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Reproductive biology and assisted reproductive technologies in felids

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Abstract

Reproductive biology has been thoroughly described in several felid species. Specifically, patterns of ovarian steroid hormones have been reported for more than half of 39 felids, and it has been observed that there is a high degree in reproductive diversity among species. For example, although females of most felids are polyestrous with induced ovulation, some species such as the clouded leopard (*Neofelis nebulosa*) and fishing cat (*Prionailus viverrinus*) spontaneously ovulate. To date, assisted reproductive technologies, including sperm cryopreservation, artificial insemination and in vitro embryo production have been developed in both domestic and wild felids that result in live offspring. This paper reviews the diversity of reproductive mechanisms and discusses the status of reproductive technologies that have been applied to wild felid conservation.

Keywords: Felids, reproductive cycle, seasonality, assisted reproductive technologies

Introduction

Currently, there are 39 extant species within the family *Felidae*, including the domestic cat (*Felis catus*). Due to ongoing habitat destruction and persecution, half of wild felid species are listed as vulnerable or 'endangered' by the International Union for Conservation of Nature (Table 1).[1] Therefore, ex situ management has become increasingly important in the conservation of several wild felid species, such as the cheetah (*Acinonyx jubatus*), clouded leopard (*Neofelis nebulosa*), and Iberian lynx (*Lynx pardinus*). Ex situ individuals serve as a 'genetic repository' for retaining all existing heterozygosity in the case of an unexpected catastrophic event impacting wild counterparts, as well as a source population for future reintroduction. Furthermore, these individuals also are invaluable resources for basic and applied research to generate information that can be challenging to obtain from free-ranging populations. Finally, zoo animals also serve as ambassadors for raising public awareness and garnering financial and political supports for conservation of wild populations.[2]

Reproductive science has had pivotal roles in genetic management of ex situ wildlife.[3] For felids, reproductive studies began in the early 1980s due to the interest in the potential benefit of assisted reproductive technologies (ARTs) in maintaining heterozygosity of small, isolated captive populations, and to advance the understanding about species-specificity in reproductive mechanisms.[4] To date, reproductive biology has been described in several felids, and patterns of ovarian steroid hormones have been reported for more than half of the 39 species. Studies to date indicate that there are variations in reproductive characteristics among felid species, including age at sexual maturation, reproductive seasonality, seminal traits, and ovulatory pattern.[5] Despite these differences, ARTs developed in domestic cats have been successfully applied to several wild felids.[6] This paper summarizes the diversity of reproductive mechanisms within the family Felidae and discusses the status of ARTs that have been applied to wild felid conservation.

Felid reproductive biology

Reproductive cycle of felids presents a great variation in terms of seasonality and ovulation mechanisms (Table 1).[7-23] The reproductive cycle consists of 4 stages: proestrus, estrus, diestrus, and anestrus.[5] Unlike canids,[24] proestrus is rather short in felids, lasting < 1 day. Estrus is characterized by the presence of an estrogen peak from preovulatory follicles. After ovulation (induced or spontaneous), female felids enter diestrus, characterized by elevated progesterone concentrations lasting throughout pregnancy or nonpregnant luteal phase. Anestrus is the period of ovarian quiescence when circulating estrogens remain at basal concentrations. Duration of interestrous interval varies among species, ranging from 1 - 145 days.[25] Age at sexual maturation of male and female felids varies among species depending on body size. Specifically, small felids such as *Leopardus* spp. reach sexual maturity between 1 - 2 years of age, whereas larger felids, such as lions and tigers reproduce at 3 - 4 years of age.[5,26]

Reproductive biology (i.e. reproductive cycle, folliculogenesis, oogenesis, and spermatogenesis) has been thoroughly studied in the domestic cat.[27-30] Development of noninvasive methods to assess gonadal hormone metabolites in feces[31] has allowed studies to determine reproductive cycles of wild felids. Through these studies, reproductive cycles of 24 wild felid species have been described.[5,25,32] We now know that there are differences in reproductive seasonality and ovulatory patterns among species or individuals within the same species. For example, reproductive seasonality of domestic cat is influenced by the duration of daylight. Under natural light, cats living in areas from Equator to Tropic of Cancer can produce litters throughout the year, whereas those living from Tropics toward the polar circle breed seasonally (between January and July).[33,34] Female

Table 1. Wild felids species reproductive characteristics and 'red list' status

Genetic lineage	Common name	Scientific name	Red list status	Reproductive seasnonality	Ovulatory pattern
Panthera	Tiger[5]	*Panthera tigris*	En	Seasonal	Induced
	Snow leopard[7]	*P. uncia*	Vu	Seasonal	Induced
	Leopard[5]	*P. pardus*	Vu	Year-round	Occasionally spontaneous
	Lion[5,8]	*P. leo*	Vu	Year-round	Occasionally spontaneous
	Jaguar[9]	*P. onca*	NT		Induced
	Clouded leopard[5]	*Neofelis nebulosa*	Vu	Seasonal in temperate zone, but year-round if housed under long-day light cycle	Spontaneous
	Sunda clouded leopard	*N. diardi*	Vu	d.d	dd
Bay cat	Borneo bay cat	*Catopuma badia*	En	d.d.	d.d.
	Asian golden cat[73]	*Pardofelis temminckii*	NT	Year-round	d.d.
	Marble cat	*P.Marmorata*	NT	d.d.	d.d.
Caracal	Caracal[10]	*Caracal caracal*	LC	Year-round	d.d.
	African golden cat	*C. aurata*	Vu	d.d.	d.d.
	Serval	*Leptailurus serval*	LC	d.d.	d.d.
Ocelot	Ocelot[5]	*Leopardus pardalis*	LC	Year-round	Induced
	Margay[5]	*L. wiedii*	NT	Year-round	Spontaneous
	Pampus cat[11]	*L. colocolo*	NT	Year-round	d.d.
	Andean cat	*L. jacobita*	En	d.d.	d.d.
	Northern tiger cat[5]	*L. tigrinus*	Vu	Year-round	Induced
	Southern tiger cat[5]	*L. guttulus*	Vu	Year-round	Induced
	Geoffroy's cat[12]	*L. geoffroyi*	LC	Year-round	d.d.
	Guina	*L. guigna*	Vu	d.d.	d.d.
Lynx	Bobcat[13]	*Lynx rufus*	LC	Seasonal	d.d.
	Canadian lynx[13,14]	*L. canadensis*	LC	Seasonal	Occasional spontaneous
	Iberian lynx[13]	*L. pardinus*	En	Seasonal	Induced
	Eurasia lynx[13]	*L. lynx*	LC	Seasonal	Induced
Puma	Cheetah[15]	*Acinonyx jubatus*	Vu	Year-round	Induced
	Puma[16,17]	*Puma concolor*	LC	Year-round	Induced
	Jaguarundi	*Herpailurus yagouaroundi*	LC	d.d.	d.d.
Leopard cat	Pallas's cat[18,19]	*Otocolobus manul*	LC	Seasonal	Induced
	Rusty-spotted cat[20]	*Prionailus rubiginosus*	NT	Year-round	d.d.
	Leopard cat	*P. bengalensis*	LC	d.d.	d.d.
	Fishing cat[21]	*P. viverrinus*	Vu	Year-round	Spontaneous
	Flat headed cat	*P. planiceps*	En	d.d.	d.d.
Domestic cat	Domestic cat[5]	*Felis catus*	NA	Seasonal	Occasionally spontaneous
	Wild cat[22]	*F. silvestris*	LC	Seasonal	d.d.
	Chinese Mountain cat	*F. bieti*	Vu	d.d	d.d.
	Sand cat[23]	*F. margarita*	LC	Seasonal	d.d.
	Black footed cat	*F. nigripes*	Vu	d.d.	d.d.
	Jungle cat	*F. chaus*	LC	d.d.	d.d.

En: Endangered; Vu: Vulnerable; NT: Near threatened; LC: Least concern; NA: Not assessed; d.d.: data deficiency cats can cycle year-round when housed indoors under 12 - 14 hours light cycle.[5] Furthermore, the ability of cat oocytes to complete nuclear maturation and develop into embryos after in vitro fertilization (IVF) is lower during August to October compared to other months.[35] However, seasonal variations in seminal characteristics have not been consistently demonstrated in domestic cat.[34-38] Specifically, epididymal sperm obtained from cats castrated during increasing light (winter and spring) tended to have higher motility and total sperm cells than samples obtained during decreasing light (summer and fall), although variations in testosterone concentration were not observed.[36] Furthermore, motility and the proportion of morphologically intact epididymal sperm were higher in samples obtained in spring than in winter and testicular testosterone concentrations significantly reduced in autumn compared to spring.[34] However, there were no differences in sperm concentration, motility, morphology, acrosome integrity, and ability to penetrate an oocyte among samples obtained throughout the year. [35] Finally, there is no clear seasonal effect on the quality of ejaculated samples, although variations in plasma testosterone and luteinizing hormone (LH) concentrations between breeding versus nonbreeding season are observed.[37]

Influence of seasonality on seminal quality is more pronounced in some wild felids, including Pallas's cat (*Otocolobus manual*),[18,39] ocelot (*Leopardus pardalis*), margay (*L. wiedii*), Northern tiger cat (*L. tigrinus*),[40] Eurasian lynx (*Lynx lynx*),[41] and snow leopard (*Panthera uncia*).[42] For example, serum LH and seminal characteristics were higher during breeding than nonbreeding season in Pallas's cat, although there is no seasonal effect on circulating testosterone concentrations.[39] Furthermore, reproductive season can be simulated when housing male Pallas's cats indoors under artificial light, although mating attempts do not result in offspring production.[18] Conversely, there is no seasonal effect on seminal characteristics and testosterone concentrations in some wild felids, including tiger (*Panthera tigris*).[43] Some nonseasonal breeders, including the leopard, can breed year-round,[44,45] despite reductions in seminal quality and fertility during some months of the year.[46]

Generally, female felids are polyestrous animals. However, studies in Eurasian and Iberian lynx have demonstrated a 'noncat-like' ovarian cycle in these species.[47] Specifically, Eurasian and Iberian lynx (*L. pardinus*) exhibit seasonal monoestrus[47,48] that differs from their cousins, bobcat (*L. rufus*)[47] and Canadian lynx (*L. canadensis*).[14] Another unique aspect of Eurasian and Iberian lynx is the presence of persistent corpora lutea (CLs) that remain active (producing progesterone) for at least 2 years, and cooccur with new CLs of the next cycle.[13,48] To date, mechanisms supporting persistent CLs are unknown. However, it has been suggested that persistent CLs stimulate negative feedback to suppress folliculogenesis outside the breeding season.[48]

Traditionally, domestic cat has historically been described as an induced ovulator and ovulation is induced by multiple mating events. Specifically, mating stimulates the release of gonadotropin releasing hormone that, in turn, triggers the release of LH from the anterior pituitary that causes final follicle maturation and ovulation.[44] However, physical contact through mating may not be the only mechanism that stimulates ovulation in felids. Specifically, ovulation can be induced via visual, olfactory and/or auditory cues in the jaguar (*P. onca*).[49] There is also evidence that noncontact mechanisms can induce ovulation in Pallas's cat, as ovulations are observed in females housed adjacent to a male.[19] Spontaneous ovulation has been observed occasionally in the lion (*P. leo*) and leopard (*P. pardus*), especially when females are housed together.[44] In the clouded leopard, margay and domestic cat, spontaneous ovulation occurs more regularly.[5] Therefore, the ovulatory pattern in the family *Felidae* appears to be species- and/or individual-specific response to physical, visual, auditory, chemical and/or social stimuli.[5]

Seminal characteristics have been thoroughly assessed in domestic cat. Typically, domestic cat ejaculates obtained by electroejaculation (EEJ) are 100 to 738 µl in volume with 60.3 - 190 x 10^6 sperm/ml and 44 - 85% motility. Samples obtained via artificial vagina are smaller (< 10 µl) in volume with higher concentration (541 - 1,730 x 10^6 sperm/ml) than via EEJ, but with comparable motility.[50] To date, seminal traits have been characterized in 25 wild felid species demonstrating large variations in seminal quality, especially, morphologically abnormal sperm percentage within an ejaculate ranging 30 - 85% (Table 2). Teratospermia (i.e. > 60% morphologically abnormal sperm in an ejaculate) is common in felids, and this phenomenon is linked to reduced genetic variability. Specifically, species lacking heterozygosity tend to produce more malformed sperm than genetically diverse counterparts. A clear example is the case of the Florida panther (*Puma concolor coryi*), a subspecies of *Puma concolo*r. Due to human encroachment and agricultural expansion, the Florida panther has experienced severe population declines that resulted in significant reductions in heterozygosity compared to other puma subspecies.[51] Consequently, Florida panthers' reproduction is severely compromised.[51,52] Florida panther ejaculates are smaller (< 1 versus 3 ml) and contain fewer morphologically normal sperm (6 versus 40%) than their counterparts in Latin America.[52] Teratospermia was also observed in some domestic cats.[53] Teratospermic cats have a higher percent of morphologically abnormal sperm and lower circulating testosterone concentrations than normospermic individuals.[53] Sperm from teratospermic donors are compromised in metabolic function,[54] and ability to undergo capacitation and acrosome reaction, that in turn, reduce fertilizing ability.[55] Furthermore, teratospermic samples are sensitive to osmotic stress, and thus are highly susceptible to cryopreservation compared to normospermic counterparts.[56]

Finally, a recent study has demonstrated that cGMP and kinase phosphorylation pathways are downregulated in cat sperm from teratospermic donors, findings that further explain the mechanisms underlying reduced metabolic function and fertilization capacity.[57]

Table 2. Seminal traits (mean ± SEM) of wild felids

Species	No. ejaculates	Volume (ml)	Concentration (x 10^6 sperm/ml)	Motility (%)	Morphologically abnormal sperm (%)
Lion*[58]	7	0.423 ± 0.112	1,940 ± 606.0	84.1 ± 7.7	54.0 ± 9.7
Tiger[59]	13	7.0 ± 1.3	31.9 ± 8.6	81.5 ± 3.7	37.5 ± 6.9
Tiger**[60]	2	NA	52.3 ± 12.4#	38.3 ± 13.2	48.8 ± 13.9
Leopard[59]	13	5.1 ± 0.6	46.2 ± 9.8	43.8 ± 5.7	79.5 ± 2.0
Amur leopard*[61]		0.0067 ± 0.0037	1,698 ± 758	84.1 ± 9.7	NA
Jaguar*[62]	11	0.35 ± 0.09	2,635.2 ± 482.8	77.0 ± 3.44	
Snow leopard[42]	36	1.54 ± 0.1	29.2 ± 5.7	76.5 ± 2.4	65.0
Clouded leopard[63]	48	0.64 ± 0.03	27.5 ± 2.3	71.0 ± 2.1	38.9 ± 1.7
Cheetah[59]	15	1.8 ± 0.3	27.3 ± 5.8	69.0 ± 5.8	64.6 ± 4.9
Iberian lynx[64]	5	0.483 ± 63.0	7.6 ± 2.2	73.5 ± 5.6	82.3
Iberian lynx**[65]	4	NA	10.2 ± 1.78#	47.5 ± 2.5	69.3
Eurasian lynx[41]	3	0.34 ± 0.06	8.86 ± 4.6	60.0 ± 30.0	NA
Canadian lynx[66]	9	NA	13.0 ± 3.6	46 ± 12.0	71.0
Bobcat[67]	13	0.36 ± 0.08	24.4 ± 7.8	55.7 ±5.7	85.3
Ocelot[40]	42	1.4 ± 0.1	101.2 ± 10.6	81.4 ± 1.2	16.7 ± 1.5
Margay[40]	41	0.5 ± 0.01	75.6 ± 11.0	73.5 ± 1.3	42.6 ± 4.8
Northern tiger cat[40]	52	0.3 ± 0.1	411.9 ± 46.3	71.4 ± 2.3	40.8 ± 4.5
Serval[66]	6	NA	46.0 ± 14.7	72 ± 8.2	44.0
Pallas's cat18	4	0.164 ± 0.030	51.3 ± 30.7	70.8 ± 2.6	48.0
Leopard cat[68]	24	0.143 ± 0.015	37.0 ± 5.4	73.8 ± 2.6	34.6 ± 9.4
Fishing cat[69]	8	0.5 ± 0.1	108 ± 29	73.0 ± 4.0	66.7 ± 15.9
Flat headed cat[70]		0.121 ± 0.072	56.7 ± 18.7	56.3 ± 19.0	63.2
Jungle cat*[71]	4	0.069 ± 0.016	75.13 ± 9.84	77.13 ± 8.16	26.2 ± 3.51
Black footed cat[72]	12	NA	NA	85.0 ± 1.21	52.8
Sand cat72	18	NA	NA	78.6 ± 1.61	60
Asian Golden cat*[73]	2	0.089	88.4	62.5	62

*Samples were collected via urethral catheterization; **Samples were collected via epididymal slicing; #Total sperm; NA: not assessed

Assisted reproductive technologies

Assisted reproductive technologies such as artificial insemination (AI) and in vitro embryo production developed for the domestic cat has been successfully applied to many wild felid species,[6] with pregnancy and/or live offspring after AI with fresh or frozen-thawed sperm reported in 14 felids.[6] Furthermore, embryos have been produced by IVF in 16 wild felid species, nine of them resulted in births of live offspring.[6] Excellent reviews on reproductive technologies, including somatic cell nuclear transfer and stem cell technologies in felids have been published recently.[6,74] Therefore, this section will focus only on technologies that have been applied to wild felid conservation.

Semen collection

With the exception of the domestic cat where semen can be obtained via artificial vagina,[50] sperm recovery from wild felids has been traditionally done with EEJ (Table 2).[25,75,76] However, during the past decade, urethral catheterization after medetomidine treatment has been widely applied to recover semen from domestic and wild felids, including lions,[58] jaguars,[62] Amur leopards (*P. pardus orientalis*),[61] jungle cats (*Felis chaus*),[71] and Asian golden cats (*Pardofelis temminckii*).[73] Comparisons between EJJ and urethral catheterization had varying results. Specifically, there were no differences in fertilizing ability and cryosurvival between samples obtained via urethral catheterization and EEJ, although the former had lower volume and higher concentration than latter.[77] However, among urethral catheterization, EEJ, and epididymal slicing, seminal quality was lowest in samples obtained via urethral catheterization.[78]

Difference between these 2 studies may be due to the variation in the interval between the sample collection methods, as ejaculation frequency impacts seminal quality.[50] Specifically, in the first study, there was a 4-day interval between urethral catherization and EEJ,[77] whereas in the second study, all 3 methods were performed once on the same day with urethral catherization being conducted prior to EEJ and then epididymal slicing.[78] Despite the inconsistent results in seminal quality, urethral catheterization is simple and requires minimum equipment, and thereby can be applied to free-ranging felids living in a remote area.[62]

The ability to recover sperm postmortem allows preservation of valuable genetics of individuals that die unexpectedly. Postmortem recovery of sperm has been attempted for at least [73] individuals across 16 species of wild felids.[60,65,79] There were no differences between EEJ and epididymal slicing in seminal quality of domestic cat samples recovered from the same individual.[78] Such direct comparison is not feasible in wild felids as most samples were obtained after death,[79] Yet, information available to date has indicated that the quality of epididymal sperm of tigers[60] and Iberian lynx[65] obtained postmortem appears to be slightly lower than those obtained via EEJ as reported before.[59,64] Finally, a recent study[79] collected[42] spermic samples from 67 gamete rescue attempts in 15 wild felid species. Of the 42 samples, 14 (33.3%), 28 (66.6%) and 35 (83.3) were suitable for AI, IVF, and intracytoplasmic sperm injection (ICSI), respectively.[79]

Sperm cryopreservation and artificial insemination

Sperm cryopreservation has been broadly applied to preserve valuable genetic of several wild felids living both ex situ and in situ.[60,74,79-83] Egg yolk-based extenders containing glycerol as a cryoprotectant have been commonly used to cryopreserve sperm from both domestic and wild felids.[79,80,82,84] However, egg yolk-based extenders are not chemically defined and can vary from batch to batch. Furthermore, commercial egg yolk-based extenders had frequent bacterial or mycoplasma contamination.[85] Therefore, studies were conducted to explore the value of soy lecithin-based extender (SOY) in cryopreservation of felid sperm. Domestic cat sperm cryopreserved in SOY fertilized cat oocytes at a rate similar to those frozen in egg yolk-based extender.[86] Furthermore, in a study in the black-footed cat (*F. nigripes*), sand cat (*F. margarita*), fishing cat, and Pallas's cat, there were no differences in postthaw motility and acrosome status between sperm cryopreserved in SOY versus in egg yolk-based medium.81 Furthermore, cryopreserved sperm from both treatments were able to fertilize in vivo matured domestic cat oocytes at a similar rate, although fertilization rates varied among species.[81]

To date, AI with fresh or frozen semen has been applied to both domestic and wild felids, resulting in pregnancies and live births.[6] Like other mammalian species, there are several factors that influence AI success. These include responses to ovarian stimulation, seminal quality, and time and site of insemination.[6] Induction of estrus and ovulation in felids commonly involves equine chorionic gonadotropin (eCG) treatment to stimulate ovarian follicle growth, followed by human chorionic gonadotropin (hCG) treatment 80 - 84 hours later to induce ovulation.[75] An advantage of this ovarian stimulation regimen is that it minimizes stress associated with animal handling. However, eCG and hCG are large, foreign glycoproteins that persist in the circulation and, thereby induce production of gonadotropin-neutralizing antibodies.[75] Because of the refractory effect of eCG/hCG, it has been recommended that this hormone regimen is given to the same animal not more than once every 6 - 12 months.[75] Porcine follicle stimulating hormone (pFSH) also has been used to stimulate ovarian response in felids.[75,87] However, this gonadotropin stimulation protocol requires multiple injections that present logistical challenge when applied to wild felids.[75] Furthermore, although pFSH treatment has been successfully used for recovery of mature oocytes and embryos in domestic cats and wild felids,[87] pregnancies after AI have not been achieved in cheetahs, leopards, lions, and tigers.[75] Some felid species are insensitive to eCG/hCG treatment.[75] Interestingly, the variation in the response to exogenous gonadotropin is independent of body size. For example, domestic cat, leopard cat and Northern tiger cat are the same size; yet, the latter species require twice the dosages of eCG compared to the 2 former felids.[75]

Although intravaginal insemination with fresh and frozen semen has resulted in live births in the domestic cat, this AI method has not resulted in pregnancies when applied to wild felids, including cheetahs, tigers and clouded leopards.[75] To date, successful pregnancies after AI with fresh or frozen semen in wild felids have been achieved by transcervical, intrauterine (either laparotomy or laparoscopy) or laparoscopic oviductal insemination.[6,88] Ejaculates of many small felids contain $< 50 \times 10^6$ motile sperm, and thus limit the use of cervical or uterine AI.[88] However, the recent development of laparoscopic oviductal insemination has allowed AI to be successfully applied to small felids, including Pallas's cat, fishing cat, sand cat, and ocelot.[88] To date, live offspring has been produced after AI with fresh or frozen sperm in these species. Oviductal AI also has been applied to larger felids, including tigers and clouded leopard.[88]

In vitro embryo production

Extrapolation of reproductive technologies developed in the domestic cat has yielded encouraging outcomes in several wild felids. Specifically, currently, in vitro embryo production from in vivo or in vitro matured oocytes has been reported in 16 wild felids, of which 9 resulted in live offpring.[6,76] Intracytoplasmic sperm injection with cryopreserved ejaculated or epididymal sperm has also been attempted in the jaguarundi, lion and fishing cat, yielding 56 - 70% cleavage rate.[76] However, transfer of preimplantation stage embryos did not result in pregnancy.[76] Although live offspring have been produced in a number of wild felids, in vitro embryo production has not been widely used in captive breeding programs for several reasons. First, there is limited information on species-specific reproductive endocrinology, gamete biology and embryogenesis. Second, the complexity of the procedure and the need for specialized equipment and facility to recover oocyte, perform IVF or ICSI and culture resulting embryos. Third reason is the limited availability of developmentally competent oocytes, especially in aging females or those with poor health. Recently, live births have been reported after transferring in vitro derived embryos produced from a 6 year old female cheetah into a younger recipient (3 year old).[89] Uterine pathologies can be frequently observed in cheetah when females reach 6 years of age resulting in infertility.90 Nevertheless, these older females can produce developmentally competent oocytes that can be fertilized and developed into embryos in vitro.[90] Therefore, recent success in cheetah clearly demonstrates the potential benefit of IVF technology in preserving fertility of genetically valuable, and underrepresented females whose genetic would be lost otherwise.

Conclusion

Domestic cat serves as a valuable model for establishing reproductive technologies in wild felids. Yet, due to the large diversity in reproductive mechanisms within the family *Felidae*, there are still needs for species-specific research, especially on how to effectively manipulate female reproductive cycles for AI, oocyte retrieval or embryo transfer. Although applied research such as characterization of seminal traits and reproductive cycles in understudied species remains a high priority, fundamental research also should be conducted in parallel to advance our understanding of mechanisms regulating gamete and embryo development. Such information is crucial for successful implementation of 'high-tech' approaches including somatic cell nuclear transfer to wild felid conservation, as these technologies would provide opportunities to produce offspring from genetically valuable individuals when natural breeding or a more conventional ARTs are not feasible.

Conflict of interest

There are no conflicts of interest to declare.

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