs, Canadian lynx, margay *Leopardus wiedii*, and tigrinas *Leopardus tigrinus* have all been found to exhibit higher faecal glucocorticoid metabolite concentrations than their wild conspecifics, suggesting that the captive environment is stressful for these species (Wielebnowski et al. 2002, Terio et al. 2004, Moreira et al. 2007, Fanson et al. 2012). Glucocorticoids have been shown to act on all levels of the hypothalamic-pituitary-gonadal axis to suppress ovarian and testicular function (Orr & Mann 1992, Yazawa et al. 2000, Breen & Karsch 2004, Gore et al. 2006, Kirby et al. 2009), so it is not surprising that the elevated faecal glucocorticoid concentrations observed in captive felids have been linked to impaired reproductive activity (decreased testosterone concentrations and reduced ovarian activity, or even ovarian quiescence; Wildt et al. 1993, Jurke et al. 1997, Terio et al. 2004, Moreira et al. 2007, Fanson et al. 2012). From a management perspective, it is encouraging that moving reproductively inactive females to larger, more enriched enclosures has led to decreased faecal glucocorticoid concentrations and the resumption of ovarian cyclicity (Jurke et al. 1997, Moreira et al. 2007). The reproductive activity of male cheetahs also appears to be higher when they are held off-exhibit than when they are on-exhibit (Koester et al. 2015, 2017). Given that much of the research available on the reproductive biology of felids has been conducted in captivity, it is tempting to speculate that differing enclosure conditions may have contributed to the variability in oestrous cycles and ejaculate traits of the different species.

Implications for management in captivity

It is evident that further research into the reproductive biology of many felid species is required to optimise the success of both captive breeding programmes and *in situ* conservation strategies. Furthermore, studies investigating the reproductive biology of felids should be conducted on larger sample sizes to capture the high degree of variability in the oestrous cycles and ejaculate characteristics of felids.

The high degree of variability of natural felid oestrous cyclicity potentially provides support for the use of exogenous gonadotrophins to stimulate follicular growth (e.g. equine chorionic gonadotrophin) and trigger ovulation (e.g. human chorionic gonadotrophin), especially for the purpose of assisted reproductive technologies (ART; Thongphakdee et al. 2018). However, responses of felids to exogenous gonadotrophin treatment also appear to be highly variable (Thongphakdee et al. 2018). In fact, Thongphakdee et al. (2018) stated that the highly variable ovarian response of felids to exogenous gonadotrophins is a 'major restriction' for ART. Pre-treatment with a

follicular inhibitor (e.g. levonorgestrel or altrenogest) has been shown to result in a more consistent ovarian response (i.e. greater follicular development) to exogenous gonadotrophins (Pelican et al. 2003, 2006, 2010, Stewart et al. 2012). A more consistent ovarian response could also be achieved by stimulating oestrus during the non-breeding season, when all follicles are undeveloped, although this remains to be tested in felids.

The effect of seasonality on reproductive activity should be considered when breeding felids in captivity, since season can affect ovarian cyclicity and responsiveness to exogenous gonadotrophins (Thongphakdee et al. 2018). It is important in the context of captive management and breeding that felids are exposed to a photoperiod that they would experience in their natural geographic range. Staff should consider the latitudinal ranges of free-living felid populations, since moving felids outside their natural latitudinal range (i.e. seasonal photoperiod) may affect their seasonality and thus reproductive performance.

It is recommended that ejaculates/sperm samples are collected during the breeding season, since ejaculates collected during the breeding season generally have a higher concentration of sperm and fewer morphological abnormalities than those collected outside the breeding season (Appendix S3). The method used to collect ejaculates/ sperm samples is also important. Electroejaculation is by far the most commonly used method to collect sperm from felids, but UC with pharmacological induction (i.e. intramuscular injection of medetomidine) yields much higher sperm concentrations (Appendix S3). The natural compensatory mechanism for teratospermia is to produce an ejaculate with higher concentrations (and a higher total number) of sperm (Müller et al. 2012), thus UC may be a better method for collecting sperm from felids. To date, UC has been used to collect sperm from only four felid species. Further investigation into the use of this method as an alternative to EE is required. Irrespective of collection method or seasonality, teratospermia is likely to remain a significant problem for many felid species. Since low GD is likely to be a major cause of teratospermia in felids, it is important that efforts to reduce further losses of GD are prioritised, especially for species facing severe habitat fragmentation and thus the formation of genetically isolated subpopulations. A long-term management goal for both *in situ* and *ex situ* conservation efforts for felids must be to maintain and enhance GD.

Assisted reproductive technologies such as artificial insemination and *in vitro* fertilisation/embryo transfer are likely to be critically important in maintaining and improving the GD of both captive and wild felid populations. Unfortunately, ART have had little success in felids (<25% success rate; Barone et al. 1994, Roth et al. 1997, Howard et al. 1997, Pope et al. 2006). Several factors appear to

contribute towards this. For example, the widespread use of ketamine-hydrochloride as the primary anaesthetic is problematic, given that its use can prevent ovulation and even cause abortions in some felids (Howard et al. 1992, Brown & Wildt 1997, Braun et al. 2009). Alternative anaesthetic drugs that do not significantly affect the reproductive cycles of female felids are required. In addition, a combination of sedative and anaesthetic should be used to minimise stress associated with artificial insemination procedures.

Captivity-related stress is problematic for the captive management and breeding of felids, and stress may reduce or completely disrupt the reproductive cycles of female felids. Captivity-related stress also decreases testosterone concentrations and the ejaculate quality in felids, although the effects of the captive environment on ejaculate quality are difficult to assess due to the limited number of control samples collected from wild felids (Appendix S3). More studies are required that compare the ejaculate traits and testosterone concentrations of captive and wild felids.

Environmental enrichment of enclosures can improve the welfare and reproductive activity of captive felids (Moreira et al. 2007), and is encouraged. Enclosure complexity appears to be of greater importance than enclosure size. Individuals selected for breeding should also be kept off-exhibit and have their exposure to humans minimised (Koester et al. 2015, 2017).

CONCLUSIONS

1. This is the first detailed review of the reproductive biology of extant felids.
2. Reproduction-focused literature is biased towards the domestic cat, cheetah, and panthera lineages (83% of papers reviewed); there is a paucity of research on many species in the domestic cat, leopard cat, ocelot, caracal, and bay cat lineages. Research effort is largely biased towards the larger cat species (e.g. *Panthera* spp.), possibly because these species are more common in captivity.
3. The oestrous cycles of female felids are highly variable. Studies of oestrous cycles of felids should therefore use the largest possible sample sizes. The exact causes of the variability are unclear, but seasonality, dietary factors, group housing and captivity-related stress are likely to contribute.
4. Many felids have highly seasonal reproduction that is driven largely by photoperiodic signalling. Other factors that affect the reproductive seasonality of felids include body weight and prey (food) availability.
5. Teratospermia is a severe problem in felids and has been reported in all but one felid lineage. All species in the puma and lynx lineages appear to produce severely

teratospermic ejaculates. The aetiology of the high prevalence of teratospermia in felids is unclear and requires further research.

6. Reduced fertility in felids appears to be linked to loss of GD. The maintenance and enhancement of GD should be a long-term goal for both *in situ* and *ex situ* felid conservation management. Habitat protection and the use of ART are encouraged, although the success rates of ART in felids are currently low.

7. A short-term management goal should be to improve the success of ART in felids by regulating dietary intake and minimising captivity-related stress, both of which can adversely affect fertility. The ejaculate quality could be improved by collecting sperm during the breeding season. The use of UC rather than EE warrants further investigation. Captivity-related stress can be reduced through enriching and increasing the complexity of enclosures.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Appendix S1. The main threats, population trends, International Union for Conservation of Nature (IUCN) Red List of Threatened Species status, and Convention for International Trade of Endangered species (CITES) rankings of the 38 extant felid species.

Appendix S2. Summary of the existing literature (84 publications) on the female reproductive biology of the 38 extant felid species.

Appendix S3. Summary of the existing literature (93 publications) on the ejaculate traits of the 38 extant felid species.

Appendix S4. Methods used for semen collection and assessment in felids.

Appendix S5. The weighted averages of ejaculate characteristics of captive and wild puma, cheetah, Iberian lynx, jaguars, and lions.